Acetone Concentration in Gas Emanating from Tails of Diabetic Rats

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This study investigated the effects of diabetic rats induced by streptozotocin (STZ) on acetone concentration emanating from the tail of a rat. Experiments were carried out with male Wistar rats (9 weeks of age, 220 – 250 g body weight). Glucose concentration in the blood was 10.8 ± 0.7 mmol/l for the control group and 39.6 ± 2.4 mmol/l for the diabetic group. β-Hydroxybutyrate concentration in blood was 218 ± 52 μmol/l for the control group and 1439 ± 101 μmol/l for the diabetic group. Both glucose and β-hydroxybutyrate concentrations in the blood of the diabetic group were significantly higher than those of the control group ($p < 0.001$). Skin gas acetone concentration emanated from rat tail was 124 ± 46 ppb for control and 1134 ± 417 ppb for diabetic. Skin gas acetone concentration emitted from the tail of a rat with diabetes was significantly higher than that from a rat in the control group ($p < 0.001$). The result indicates that skin acetone emanating from a rat tail is a useful parameter to use for insulin-dependent diabetes (type I).

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Introduction

The vapor exhaled by humans contains numerous volatile organic compounds (*e.g.* acetone, ethanol, isoprene).[1] Ketones (β-hydroxybutyrate, acetoacetate and acetone) are generated in the liver, mainly from the oxidation of fatty acids, and are exported to peripheral tissues, such as the brain, heart, kidney and skeletal muscle for use as energy fuels.[2,3] Acetone is mainly generated from decarboxylation of acetoacetate.[4] Production of acetone is known to increase with diabetic ketoacidosis,[4,5] fasting[5,6] and exercise.[7,8] Since plasma acetone concentration is significantly related to breath acetone,[4] breath acetone is potentially useful as an indicator of ketosis.[9]

A few studies have been reported in reference to acetone in exhaled air and skin gas in humans. It has been reported that skin gas acetone concentration of patients with diabetes was significantly higher than those of the control subjects.[10] It has also been reported that concentrations of acetone in both skin gas and exhaled air increased according to the length of fasting periods;[11] researchers also demonstrated that there is a good relationship between skin and exhaled air acetone concentration.[1,11] From these results, we can see that the skin gas acetone concentration reflects that of exhaled air.

In human diabetic patients, treatments such as dietary control, oral medication, and insulin therapy should be the highest priority. With regard to the study of human diabetic patients, measurement of skin gas acetone is limited after treatments.[10] Thus, we investigated acetone concentration emanated from the tails of rats with non-treated diabetes. A previous study observed the hydrocarbons of exhaled air and air emanated from the whole body of rats enclosed in a glass desiccator.[12] In that experimental system, animals produced feces and intestinal gas, which themselves effect the concentrations of hydrocarbons. Although collection systems that can remove hydrocarbon contaminant (feces and intestinal) gas have been developed,[13] they are very complicated.[13–15] In this study, skin gas is easily collected from a rat tail, which can avoid hydrocarbon contaminants. The method we used in this study is the first collecting system for the skin gas acetone concentration from diabetic rats. We investigated the effects of diabetes on acetone concentration emanated from rat tails.

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Experimental

Experimental protocol

The protocols of animal experiments were approved by the Ethical Committee of Nagoya Institute of Technology. Experiments were carried out with male Wistar rats (9 weeks of age, 220 – 250 g body weight), which were housed individually in a temperature-controlled room (22 ± 1°C), given food and water *ad libitum* throughout the study, and maintained on a 12/12 h light-dark cycle. Rats were accustomed to entering the pipe (22.5 cm length and 6.2 cm diameter) voluntarily before the experiment (Fig. 1). Four days prior to the experiment, the rats were anesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg body weight) and catheterized in the right atrium. The catheter was led subcutaneously and exteriorized between the ears. Five days after the operation, the rats were divided into a diabetic ($n = 8$) or control group ($n = 7$) for the weight to become the same. The mean and standard error (M ± SE) of the body weight of the control group was 266 ± 1.2 g, and the values for diabetic group was 267 ± 2.3 g. Skin gas was obtained from the tail in all rats, and blood samples

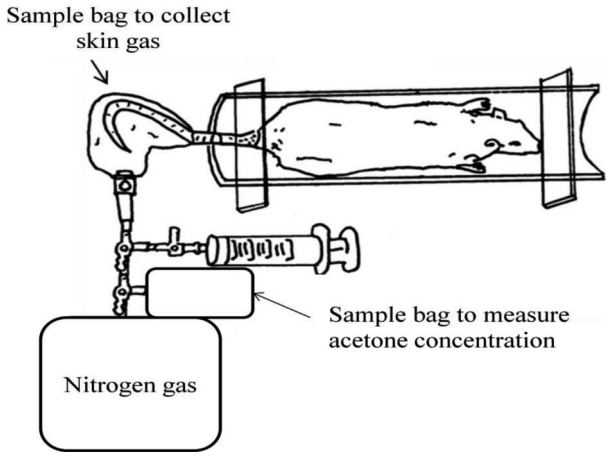

Fig. 1 Collecting system of skin gas emanating from rat tails.

(0.3 ml) were drawn from a catheter. Blood glucose and β-hydroxybutyrate were determined by glucose analyzer (Pirot, Technicon International, CA) and ketometer (Arkry, Kyoto), respectively. To induce diabetes, streptozotocin (STZ) dissolved in the physiological saline (100 mg/kg body weight), was injected in the right atrium after fasting for 14 h. Control rats received an equal volume of the physiological saline. Two days after STZ administration, body weight of rats was measured, skin gas was obtained from the tails of all rats, and blood was collected from the catheter in the right atrium.

Sampling procedure for rat tail skin gas

The concentration of acetone emanated from rat tail was measured using modified versions of the methods of Nose *et al*.[16] The sampling bag was made from a polyvinyl fluoride (PVF) sheet purchased from GL Sciences (Tokyo). After a rat entered the pipe (Fig. 1), the tail was washed with paper (Kimwiper S-200, Kimberly-Clark Co., supplied by Kulesia, Tokyo) dampened with distilled water, and the tail was inserted into the sampling bag, which was fixed to 12 cm from the tail end with a flexible sealing film (GL Sciences, Tokyo). All the air in the bag was sucked out with a glass syringe; subsequently, 25 ml of nitrogen gas was infused into the bag. After 3 min, 20 ml of skin gas was sampled from the bag and injected into another empty bag; this gas was used to measure acetone concentration by gas chromatography. The sampling gas was analyzed with a cold trap gas chromatographic system.[10,17] A 15-ml volume of skin gas sample was automatically sucked into the stainless-steel tube from the sampling bag. In this process, the sample was fed into the stainless-steel sample loop cooled with liquid nitrogen. After the sample injection valve was rotated from the trapping position to the injection position, the trap tubing was heated directly to aid thermal desorption of the acetones in the sample.

Analytical conditions

We used a Type GC-14B gas chromatograph (FID, Shimadzu, Kyoto, Japan). The separation conditions for measuring the acetone were as follows: a Porapack Q open tubular column (Type G-950: 1.2 mm internal diameter and 40 m length, Chemical Evaluation and Research Institute, Tokyo, Japan); the injection and detection temperatures were 150°C, and the column temperature was 80°C. Helium gas is used as carrier gas, and the flow rate was 20 ml/min. The retention time was 8.1 min. Calibration was performed using five different acetone calibration gases (0.12, 0.19, 0.53, 0.97 and 1.99 ppm).

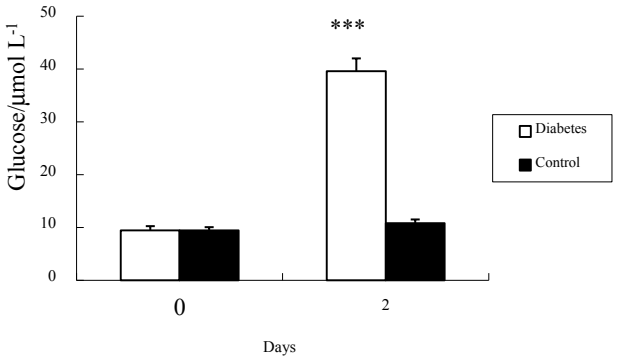

Fig. 2 Comparison of glucose concentration in blood between diabetic and control groups. ***, $p < 0.001$ significant difference compared with control group.

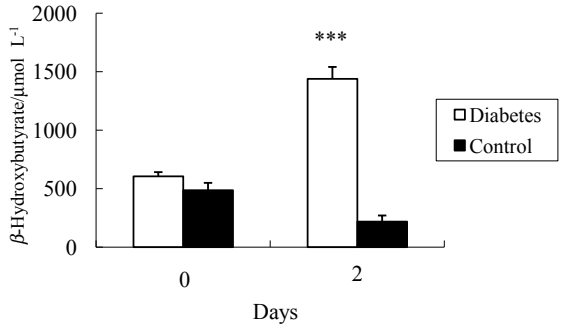

Fig. 3 Comparison of β-hydroxybutyrate concentration in blood between diabetic and control groups. ***, $p < 0.001$ significant difference compared with control group.

Statistical analysis

Values were expressed as M ± SE. Data were analyzed using a one-way analysis of variance with repeated measures with $p < 0.05$ determined to be statistically significant. When differences were obtained, post hoc analysis was performed using Fisher's PLSD (Protected Least Significant Difference). Statistical analysis was calculated with StatView (Ver. 5.0, Abacus Concepts). Linear correlation coefficients were determined using linear regression analysis.

Results

The M ± SE of the body weight of diabetic rats was 267 ± 2.3 g and that of the control group was 266 ± 1.2 g before STZ injection. Two days after STZ administration, body weight of rats with diabetes was 235 ± 4.2 g and that of the control rats was 252 ± 6.9 g. The body weight of the diabetic group was significantly decreased compared with the control ($p < 0.01$).

Figure 2 shows a comparison of blood glucose concentration between the diabetic and the control groups. The blood glucose level in the diabetic rats was significantly higher than the value in the control ($p < 0.001$). Figure 3 shows a comparison of β-hydroxybutyrate concentration in blood between diabetic and control groups. The β-hydroxybutyrate level of blood in the diabetic group was significantly higher than that in the control

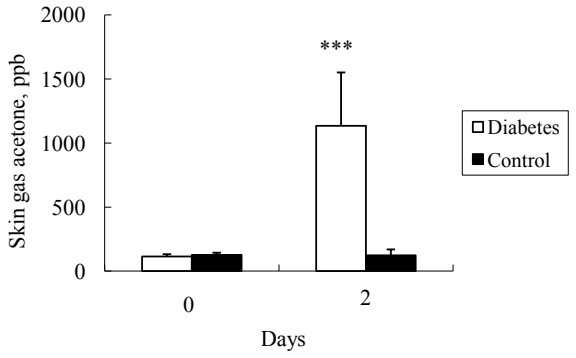

Fig. 4 Comparison of acetone concentration from rat tails between diabetic and control groups. ***, $p < 0.001$ significant difference compared with control group.

($p < 0.001$). Figure 4 shows the skin acetone concentration from the rat's tails. The skin gas acetone concentration in the diabetic rats significantly increased compared to the value for the control ($p < 0.001$).

Discussion

Blood glucose markedly increased due to STZ administration (Fig. 2). STZ has been used as a diabetogenic agent in rats and other animals.[18,19] STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly (ADP-ribose) synthetase, and thus depletes the intracellular NAD and NADP levels. NAD depletion by STZ inhibits proinsulin synthesis and thus induces diabetes. Animal models of diabetes mellitus can be produced by use of chemicals or by an immune reaction on the pancreatic β-cells. This model may be comparable to type I (insulin-dependent) diabetes in humans.

In the present study, β-hydroxybutyrate concentration of blood in the diabetic group significantly increased compared to that in the control ($p < 0.001$). The data in the present study coincided with the results of Wahren *et al.*,[20] who reported that β-hydroxybutyrate concentration was higher in diabetes rats compared to that in control. In the control group, blood β-hydroxybutyrate concentration at day 2 compared to day 0 decreased (Fig. 3). This result might be due to decrease in body weight, because Féry and Balasse[21] reported that plasma β-hydroxybutyrate concentration positively related with body weight. In the present study, the body weight of the control group decreased from 266 ± 1.2 g at day 0 to 252 ± 6.9 g at day 2.

Acetone is derived from acetoacetate through spontaneous decarboxylation of enzymatic conversion (*via* acetoacetate decarboxylation).[3,4] The present study clearly demonstrated that the skin gas acetone emanating from rat tails was markedly enahanced in diabetic rats compared to control rats. Acetone concentration emanating from rat tails has not been previously reported. It has been reported that skin gas acetone concentrations of human patients with diabetes were significantly higher than those of the control subjects.[10] Our result basically agrees with the results of the previous investigation,[10] which reported that levels of skin acetone markedly decreased with the decrease of β-hydroxybutyrate level due to insulin therapy in insulin-dependent diabetes. The mechanisms of an increase in free fatty acid and ketone metabolism were studied: the insulin deficiency induced an increase of lipolysis.[21] This might resulted in an increase in blood ketones, which occurs in diabetes. Yamane *et al.*[10] have reported that skin acetone is a useful parameter to regularly measure in diabetic patients, especially in insulin-dependent diabetes (type I). These results suggest that skin acetone measurement may be useful for assessment of diabetes, especially in type I diabetes.

In the present study, we revealed acetone concentrations emanating from rat tails. Some researchers previously examined volatile hydrocarbons, such as ethane and pentane, with reference to lipid peroxidation in exhaled air and from the whole body in rats.[12-15] However, there are no reports referencing the volatile organic compounds from rat tails. The collection of skin gas from rat tail has advantages compared to that of skin gas from humans which has been previously reported.[10] In human diabetic patients, the treatments should be the highest priority. Accordingly, measurement of skin gas acetone of diabetes in humans is limited to after treatment, such as dietary control, oral medication, and insulin therapy. However, in animal studies, skin gas acetone concentration can be observed without any treatments for diabetes. The present study clarifies that acetone concentration accompanies the progress of diabetes when the influence of treatments can be ignored.

In conclusion, the result suggests that skin acetone emanated from rat tails is a useful parameter in diabetic patients, especially in insulin-dependent diabetes (type I).

Acknowledgements

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