Investigation of metabolism of exogenous glucose at the early stage and onset of diabetes mellitus in otsuka long-evans tokushima fatty rats using [1,2,3-13c] glucose breath tests

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Abstract

Breath tests using glucose with ^{13}C at a specific carbon site aids in the evaluation of just one glucose metabolism pathways. However, when glucose labeled at three carbon sites ([1-13C], [2-13C], and [3-13C] glucose) is used in an individual, almost all pathways can be evaluated noninvasively. The glucose clamp test is the gold standard for evaluating various aspects of glucose metabolism, including insulin resistance. However, it is not possible to perform invasive and labor-intensive insulin clamp tests on pre-diabetes patients, because there are more than 30 million such patients in the US alone. Although the 75-g oral glucose tolerance test is widely used in clinical practice, it poses an inevitable risk of hyperglycemia. Therefore, the use of a noninvasive and safe method for the management of impaired glucose metabolism is desirable, especially in longitudinal studies. Ideally, longitudinal studies should use the same individuals as subjects throughout their lives because type 2 diabetes progresses from an early asymptomatic stage (with hyper-insulinemia associated with metabolic syndrome and insulin resistance) to mild hyperglycemia and finally frank diabetes, which requires pharmacological treatment. Several animal models of diabetes have been created to investigate insulin resistance and glucose metabolism. However, with animal models of diabetes, the same animal cannot be studied longitudinally because it is difficult to guarantee survival after repeated blood sampling to measure insulin and glucose. In practice, most researchers collect data on plasma glucose and insulin levels at multiple time points from different individuals to examine changes in glucose metabolism with advancing age. Therefore, alternatives to blood sampling methods are required to examine the same animal throughout its life. Compounds labeled with the stable isotope 13C have been extensively used for the diagnosis of several metabolic conditions. 13C-glucose was the first substrate used for breath tests in human metabolic studies. Briefly, a small amount of orally administered 13C-glucose is absorbed in the small intestine and reaches the liver via the blood stream. Therefore, the pattern of 13CO2 levels in exhaled breath after the oral administration of 13C-glucose reflects glucose metabolism. Specifically, a different carbon position has a different metabolic pathway (Fig 1), as stated by Ruzzin et al. [3-^{13}C] glucose (carbon-13 isotope at carbon position 3) provides 13C at position 1 in the pyruvate molecule, which produces 13CO2 when pyruvate is decarboxylated. In contrast, [1-13C] glucose produces 13C at carbon 2 of acetate, which produces 13CO2 at the beginning of the third turn of the TCA cycle. [2-13C] glucose provides 13C at carbon 1 of acetate, which produces 13CO2 at the beginning of the second turn of the TCA cycle. The recovery of 13CO2 from labeled 13C-acetate entering into the TCA cycle is incomplete because some 13C carbons are incorporated into fatty acids and amino acids. This phenomenon is the scientific basis on which these experiments are interpreted. This study aimed to compare the breath 13CO2 excretion rates after orally administering [1-13C], [2-13C], and [3-13C] glucose to Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO, control) rats of different ages. We also attempted to determine the age (in weeks) at which the breath 13CO2 recovery pattern following the oral administration of 13C-glucose noticeably differed between diabetic and control rats. OLETF rats were first used as an animal model of metabolic syndrome in 1984 in Tokushima, a rural Japanese town, and have since become an animal model of noninsulin dependent diabetes mellitus (NIDDM) because of their characteristic features. Their food intake increases over time, resulting in obesity and increased insulin resistance. Hyperglycemia occurs late, developing after 18 weeks of age. The disease progresses with age, and exercise and pair-feeding can be used to modulate the degree of obesity and hyperglycemia. These characteristics of OLETF rats have been reported to result from the absence of cholecystokinin-1 (CCK-1) receptors. OLETF rats are widely used as a model of type 2 diabetes mellitus.

Summary and Conclusion: It is difficult to determine which process of glucose metabolism is impaired in diabetic and prediabetic patients. The aim of this study was to evaluate the changes in glycolysis, gluconeogenesis, glucose uptake, and oxidation of exogenous glucose separately at the early stage and onset of diabetes mellitus in Otsuka Long-Evans

Tokushima Fatty (OLETF) rats using [1, 2, 3-13C] glucose breath tests. The three types of 13C-glucose breath tests were performed thrice in each period, i.e., 6–12 weeks, 15–18 weeks, and 21–24 weeks after birth at one-week intervals. The 13CO2concentration was measured and was expressed as delta per mil, and a breath 13CO2excretion curve was obtained. The maximal values during breath test time were significantly higher in OLETF rats of all ages and the increases in13CO2 excretions were delayed in OLETF rats in all types of breath tests. This suggests that OLETFs had lower glucose metabolism than control rats, and overall glucose metabolism is enhanced with age in both types of rats. Utilization of [2-13C] glucose was suppressed in the early stage of pre-diabetes, and that of [3-13C] glucose was enhanced just before the onset of diabetes. For the [1-13C] glucose breath test, no significant differences in area under the curve until 180 min were observed between OLETF and control rats at any age. We conclude that reduced gluconeogenesis might play a greater role in regulating plasma glucose levels in the primary stage of pre-diabetes, whereas increased glucose uptake might begin at the initial stage, and be enhanced at the onset of diabetes. Glucose oxidation was found to not change to a great extent in this diabetic animal model.