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Diets influence the diabetic phenotype of transgenic mice expressing a dominant negative glucose-dependent insulinotropic polypeptide receptor (GIPR^{dn})

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Abstract

Transgenic mice overexpressing a dominant negative glucose-dependent insulinotropic polypeptide receptor (GIPR^{dn}) have recently been shown to develop diabetes mellitus due to disturbed postnatal development of the endocrine pancreas. In this study, the effects of feeding a high fibre/low calorie diet on the diabetic phenotype of GIPR^{dn} transgenic mice were examined. Transgenic and control animals received either a conventional breeding diet (BD) or a high fibre diet (HF). Both fasting and postprandial blood glucose levels and HbA1C levels were largely elevated in transgenic mice vs. controls ($p<0.05$), irrespective of the diet fed. Food and water intake and the daily urine volume of GIPR^{dn} transgenic mice were higher than that of control mice ($p<0.05$). Transgenic animals receiving the HF diet showed significantly lower blood glucose and HbA1C levels as well as less food and water intake than transgenic mice fed BD. The 365-day survival of transgenic mice was significantly lower than that of control mice. Transgenic animals fed the HF diet lived significantly longer than their counterparts receiving BD. GIPR^{dn} transgenic mice develop a severe diabetic phenotype which can be ameliorated by a HF diet, thereby resembling some aspects of the pathophysiology of human type 2 diabetes mellitus.

Keywords: Diabetes mellitus; Incretin hormones; GIP; GIP receptor, High fibre diet

1. Introduction

The prevalence rate of type 2 diabetes mellitus is escalating throughout the world. Dietary behaviour clearly contributes to this phenomenon and is obviously implicated in the aetiology of type 2 diabetes mellitus. Energy intake in excess of requirements, high intake of saturated and *trans* fatty acids and high intake of fibre-depleted refined grain foods have been proposed as contributing factors [1]. Consequently, whole grain foods have been suggested to protect against the development of diabetes mellitus and as helpful to foster the management of diabetic patients [2,3]. Insoluble dietary fibre improves glucose tolerance and accelerates the meal-dependent release of the gastrointestinal hormone glucose-dependent insulinotropic polypeptide (GIP) [4], one of the important gluco-regulatory incretin hormones. The "incretin concept" postulates endocrine transmitters which are released from the intestinal mucosa in response to nutrients, especially carbohydrates, and stimulate insulin secretion [5,6]. Together with glucagon-like peptide 1 (GLP-1), GIP, which is postprandially released from duodenal K-cells, is such an established incretin hormone.

The insulinotropic actions of GIP are impaired in diabetic patients [7–12]. Whereas exogenous GLP-1 administration augments the insulin response and normalizes fasting hyperglycemia in diabetic patients and rodent models of diabetes, GIP administration has no effects [13–17]. The diminished effect of GIP in diabetic patients, despite normal or even elevated GIP levels, is consistent with the idea of a homologous desensitization of the GIP receptor (GIPR) [18–21].

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To investigate the importance of the GIP/GIPR axis for the enteroinsular axis *in vivo*, mouse models were generated by means of gene targeting. GIP receptor-deficient mice ($GIPR^{-/-}$) exhibit relatively mild changes in glucose homeostasis [22]. The same holds true for GLP-1 receptor knockout mice ($GLP\text{-}1R^{-/-}$), and double mutant mice ($GIPR^{-/-}/GLP\text{-}1R^{-/-}$) [23–25]. A potential explanation for these unexpectedly mild phenotypic consequences might be compensation of the loss of incretin signalling by e.g. enhanced sensitivity to other insulinotropic peptide hormones and/or upregulation of signalling molecules [25]. The compensation of the lack of GLP-1 action in $GLP\text{-}1R^{-/-}$ mice was suggested by glucagon [26] as well as by increased secretion and action of GIP [27,28]. Likewise, $GIPR^{-/-}$ mice exhibited an increased sensitivity of the beta cell to GLP-1 [29]. To avoid these complicating mechanisms, we chose expression of a dominant negative mutant of the GIPR in transgenic mice as an alternative strategy to elucidate the role of the GIP receptor within the enteroinsular axis *in vivo* [30,31]. The cDNA of the human GIPR was mutated at the third intracellular loop, where a deletion of eight amino acids (pos. 319–326) and a point mutation at position 340 was introduced. The loss of function of the mutated GIPR was demonstrated *in vitro*, using stably transfected CHL (Chinese hamster lung) cells: the $GIPR^{dn}$ bound GIP with normal affinity but failed to increase intracellular cAMP levels. Transgenic mice were then generated, expressing the mutated human GIPR cDNA under the control of the rat pro-insulin 2 gene promoter in pancreatic beta cells. These $GIPR^{dn}$ transgenic mice demonstrated a disturbed development of the endocrine pancreas and showed severe diabetes mellitus. The severe reduction of the beta cell mass and the disruption of the islet architecture led to largely reduced glucose-induced insulin secretion in these animals which could not be augmented by administration of pharmacological doses of GIP or GLP-1 [30]. The difference in the phenotype of $GIPR^{-/-}$ and $GIPR^{dn}$ transgenic mice may result from triggering of compensatory mechanisms by the ubiquitous knockout of the GIPR which does not occur in $GIPR^{dn}$ transgenic mice, harbouring a functional intact endogenous GIPR. However, recruiting of signalling molecules and therefore general impairment of G-protein coupled receptor signalling of the beta cell could also contribute to the early onset diabetes of $GIPR^{dn}$ transgenic mice.

In type 2 diabetic humans, the major abnormality of the enteroinsular axis was suggested to be the reduced insulinotropic activity of GIP [32]. Further, the volume density of beta cells in the pancreas [33,34] as well as beta cell mass [34] were reported to be reduced in type 2 diabetic humans. Therefore, $GIPR^{dn}$ transgenic mice resemble at least some aspects of the clinical situation in human type 2 diabetes.

In the present study, we have characterized the phenotype of $GIPR^{dn}$ transgenic mice in more detail as well as the effects of a high fibre/low disaccharide diet on the metabolic control of $GIPR^{dn}$ transgenic mice as compared to mice fed standard breeding diet. This study was designed to mimic dietary set ups discussed in the clinical situation in order to improve the metabolic control of diabetic patients.

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with institutionally approved and current animal care guidelines. Transgenic mice were generated as previously described [30]. Transgenic males were bred onto a CD1 background (Charles River-Wiga, Germany). DNA was extracted from tail tips as previously described, and transgenic mice were identified by polymerase chain reaction [30]. Animals investigated in this study were hemizygous male and female transgenic mice and age- and sex-matched non-transgenic littermate controls. Mice were maintained on a 12-h light and 12-h dark cycle and received tap water *ad libitum*.

One group of mice was fed a standard breeding diet (BD: Altromin C1314, Germany, Table 1A). In order to study the influence of fibre and disaccharide intake on the metabolic state, a second group of mice was fed a high fibre diet (HF) from weaning until 120 days of age. Afterwards they were fed the breeding diet (see above). The HF is rich in insoluble dietary fibre, contains a restricted amount of disaccharides, and a restricted amount of calories (Altromin C1009, Germany, Table 1A). Numbers of animals fed BD or HF, used for the different investigations are listed in Table 1B.

In addition, one group of animals ($n=21$) permanently receiving HF was examined at 140, 170 and 230 days of age.

2.2. Urine glucose

Urinary glucose excretion was determined semiquantitatively, using the glucose–oxidase–peroxidase method (Combiscreen® Glucose, Germany).

Spot urine samples were taken from 110- and 140-day-old animals of the different diet groups both after a 12-h fasting period and 2 h after refeeding.

2.3. Blood and serum parameters

Blood glucose levels were determined, using the Precision QID system (Medisense, Germany).

Table 1A
Composition of the different diets

	BD: Altromin 1314	HF: Altromin C1009
Protein % (w/w)	23.6	17.3
Fat % (w/w)	4.9	7.0
Fibre % (w/w)	3.7	63.0
Ashes % (w/w)	6.4	5.6
Disaccharides % (w/w)	5.8	1.3
Nfe % (w/w)	44.6	3.9
Energy (kcal/kg)	2,826	1,374
Protein ME (%)	29.2	44.1
Fat ME (%)	15.6	45.9
Nfe ME (%)	55.2	10.0

BD, breeding diet: fibre from wheat products; HF, high fibre diet: fibre from cellulose; Nfe, nitrogen free extractives; ME, metabolizable energy.