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Changes in Glycolipid Metabolism During A High-Sucrose Feeding in Spontaneously Diabetic Torii (SDT) Rats, A Genetic Model of Nonobese Type 2 Diabetes

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Abstract: A high-sucrose diet induces insulin resistance and dyslipidemia in normal rats. The purpose of the present study was to investigate the effects of a high-sucrose diet on glycolipid metabolism in Spontaneously Diabetic Torii (SDT) rats, a genetic model of nonobese type 2 diabetes. Male SDT rats were fed with a high-sucrose (68% of energy) diet from 8-24 weeks of age. SDT rats fed a high-sucrose diet showed hypercholesterolemia, hyperinsulinemia and hyperleptinemia but the body weight and the serum triglyceride level were not increased. Unexpectedly in SDT rats fed a high-sucrose diet, we observed a suppress of the incidence of diabetes mellitus. In computed tomography analysis, SDT rats fed a high-sucrose diet showed an increase of visceral or subcutaneous fat tissue weight. In pathological analyses, slight or mild fatty liver was observed by high-sucrose feeding. A high-sucrose diet in SDT rats induced dyslipidemia, insulin resistance and fat storage but not development of diabetes. High sucrose-fed diabetic rats are considered to be useful for further elucidation of complex mechanisms in glycolipid metabolic abnormality.

Key words: Diabetes, high-sucrose diet, rat, abnormality, fatty liver, serum

INTRODUCTION

Prevalence of diabetes in Asian populations has increased rapidly in recent decades. Similarly, rates of overweight and obesity are increasing sharply, driven by economic development, nutrition transition and increasing sedentary life styles (Chan *et al*., 2009; Zimmet *et al*., 2001). Therefore, quality or content of diet is a critical point in the investigation of etiology of diabetes mellitus. An increased intake of fat or fructose/sucrose is associated with increased weight gain, elevated circulating Triglyceride (TG) levels and insulin resistance in human and animal models (Basciano *et al*., 2005; Daly *et al*., 1997). It has been reported that diets enriched in sucrose reduced insulin suppression of glucose production and increased capacity for gluconeogenesis in Sprague-Dawley (SD) rats (Bizeau *et al*., 2001; Commerford *et al*., 2002; Pagliassotti *et al*., 1996; Pagliassotti and Prach, 1997). The Spontaneously Diabetic Torii (SDT) rat is a model of non-obese spontaneous diabetes which was developed by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan) (Masuyama *et al*., 2004; Shinohara *et al*., 2000). Male SDT rats develop hyperglycemia, hypoinsulinemia and hyperlipidemia with age and express of urinary glucose from about 20 weeks of age. SDT rats also develop ocular complications (cataract and retinopathy) and nephropathy from about 40 weeks of age (Sasase *et al*., 2006; Ohta *et al*., 2007). There are few reports in which effects on a high-sucrose diet in diabetic models are investigated. In this study, we examined the effects of a high-sucrose diet on glycolipid metabolism in SDT rats, a genetic model of nonobese type 2 diabetes.

MATERIALS AND METHODS

Animals and diets: Male SDT rats (Clea Japan, Tokyo, Japan) were used for the study. The rats were divided into two groups, a control group and a high-sucrose diet group (HS group). Rats in a control group were fed with a standard diet (CRF-1, Charles river Japan, Yokohama, Japan) and rats in a HS-group were fed with a high-sucrose diet (68% sucrose based on percentage of total calories, D11725, Research Diet Inc., New Brunswick, NJ)

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from 8-24 weeks of age. The energy content of the standard and the high-sucrose diets were 3.59 and 3.90 Kcal g^{-1}, respectively. Since, there were multiple rats in each cage, the calorie intake was calculated by dividing the total cage calorie intake by the number of animals per cage. The animals were housed in a controlled room (a 12 h lighting cycle) and allowed free access to water.

Biological parameters: Food intake and body weights of the rats from 8-24 weeks of age were measured at every 2 weeks. Blood chemical parameters such as glucose, insulin TG and Total Cholesterol (TC) levels were examined at every 2 weeks. Blood samples were collected from the tail vein of non-fasted rats. Serum glucose, TG and TC levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and automatic analyzer (Hitachi, Tokyo, Japan).

Serum insulin, leptin and adiponectin levels were examined at every 4 weeks. Serum insulin or leptin levels were measured with a rat insulin or leptin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). Serum adiponectin levels were measured with a mouse/rat adiponectin ELISA kit (Otsuka pharmaceutical Inc, Tokyo, Japan). In refed condition, the glucose levels were examined. After SD rats at 12 weeks of age were fasted for 16 h, the rats in each group were fed CRF-1 or a high-sucrose diet. Serum glucose levels were measured at 30, 60, 120 and 180 min after refeeding.

Fat tissue weight: Visceral and subcutaneous fat tissue weights in each rat were determined at 16 weeks of age by Computed Tomography (CT) analysis. The fat weights were measured by a laboratory X-ray CT device (LATheta, ALOKA Co., LTD., Osaka, Japan). Rats were anesthetized with an intraperitoneal injection of 50 mg kg^{-1} pentobarbital (Tokyo chemical industry, Tokyo, Japan) and about 20 CT photographs per rat were taken at 5 mm intervals between diaphragm and lumbar vertebrae. Total fat weights and Visceral/Subcutaneous (V/S) ratios were calculated from visceral and subcutaneous fat weights.

Tissue sampling and histopathology: Necropsy was performed at 24 weeks of age and organ weights such as liver and pancreas were measured. The liver and pancreas were fixed in 10% neutral buffered formalin. After resection, the tissue was paraffin-embedded by standard techniques and thin-sectioned (3-5 μm). The sections were stained with Hematoxylin and Eosin (HE).

Statistical analysis: Results of biological parameters and fat tissue weights were expressed as the mean±Standard Deviation (SD). Statistical analysis of differences between mean values was performed using the F-test followed by the Student's t-test or Aspin-Welch's t-test. Differences were defined as significant at $p<0.05$.

RESULTS AND DISCUSSION

Biological parameters: The caloric intake was comparable from 10-18 weeks of age between SDT rats on high-sucrose diets (HF group) and a standard diet (control group) however, the caloric intake of control group was increased from 20-24 weeks of age (Fig. 1a). Body weights were not different from both diet groups from 8-20 weeks of age and the body weights in HS group significantly increased after 20 weeks of age (Fig. 1b). Serum glucose levels in control group began to increase in a time dependent manner after 12 weeks of age. However, the elevation of glucose levels in HS group was not observed (Fig. 2a). Serum TG levels were not significantly different from both diet groups during the experimental period and a sustained increase of TC levels was observed in HS group (Fig. 2b and c). The serum insulin levels were higher in HS group than in control group with a

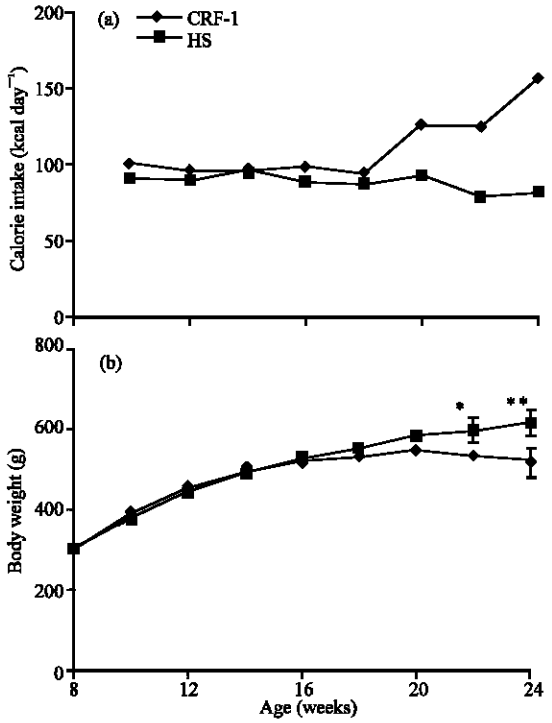

Fig. 1: Changes of calorie intake (a) and body weight (b) in SDT rats fed CRF-1 (control group) or a high sucrose diet (HS group). Data represent means±SD (n = 5 or 6). *$p<0.05$, **$p<0.01$; significantly different from the SDT rat fed CRF-1

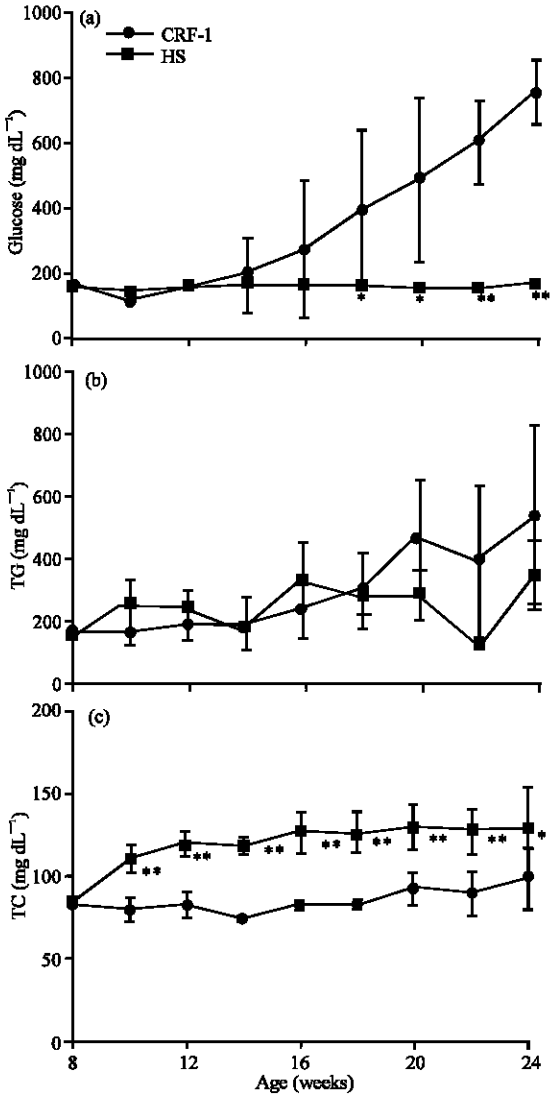

Fig. 2: Changes of serum glucose (a) triglyceride (b) and total cholesterol (c) levels in SDT rats fed CRF-1 (control group) or a high sucrose diet (HS group). Data represent means±SD (n = 5 or 6). *p<0.05, **p<0.01; significantly different from the SDT rat fed CRF-1

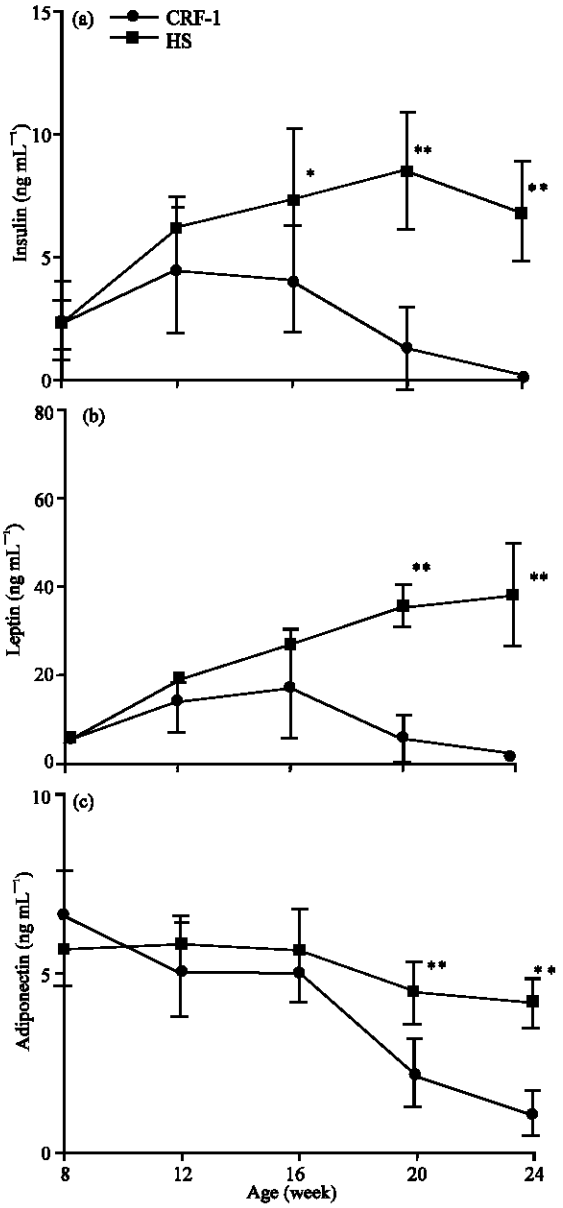

Fig. 3: Changes of serum insulin (a) leptin (b) and adiponectin (c) levels in SDT rats fed CRF-1 (control group) or a high-sucrose diet (HS group). Data represent means±SD (n = 5 or 6). *p<0.05, **p<0.01; significantly different from the SDT rat fed CRF-1

significant difference observed from 16-24 weeks of age (Fig. 3a). The serum leptin levels significantly increased from 20-24 weeks of age in HS group compared to control group (Fig. 3b). The serum adiponectin levels in control group began to decrease in a time-dependent manner after 16 weeks of age but the decrease in adiponectin levels was suppressed in HS group (Fig. 3c). In refed condition, the serum glucose level at 30 min after refeeding was significantly lower in HS group compared to control group (Fig. 4).

Fat tissue weights: Visceral and subcutaneous fat tissue weights in HS group were significantly increased as compared with those in control group (Table 1). Also, a total fat tissue weight in HS group was significantly increased. The V/S ratio were not different from both diet groups (Table 1).

Histopathology: The organ weight of liver in HS group significantly increased in HS group compared to control group (21.47±2.54 and 18.83±0.98 g). The organ weight of pancreas did not change in both diet groups. Severe histopathological changes (+++) in the pancreas including atrophy of islets, multiple irregular projection of islets and islet fibrosis were observed in control group.

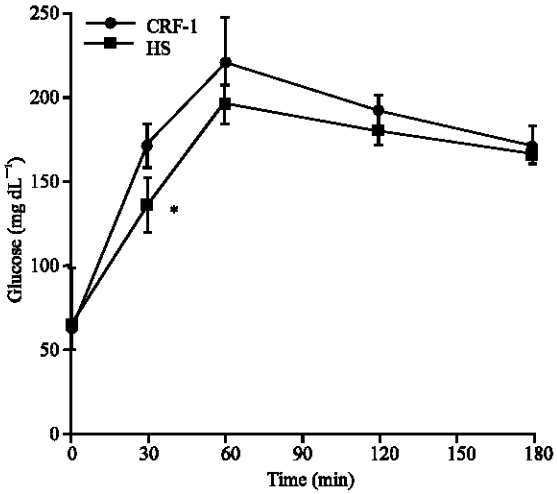

Fig. 4: Changes of serum glucose levels after refeeding. Data represent means±SD (n = 4). *p<0.05; significantly different from the SDT rat fed CRF-1

Table 1: Changes of fat tissue weights at 16 weeks of age of SDT rats incontrol (CRF-1) group and high sucrose group. Visceral and subcutaneous fat tissue weights were determined by computed tomography analysis

Groups	Visceral fat (g)	Subcutaneous fat (g)	Total fat (g)	V/S (ratio)
RF-1	27.6±8.1	11.8±4.5	39.37±12.4	2.40±0.31
High sucrose	38.8±6.0*	16.9±2.5*	55.6±8.0*	2.31±0.26

Data represents means±SD (n = 6), *p<0.05; significantly different from CRF-1 group

However, these changes were hardly noticeable in HS group (Table 2, Fig. 5). Histopathological changes of hemosiderin deposition and vacuolation of islets also tended to minor in HS group.

Slight or moderate fatty changes in hepatocyte were observed in HS group but those changes were not observed in control group (Table 2, Fig. 6). In SDT rats, a new model for non-obese diabetes, body weight, blood glucose, insulin and lipids are completely normal compared with SD rats until about 16 weeks of age and thereafter, these rats gradually increase blood glucose levels with advancing age (Shinohara *et al*., 2007; Matsui *et al*., 2008).

Calorie intake and body weights in SDT rats fed a high-sucrose diet were not different from those in SDT rats fed a standard diet from 8-18 weeks of age (Fig. 1a and b). It has been reported that a high-sucrose diet did not affect body weight in SD rats (Commerford *et al*., 2002; Pagliassotti *et al*., 1996) and the result in SDT rats is also similar.

However, calorie intake in control group showed a tendency to increase after 20 weeks of age and the body weights decreased after 22 weeks of age (Fig. 1a and b). The reason why is considered that the rats in control group developed diabetes mellitus after 16 weeks of age (Fig. 2a). Serum insulin levels in HS group were significantly increased after 16 weeks of age (Fig. 3a).

Also in SD rats a high-sucrose diet induces hyperinsulinemia and insulin resistance (Kim *et al*., 1999; Pagliassotti *et al*., 1996). Moreover, a sustained increase of TC levels was observed in HS group (Fig. 2c). High sucrose feeding in rats induces plasma dyslipidemia and hepatic steatosis (Huang *et al*., 2007; Pagliassotti *et al*., 1996). In this study, a high-sucrose diet in SDT rats showed slight or moderate fatty change in liver (Table 2,

Table 2: Histopathological findings of pancreas and liver at 24 weeks of age in SDT rats fed CRF-1 or a high-sucrose diet

		Animal no.											
		CRF-1						High sucrose					
Organs	Pathological findings	1	2	3	4	5	6	1	2	3	4	5	6
Pancreas													
	Atrophy, islet	+++	+++	+++	NE	+++	+++	+	+	+	+	+	±
	Multiple irregular projection, islet	+++	+++	+++	-	+++	+++	+	++	+	+	+	±
	Fibrosis, islet	+++	+++	+++	-	++	+++	+	+	+	+	±	±
	Mononuclear cellinfiltration	+	+	±	-	++	+	+	+	+	+	+	±
	Hemosiderin deposit, islet	++	++	++	-	++	++	+	++	+	+	±	+
	Vacuolation, islet cell, focal	++	+	±	-	±	+	+	+	±	±	±	±
Liver													
	Fatty change, hepatocyte, periportal	-	-	-	NE	-	-	+	±	±	+	+	+
	Clear change, hepatocyte, diffuse	-	-	-	-	-	-	++	++	++	++	++	++
	Fibrosis, focal	-	-	-	-	±	-	-	-	-	-	-	-

Negative, ±: Very slight, +: Slight, ++: Moderate, +++: Severe, NE: Not Examine

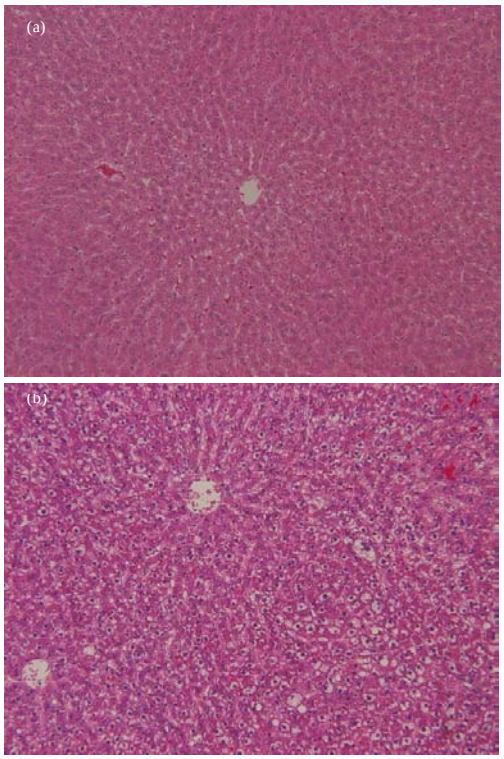

Fig. 5: Histopathological analysis of pancreas. HE stain. Original magnification x 200. a) SDT rats fed CRF-1 (control group) (24 weeks of age); b) SDT rats fed a high sucrose diet (HS group) (24 weeks of age). Severe changes in pancreas, including atrophy of islets and multiple irregular projection of islets and islet fibrosis were observed in control group. However, these changes were minor in HS group

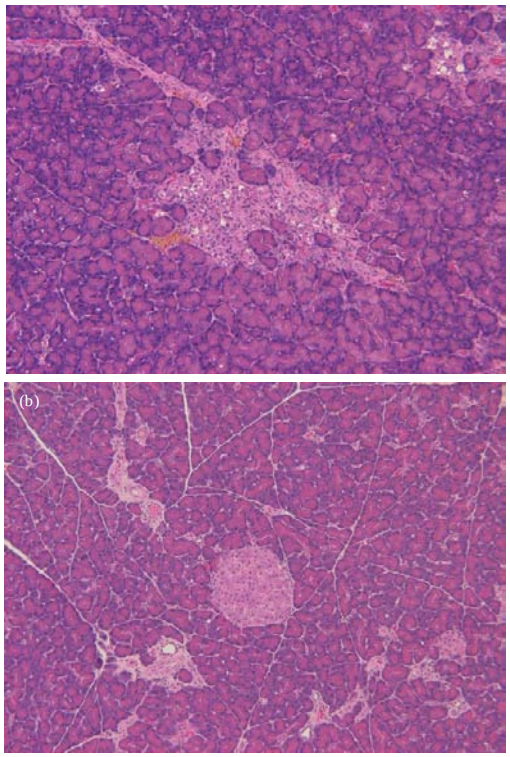

Fig. 6: Histopathological analysis of liver. HE stain. Original magnification x 200. a) SDT rats fed CRF-1 (control group) (24 weeks of age); b) SDT rats fed a high sucrose diet (HS group) (24 weeks of age). Slight or moderate fatty change in hepatocyte was shown in SDT rats fed a high-sucrose diet

Fig. 6). In SDT rats fed a high-sucrose diet, insulin resistance, dyslipidemia and fatty liver were induced but unexpectedly, the incidence of diabetes mellitus was inhibited (Fig. 2a). In refed condition, serum glucose levels in HS group were decreased (Fig. 4). It has been reported that an inhibition of postprandial hyperglycemia prevented development of diabetes (Ohta *et al*., 2003). Also in human an improvement of postprandial hyperglycemia in prediabetes prevents or delays progression to diabetes (Chiasson *et al*., 2002). A decrease of adiponectin in control group might be caused by the development of diabetes (Fig. 3c).

SDT rats fed a high-sucrose diet showed an increase of visceral and subcutaneous fat tissue weights (Table 1). It has also been reported that an increase of visceral fats such as epididymal, retroperitoneal and mesenteric fats was observed in SD rats on a high-sucrose diet (Pagliassotti *et al*., 1996). In this study, we determined visceral and subcutaneous fat tissue weights using CT device. Troglitazone or pioglitazone treatment in diabetic rats increases fat tissue weights and the number of small adipocytes. The increase of small adipocyte induces an improvement of glycolipid metabolism (Okuno *et al*., 1998; De Souza *et al*., 2001). In further study, the size and the function of adipocytes in high sucrose feeding should be investigated. Serum leptin levels in HS group increased (Fig. 3b) and the reason might be related with an increase of fat tissue weights.

In histopathological analysis, slight or moderate fatty change in liver was observed in SDT rats fed a high-sucrose diet (Table 2, Fig. 6). In Zucker Diabetic Fatty (ZDF) rats, a genetic obese diabetic model, a prominent increase of liver weight and severe fatty liver were

observed on a high-sucrose diet (Ohta *et al*., 2009). It is reported that lipid accumulation in muscle also caused insulin resistance (Krebs and Roden, 2005). Further study of intramuscular lipid should be done in the future. Histopathologically severe changes of the pancreas, which are specific to the SDT strain including atrophy of islets, multiple irregular projections of islets and islet fibrosis were observed in control group (Table 2, Fig. 5). Irregular boundaries of islets in pancreas were also observed in ZF rat a genetic obese model (Ohta *et al*., 2010).

Furthermore, slight or moderate changes of hemosiderin deposition and vacuolation of islets were observed in control group but these changes were minor in HS group. The reason why is considered that the rats were prevented the development of diabetes mellitus (Fig. 2a).

CONCLUSION

In this study, SDT rats fed a high-sucrose diet showed dyslipidemia, insulin resistance, an increase of fat tissue weights and mild fatty liver but not development of diabetes. High sucrose-fed diabetic rats are considered to be useful for further elucidation of complex mechanisms in glycolipid metabolic abnormality.

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