Hyperlipidemia and Fat Absorption in Model Rats with Type 2 Diabetes Mellitus

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ABSTRACT

Type 2 diabetes mellitus is frequently complicated by hyperlipidemia, which is closely related to the occurrence of atherosclerotic disorders. The small intestine, which absorbs dietary lipids, plays an important role in the regulation of the serum lipid concentrations. We confirmed morphological changes in small intestinal villi in the progression of diabetes mellitus and evaluated intestinal lipid absorption by the $^{13}$C-trioctanoin breath test in model rats with type 2 diabetes mellitus (OLETF rats). We measured the height of the intestinal villous epithelium and serially measured the expiratory $^{13}$CO$_2$ concentration after the administration of $^{13}$C-trioctanoin in OLETF rats at the ages of 28, 36, and 44 weeks. The serum total cholesterol and triglyceride concentrations were significantly higher in the OLETF rats than in the control LETO rats. The height of the small intestinal villi was increased, indicating hyperplastic change. The $^{13}$CO$_2$ concentration was significantly increased, suggesting hyperplasia of the small intestinal villi and enhancement of lipid absorption or increased total amount absorption.

Excessive lipid absorption from the small intestine was suggested to be one of the causes of hyperlipidemia (particularly hypertriglyceridemia) complicating diabetes mellitus.

Introduction

Lifestyle-related diseases such as diabetes mellitus and hyperlipidemia are increasing with the increased availability of western-style food and associated excessive energy intake.

Particularly, type 2 diabetes mellitus, in the etiology of which genetic factors and environmental factors are intertwined, has increased until it accounts for 85% or more of diabetes mellitus. Diabetes mellitus is often complicated by abnormalities of serum lipid metabolism, and quantitative abnormalities in lipoproteins such as an increase in the serum triglyceride level, a decrease in the HDL cholesterol level, an increase in the LDL cholesterol level, and postprandial hyperlipidemia are observed. These abnormalities in lipoprotein metabolism are closely related to the development of atherosclerotic disorders such as ischemic heart disease and cerebrovascular...
disorders and are important as prognostic factors in diabetic patients. 1)

The absorption of dietary lipids by the small intestine, which is the direct intake of all nutrients, plays an important role in the regulation of serum lipid levels along with lipid metabolism in adipose tissue and the liver. Hyperplasia of the villous epithelium was observed in STZ-diabetic rats, which are a model of insulin-requiring diabetes mellitus, and a relationship between the small intestinal absorption and the blood glucose level has been reported.2-6) There have also been reports that the absorption of glucose, sucrose, and triglycerides was increased in experiments using GK rats and STZ-diabetic rats,6-9) and an increase in glucose or lipid absorption due to an increase in the area of the small intestinal epithelium is considered to be involved in the pathology of diabetes mellitus in addition to the role of apolipoprotein in the lipid transfer in small intestinal epithelial cells.

In this study, the occurrence of hyperlipidemia complicating diabetes mellitus and morphological changes in the small intestinal villi were examined in OLETF diabetic rats, which are a model of spontaneously-occurring type 2 diabetes mellitus. Moreover, to confirm increased lipid absorption, a 13C-trioctanoin breath test was performed, and the lipid absorbing function in a diabetic condition was evaluated.

Materials and Methods

I. Materials

As model rats of type 2 diabetes mellitus, 20 each of 5-week-old (body weight 180-200 g) male Otsuka Long-Evans Tokushima Fatty (OLETF) rats, and male Long-Evans Tokushima Otsuka (LETO) rats as control were used (provided by Tokushima Research Center, Otsuka Pharmaceutical Co., Ltd.).

The rats were housed at 4 per cage in an animal room adjusted to a room temperature of 20-25°C. They were given water and food (solid food for experimental animals MM-3, Funabashi Farm, Chiba), and the animal room was illuminated by 12-hour light and dark cycles.

The following items were evaluated 3 times at 2-month intervals, i.e., 28 weeks after birth (28W group, 6 animals), 36 weeks after birth (36W group, 6 animals), and 44 weeks after birth (44W group, 8 animals).

This experiment was approved by the Animal Research Committee of Osaka Medical College, and the animals received care according to the guidelines of this committee.

II. Methods

1. 13C-trioctanoin breath test

After 24 hours fasting, each rat was placed in an airtight chamber (capacity 5 L, Metabolica MC-AP, Sugiyama-Gen Iriki Co., Ltd., Tokyo) for 30 minutes. After the air in the chamber was stirred well in an airtight condition, 20 ml (baseline expiration) was collected, and the baseline value was determined. After ventilation of the chamber, 20 mg/kg solution (dissolved in 1 ml of olive oil) of 13C-trioctanoin (Trioctanoin-1, 1, 1, -13C; Cambridge Isotope Laboratories, Inc., UK) was administered into the stomach using a probe, and air (expiration) in the air tight chamber was collected after 30 minutes by the same method. Expiration was collected a total of 4 times at 30-minute intervals until 120 minutes after the administration. The chamber was sufficiently ventilated and cleaned during each interval between the measurements. The 13CO2 concentration in the collected air was determined using an isotope ratio mass spectrometer (Traser MAT; Finnigan MAT, San Jose, CA). The increase rates of the values after 30, 60, 90, and 120 minutes compared with the baseline value before the administration were calculated (Δ 13CO2 (%) = 13C-trioctanoin after administration - 13C-trioctanoin before administration). The highest value in each group was regarded as the peak value, the sum of Δ 13CO2 was calculated as the area under the curve, and each parameter was evaluated.

2. 13C-acetate breath test

The 13C-acetate (acetic[1-13C, 99AP13C1] acid as the Na salt; Mass Trace, Woburn, MA) gastric emptying breath test was performed using 6 each of 44-week-old OLETF and LETO rats. Similarly to the 13C-trioctanoin breath test, rats fasted for 24 hours were placed in an airtight chamber for 15 minutes. After the air in the chamber was stirred well, 20 ml was sampled, and the baseline value was determined. After ventilation, a 2 mg/kg-solution (dissolved with 50% glucose solution) of 13C-acetate was administered into the stomach using a stomach tube. After 15 minutes, the air in the chamber (expiration) was stirred, and 20 ml was sampled. Thereafter, expiration was sampled by the same method a total of 12 times at 15-minute intervals until 180 minutes after the administration. Increase rates in the 13CO2 concentration compared with the baseline value
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before the administration of $^{13}$C-acetate, ($\Delta^{13}$CO$_2$ (‰) = $^{13}$C-acetate after administration - $^{13}$C-acetate before administration) were calculated, the highest value in each group was regarded as the peak value, and each parameter was evaluated.

3. Blood biochemical examinations

After the breath tests, under ether anesthesia, blood was collected by percutaneous cardiac puncture. The animals were sacrificed by this blood sampling. The body weight before blood sampling, total protein (Biuret method), fasting glucose (enzymatic method), hemoglobin A1c (latex agglutination turbidimetry), serum insulin (RIA2 antibody method), total cholesterol (enzymatic method), and triglyceride (GPO/HIDAOS: Glycerol blanking method) were measured.

4. Histological examinations

1) Small intestine

After the animals were sacrificed by blood sampling, the small intestine was removed, immediately, and the length from the pyloric ring to the terminal ileum was measured. From the resected small intestine, a 3-cm segment 20 cm anal from the pyloric ring (proximal small intestine) was resected, fixed in 20% buffered formalin for 24 hours, embedded in paraffin, and sectioned at a thickness of 3-µm. The paraffin sections were stained with hematoxylin and eosin (H&E.). The length from the lamina muscularis mucosae to the end of villi (height of villi) was measured using a video micrometer (VM-30, OLMPUS, Tokyo) under light microscopy at three randomly selected sites in each sample, and the mean value was calculated (Fig. 1).

2) Pancreas

The pancreas was resected, fixed in 20% buffered formalin for 24 hours, and embedded in paraffin. The samples were examined after insulin antibody staining.

5. Statistical evaluation

Data are expressed as the mean ± standard deviation. Statistical analyses were performed using the Student’s t-test at the 5% level of significance.

Results

1. Body weight

In the 28W, 36W, and 44W OLETF groups, the body weights of rats were 588.3 ± 8.7, 623.3 ± 2.1, and 628.3 ± 9.5 g respectively. In the 28W, 36W, and 44W control LETO groups, weights were 438.3 ± 4.8, 445.0 ± 7.6, and 441.7 ± 7.5 g respectively. Rats in the 28W, 36W, and 44W OLETF groups weighed significantly heavier than those in the corresponding LETO group at each time point (Fig. 2-A). The ratio of the body weight of OLETF rats relative to that of LETO rats (OLETF/LETO ratio) increased significantly greater in the 44W group than in the 28W group (Fig. 2-B).

2. Blood biochemical examinations

1) Total protein: The total protein concentrations in the 28W, 36W, and 44W OLETF groups were 7.62 ± 0.17, 9.10 ± 1.24, and 8.36 ± 0.94 g/dl respectively; those in the 28W, 36W, and 44W LETO groups were 6.45 ± 0.19, 6.53 ± 0.05, and 6.49 ± 0.11 g/dl respectively. Each OLETF group showed a significantly higher protein concentration than the corresponding LETO group at each time point (Fig. 3-A). The OLETF/LETO ratio was not significant difference among the time points (Fig. 3-B).

2) Insulin: In the OLETF rats, the insulin concentrations in the 28W, 36W, and 44W OLETF groups were 7.62 ± 0.17, 9.10 ± 1.24, and 8.36 ± 0.94 g/dl respectively; those in the 28W, 36W, and 44W LETO groups were 6.45 ± 0.19, 6.53 ± 0.05, and 6.49 ± 0.11 g/dl respectively. Each OLETF group showed a significantly higher protein concentration than the corresponding LETO group at each time point (Fig. 3-A). The OLETF/LETO ratio was not significant difference among the time points (Fig. 3-B).
0.07, 0.13 ± 0.02, and 0.27 ± 0.08 ng/ml respectively. The difference between the two groups was not significant at 28W, but the values were significantly higher in the OLETF groups than in the LETO groups at 36W and 44W (Fig. 4-A). The OLETF/LETO ratio was significantly higher at 36W than at other time points (Fig. 4-B).

**Body weight**

![Body weight graph](image1)

Fig. 2. Body weight in each OLETF group and LETO group.
A: average are expressed as the mean ± SD.
*** p<0.005, significant deference compared with control (LETO) rats.
B: OLETF/LETO ratio are expressed as the ratio of the body weight of OLETF rats relative to that of LETO rats. * p<0.05

**Total protein**

![Total protein graph](image2)

Fig. 3. Total protein concentration in each OLETF group and LETO group.
A: average are expressed as the mean ± SD.
* p<0.05; *** p<0.005, significant deference compared with control (LETO) rats.
B: OLETF/LETO ratio are expressed as the ratio of the total protein concentration of OLETF rats relative to that of LETO rats.
3) Fasting blood glucose: Fasting blood glucose concentrations in the 28W, 36W, and 44W OLETF groups were 163.7±9.0, 234.8±14.0, and 230.6±8.2 mg/dl respectively; those in the 28W, 36W, and 44W LETO groups were 135.7±9.0, 136.0±5.2, and 174.3±5.4 mg/dl respectively. It was significantly higher in the OLETF groups than in the LETO groups at each time point (Fig. 5-A). The OLETF/LETO ratio was significantly higher at 36W than at other time points (Fig. 5-B).

**Insulin**

![Average Insulin Concentration](image1)

Fig 4. Insulin concentration in each OLETF group and LETO group.
A: average are expressed as the mean ± SD.
*** p<0.005, significant difference compared with control (LETO) rats.
B: OLETF/LETO ratio are expressed as the ratio of the insulin concentration of OLETF rats relative to that of LETO rats. * p<0.05; ** p<0.01

**Glucose**

![Average Glucose Concentration](image2)

Fig 5. Fasting blood glucose concentration in each OLETF group and LETO group.
A: average are expressed as the mean ± SD.
* p<0.05, *** p<0.005, significant difference compared with control (LETO) rats.
B: OLETF/LETO ratio are expressed as the ratio of the fasting blood glucose concentration of OLETF rats relative to that of LETO rats. ** p<0.01; *** p<0.005
4) **Hemoglobin A1c:** In the OLETF rats, the hemoglobin A1c concentrations in the 28W, 36W, and 44W OLETF groups were 3.02 ± 0.07, 3.04 ± 0.10, and 3.00 ± 0.05% respectively; those in the 28W, 36W, and 44W LETO groups were 2.33 ± 0.14, 2.55 ± 0.10, and 2.27 ± 0.09% respectively. It was significantly higher in the OLETF groups than in the LETO groups at each time point (Fig. 6-A). The OLETF/LETO ratio was no significant difference among the time points (Fig. 6-B).

![Fig. 6 Hemoglobin A1c concentration in each OLETF group and LETO group.](image)

A: average are expressed as the mean ± SD.

*** p<0.005, significant deference compared with control (LETO) rats.

B: OLETF/LETO ratio are expressed as the ratio of the hemoglobin A1c concentration of OLETF rats relative to that of LETO rats.

![Fig. 7 Total cholesterol concentration in each OLETF group and LETO group.](image)

A: average are expressed as the mean ± SD.

* p<0.05; *** p<0.005, significant deference compared with control (LETO) rats.

B: OLETF/LETO ratio are expressed as the ratio of the total cholesterol concentration of OLETF rats relative to that of LETO rats.
5) Total cholesterol: Total cholesterol concentrations in the 28W, 36W, and 44W OLETF groups were 155.0±7.2, 141.2±8.1, and 161.2±17.6 mg/dl respectively; those in the 28W, 36W, and 44W LETO groups were 116.0±3.0, 122.3±1.8, and 119.1±3.6 mg/dl respectively. It was significantly higher in the OLETF groups than in the LETO groups at each time point (Fig. 7-A). The OLETF/LETO ratio was no significant difference among the time points (Fig. 7-B).

6) Triglyceride: Triglyceride concentrations in the 28W, 36W, and 44W OLETF groups were 186.5±21.4, 310.2±121.7, and 457.6±91.2 mg/dl respectively; those in the 28W, 36W, and 44W LETO groups were 40.8±6.2, 37.8±3.3, and 43.0±4.2 mg/dl respectively. It was significantly higher in the OLETF groups than in the LETO groups at each time point (Fig. 8-A). The OLETF/LETO ratio was marked increases with age (Fig. 8-B).

![Triglyceride](image)

**Fig. 8** Triglyceride concentration in each OLETF group and LETO group.
(A) Average are expressed as the mean ± SD.
* p<0.05; *** p<0.005, significant deference compared with control (LETO) rats.
B: OLETF/LETO ratio are expressed as the ratio of the triglyceride concentration of OLETF rats relative to that of LETO rats. *** p<0.005

3. Histological changes
1) Small intestine
   (1) Total length of small intestine in the 28W, 36W, and 44W OLETF groups were 140.0±1.2, 147.2±1.3, and 147.0±2.7 cm respectively; those in the 28W, 36W, and 44W LETO groups were 121.8±1.4, 129.3±2.2, and 127.0±1.2 cm respectively. It was significantly longer in the OLETF groups than in the LETO groups at each time point (Fig. 9-A). The OLETF/LETO ratio was not significant difference among the time points (Fig. 9-B).
   (2) Height of small intestinal villi in the 28W, 36W, and 44W OLETF groups were 454.5±29.7, 521.0±21.2, and 526.2±10.3 µm respectively; those in the 28W, 36W, and 44W LETO groups were 428.6±8.9, 438.8±10.0, and 455.0±9.3 µm respectively. It did not significantly differ between the OLETF groups and LETO groups at 28W but was significantly higher in the OLETF groups than in the LETO groups at 36W and 44W, indicating hyperplastic changes (Fig. 10-A). The OLETF/LETO ratio were higher at 36W and 44W than at 28W (Fig. 10-B), but the differences among the time points were not significant.
2) Pancreas
Marked fibrosis was noted in the 44W OLETF group compared with the 44W LETO group. The percentages of $\beta$ cells in the islets of Langerhans were 11.71 ± 2.07% in the 44W OLETF group and 10.97 ± 2.79% in the 44W LETO group, showing no significant difference.

4. Functional changes
1) $^{13}$C-trioctanoin breath test
(1) $^{13}$CO$_2$ peak value: The peak value of $\Delta$ $^{13}$CO$_2$ concentration was observed after 60
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It was higher in the OLETF groups than in the LETO groups at 36W and 44W (Fig. 12-A). The OLETF/LETO ratio was no significant difference among the time points, but it was higher at 36W and 44W than at 28W (Fig. 12-B).

Fig. 11. Peak value of $\Delta^{13}$CO$_2$ concentration in the $^{13}$C-trioctanoin breath test in each OLETF group and LETO group.

Fig. 12. The $\Delta^{13}$CO$_2$ concentration after 60 min in the $^{13}$C-trioctanoin breath test in each OLETF group and LETO group.

A: average are expressed as the mean ± SD.

* $p<0.05$, significant deference compared with control (LETO) rats.

B: OLETF/LETO ratio are expressed as the ratio of the $^{13}$CO$_2$ peak value of OLETF rats relative to that of LETO rats.
(2) Area under the curve (AUC): The area under the Δ\(^{13}\)CO\(_2\) concentration-time curve in the 28W, 36W, and 44W OLETF groups were 147.0±5.8, 234.7±18.1, and 227.8±9.4‰ respectively; those in the 28W, 36W, and 44W LETO groups were 144.8±12.0, 177.2±6.1, and 180.2±14.0‰ respectively. It showed no significant difference between the LETO groups and OLETF groups at 28W but was significantly higher in the OLETF groups than in the LETO groups at 36W and 44W.

\[ \Delta^{13}\text{CO}_2(\%) \]

![Graph showing AUC values for OLETF and LETO groups.](image)

**Fig. 13** Area under the curve (AUC) in the \(^{13}\)C-trioctanoin breath test in each OLETF group and LETO group.

A: average are expressed as the mean ± SD.
* p<0.05, significant deference compared with control (LETO) rats.
B: OLETF/LETO ratio are expressed as the ratio of the \(^{13}\)C-trioctanoin AUC of OLETF rats relative to that of LETO rats.

\[ \Delta^{13}\text{CO}_2(\%) \]

![Graph showing peak value of \(^{13}\)CO\(_2\) concentration in the \(^{13}\)C-acetate breath test.](image)

**Fig. 14** Peak value of \(^{13}\)CO\(_2\) concentration in the \(^{13}\)C-acetate breath test in each OLETF group and LETO group.
(Fig. 13-A). The OLETF/LETO ratio were higher at 36W and 44W than at 28W (Fig. 13-B), but the differences among the time points were not significant.

2) 13C-acetate breath test

In the 13C-acetate breath test as a test of gastric emptying, the peak value of 13CO2 concentration was observed after 30 minutes in both the 44W OLETF group and the 44W LETO group and was 204.4 ± 10.1‰ and 157.2 ± 7.9‰ respectively. It decreased gradually at a similar rate after the peak in both the OLETF and LETO groups (Fig. 14).

Discussion

In type 1 diabetes mellitus (STZ rats), hypertriglyceridemia was observed with hyperglycemia, and exceeded lipid absorption or increased total amount absorption associated with hyperplastic changes of the small intestine were considered to be an important factor.

Type 2 diabetes mellitus is also frequently complicated by hyperlipidemia (particularly hypertriglyceridemia), is associated with a very high incidence of atherosclerotic disorders, and ischemic heart disease is an important prognostic factor in patients with diabetes mellitus. One of the causes of hypertriglyceridemia in type 2 diabetes mellitus is excessive production of very-low-density lipoprotein (VLDL) and chylomicrons, and increased synthesis of VLDL triglycerides due to increased influx of glucose and free fatty acids into the liver, where they are converted to triglycerides. In addition, diabetes is often accompanied by catabolic disorders of VLDL triglycerides, which are correlated with the severity of hyperglycemia.

To clarify the mechanism of the occurrence of hyperlipidemia in type 2 diabetes mellitus, we examined morphological changes in the small intestinal villous epithelium and evaluated lipid absorbing function by a breath test. The OLETF rats used in this study were a strain derived by repeated breeding of Long-Evans rats and they have the defect of CCK-A receptors. CCK is one of the incretin-like materials with an insulin-secretion-stimulating activity, and the defect of its receptors is a cause of overeating. Male OLETF rats all exhibit diabetic type responses on the oral glucose tolerance test at the age of 25 weeks and show characteristics including increases in the plasma insulin concentrations after the age of 8 weeks but decreases after the age of 60 weeks, and glucose-specific insulin secretion insufficiency and insulin resistance in peripheral tissues since the age of 12 weeks. Although the pathologic profile of OLETF rats is not in complete agreement with that of human type 2 diabetes mellitus, they are a model having the same risk factors as human type 2 diabetes mellitus such as overeating, obesity, insulin resistance, and insulin secretion insufficiency and are appropriate for research on the pathogenic mechanism of type 2 diabetes mellitus. In this study, male OLETF rats were examined at 2-month intervals at 28, 36, and 44 weeks of age.

Evaluation of metabolic kinetics of lipids has become possible by lipid absorption tests using compounds labeled with stable radioisotopes. In the breath tests using 13C-labeled medium-chain lipids, 13CO2, which appears in expiration after lipids absorbed by the small intestine are used in peripheral tissues is measured. The results of serial measurements of 13CO2, the final metabolic product of lipids, are considered to closely reflect the lipid absorption ability. Among 13C-trioctanoin, triolein, and palmitic acid, the 13CO2 concentration reaches a peak earliest after the administration of trioctanoin, and the mass ratio of trioctanoin in expiration is markedly higher than those of the other 2 agents. Therefore, the 13C-trioctanoin breath test is a simple and useful method that allows quick and sensitive evaluation of the lipid digestion and absorption abilities. However, as the 13C-labeled breath test is an indirect method in which the percentage of 14CO2 in expiration is determined after the processes of absorption, transport, metabolism, and excretion of the labeled agent, it is possible that its results may be affected by various factors.

Of the triglycerides that enter the lymph flow from the small intestinal villi, about 80% is derived from the diet. The triglycerides that have been ingested are emulsified by bile acids in the small intestine, degraded by lipase, and converted to fatty acids and glycerol or monotriglycerides. They are synthesized into triglycerides again in small intestinal epithelial cells, form chylomicrons as they are covered with phospholipids and cholesterols by protein, and enter lymph.

Trioctanoin, a medium-chain lipid used in this study, is scarcely affected by bile acids, is hydrolyzed in the intestine, absorbed by the small intestinal villi, and released into blood via the lymph flow primarily as chylomicrons. In blood, it is hydrolyzed again by lipoprotein lipases and used
in cells as a source of energy.

Since insulin secretion is markedly reduced in STZ rats, a model of type 1 diabetes mellitus, the activity of insulin-dependent lipoprotein lipase is reduced, presumably resulting in a decrease in triglyceride resolution. The expiratory excretion of $^{13}$CO$_2$, which is a metabolic product of $^{13}$C-trioctanoin, was also expected to decrease with a decrease in the utilization of dietary lipids. However, STZ rats showed hypertriglyceridemia, significant hyperplasia of the small intestinal villi, and a significant increase in the expiratory $^{13}$CO$_2$ concentration, suggesting that lipid absorption via the small intestine may be greater than in the controls.

In the OLETF rats at 36W, a model of type 2 diabetes mellitus, the serum insulin level was significantly higher than in the LETO rats used as controls, suggesting that the activity of insulin-dependent lipoprotein lipase was enhanced and that the triglyceride production was decreased. In the OLETF rats at 44W, the triglyceride level is higher than those at 36W, as a result of increasing endogenous triglyceride that the activity of lipoprotein lipase is reduced with decreased the serum insulin level and exogenous triglyceride provided from a diet. The expiratory $^{13}$CO$_2$ concentration was significantly higher in the OLETF rats than in the LETO rats at 36W and 44W, indicating that lipid absorption was higher than in the controls.

In the OLETF diabetic rats, the length of the small intestine and the height of the small intestinal villi increased with the onset and progression of diabetes. There have been reports on hyperplasia of small intestinal villous epithelium. Concerning in vitro studies of glucose absorption using the intestine of diabetic rats, hyperplasia of the small intestinal villi and increased glucose uptake by the small intestine with increased absorption area have been reported. Thus, postprandial hyperglycemia may be caused by increased quantity or speed of glucose absorption due to intestinal hyperplasia, exceeding the ability of insulin secreted by the pancreas to dispose of the glucose. Excessive insulin secretion is eventually depleted, allowing diabetes to progress.

Overeating increases the exposure of the intestinal epithelium to food and promotes the chylomicron production in the small intestine. In the 36W OLETF group, the insulin concentration was increased markedly about 17 times, and the triglyceride concentration about 9 times, suggesting increased triglyceride resolution due to enhancement of the activity of insulin-dependent lipoprotein lipase. However, while the insulin level decreased to about 5.7 times in the 44W OLETF group, the triglyceride production increased further to about 12 times. There was no change during the 2 months in the length of the small intestine, height of the villi, or expiratory $^{13}$CO$_2$ concentration in the two groups. The mechanism of the occurrence of hypertriglyceridemia in STZ rats was considered to be an increase in absorption surpassing the increase in the triglyceride production. In OLETF rats, hypertriglyceridemia is considered to be caused partly by increased absorption from the small intestine but also by decreased triglyceride production and, possibly, other mechanisms unlike STZ rats with type 1 diabetes mellitus.

There have been various opinions about gastric emptying in diabetes mellitus, and no consensus has been reached. Reduced gastric motility due to peripheral nerve disorders and resultant delay of emptying, alleviation of relaxation on the proximal stomach causing rapid emptying, and normal gastric emptying have been reported. Therefore, we evaluated gastric emptying in 44-week-old OLETF and LETO rats by administering glucose solution containing $^{13}$C-acetate under the same conditions as the $^{13}$C-trioctanoin breath test. In both LETO and OLETF rats, the expiratory $^{13}$CO$_2$ concentration reached a peak after 30 minutes, suggesting that there was no acceleration or delay of gastric emptying in the diabetic rats used in this study. Since the peak expiratory $^{13}$CO$_2$ concentration was observed 60 minutes after the administration in both LETO and OLETF rats also on the $^{13}$C-trioctanoin breath test, the gastric motility of the diabetic rats used in our study was considered to be normal.

In OLETF rats, hyperinsulinemia by hypertri-
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metabolism (hypertriglyceridemia). This is a phenomenon that corresponds to human metabolic syndrome, and the results of this study are considered to be informative also for pathological evaluation of this syndrome.

In this study, the total length of the small intestine was longer, villi were taller, and hyperplastic changes were observed histologically, in the 26-week-old OLETF rats that had developed diabetes compared with the control group. The expiratory $\Delta ^{13}$CO$_2$ concentration increased significantly in the OLETF rats from 36W, so that hyperplasia and enhancement of lipid absorption are considered to begin after the onset of diabetes mellitus.

Further evaluation of in what stage hyperplastic changes in the small intestinal villi begin and whether hyperplastic changes can be reversed by dietary therapy and control of the blood glucose or triglyceride concentrations by insulin therapy is considered necessary. The findings will be useful for the treatment and prevention of metabolic syndrome.

Conclusions

In OLETF diabetic rats, hyperplasia of the small intestinal villi and enhancement of lipid absorption or increased total amount absorption were observed with the onset and progression of diabetes mellitus. Excessive lipid absorption from the small intestine was considered to be one of the causes of hyperlipidemia complicating diabetes mellitus.

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