

Glucokinase, the pancreatic glucose sensor, is not the gut glucose sensor

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Abstract

Aims/hypothesis The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are released from intestinal endocrine cells in response to luminal glucose. Glucokinase is present in these cells and has been proposed as a glucose sensor. The physiological role of glucokinase can be tested using individuals with heterozygous glucokinase gene (*GCK*) mutations. If glucokinase is the gut glucose sensor, GLP-1 and GIP secretion during a 75 g OGTT would be lower in *GCK* mutation carriers compared with controls.

Methods We compared GLP-1 and GIP concentrations measured at five time-points during a 75 g OGTT in 49 participants having *GCK* mutations with those of 28

familial controls. Mathematical modelling of glucose, insulin and C-peptide was used to estimate basal insulin secretion rate (BSR), total insulin secretion (TIS), beta cell glucose sensitivity, potentiation factor and insulin secretion rate (ISR).

Results GIP and GLP-1 profiles during the OGTT were similar in *GCK* mutation carriers and controls ($p=0.52$ and $p=0.44$, respectively). Modelled variables of beta cell function showed a reduction in beta cell glucose sensitivity ($87 \text{ pmol min}^{-1} \text{ m}^{-2} [\text{mmol/l}]^{-1}$ [95% CI 66–108] vs $183 \text{ pmol min}^{-1} \text{ m}^{-2} [\text{mmol/l}]^{-1}$ [95% CI 155–211], $p<0.001$) and potentiation factor (1.5 min [95% CI 1.2–1.8] vs 2.2 min [95% CI 1.8–2.7], $p=0.007$) but no change in BSR or TIS. The glucose/ISR curve was right-shifted in *GCK* mutation carriers.

Conclusions/interpretation Glucokinase, the major pancreatic glucose sensor, is not the main gut glucose sensor. By modelling OGTT data in *GCK* mutation carriers we were able to distinguish a specific beta cell glucose-sensing defect. Our data suggest a reduction in potentiation of insulin secretion by glucose that is independent of differences in incretin hormone release.

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Abbreviations

BSR	basal insulin secretion rate
EIR	early insulin response
GIP	glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide 1
HOMA(B)	homeostasis model assessment of beta cell function
HOMA(S)	homeostasis model assessment of insulin sensitivity

IGI	insulinogenic index
ISIcomp	index of composite whole-body insulin sensitivity
ISR	insulin secretion rate
QUICKI	quantitative insulin sensitivity check index
SGLT	sodium–glucose cotransporter
TIS	total insulin secretion

Introduction

The incretin effect explains the augmentation of insulin secretion by oral glucose compared with similar glycaemia achieved by i.v. glucose and is mediated through gut-derived hormones. The most important incretin hormones are glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1), which both potentiate glucose-stimulated insulin release by pancreatic beta cells. There is controversy in the literature as to how these specialised cells sense the gut glucose load. Glucokinase [1], sodium–glucose cotransporter (SGLT) [2] and G protein-coupled receptors [3] have all been proposed as functioning chemosensors in the gut enteroendocrine cells.

The enzyme glucokinase has been proposed as the chief glucose sensor in the gut, analogous to its role in pancreatic beta cells [1, 4–6]. When GIP-producing K cells in the duodenum were engineered to produce insulin under the control of the GIP gene promoter, they functioned in place of beta cells to maintain whole-body glucose homeostasis [5, 7]. In vitro data from immortalised L cell lines suggest that GLP-1 release from L cells and insulin release from beta cells share some common glucose-sensing machinery including glucokinase [8, 9]. In addition to glucokinase-regulated enteroendocrine cells releasing GIP and GLP-1, glucose-responsive neurons in the myenteric plexus may also be regulated by glucokinase [1, 10, 11].

We aimed to study patients with naturally occurring glucokinase gene (*GCK*) mutations to assess the role of glucokinase in GIP and GLP-1 secretion. Heterozygous *GCK* mutation carriers have a rightward shift in their glucose/insulin secretion rate (ISR) dose–response curves compared with normal controls, consistent with their reduced glucose-sensing function of pancreatic beta cell glucokinase [12]. Since luminal, but not systemic, glucose is a potent trigger of incretin hormone release, if glucokinase was also the chief glucose sensor in the gut K and L cells then, we hypothesised, the secretion of incretin hormones (GIP and GLP-1) in response to an identical oral glucose load would be lower in heterozygous *GCK* mutation carriers compared with controls (despite a higher plasma glucose level in the mutation carriers). We also used these OGTT data to employ a mathematical model of beta

cell function to quantify abnormalities in pancreatic glucose sensitivity and potentiation in *GCK* mutation carriers compared with controls.

Methods

Participants for clinical studies Seventy-seven participants from *GCK* mutation families were recruited in the UK. Forty-nine carried the *GCK* mutation and 28 familial controls did not. All participants were between 16 and 70 years and had no history of type 2 diabetes in first-degree relatives. Pregnant women and those taking steroids were excluded. The study protocol was approved by local research ethics committees and the studies were performed in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Methods for clinical studies Measurements were taken according to a common protocol. Participants fasted from 22:00 hours the night before assessment, and avoided excessive exercise and alcohol for the previous 24 h. Three plasma samples for glucose, insulin, C-peptide, GLP-1 and GIP were collected from the fasting participants before an OGTT. Four further plasma samples were taken at 30 min intervals following a 75 g oral glucose load. A single blood sample was taken for DNA extraction. Body composition was assessed by bioelectrical impedance (Bodystat 1500; Douglas, Isle of Man, British Isles). Waist and hip measurements were taken.

Sample analysis All blood samples were stored at -80°C . All biochemistry was performed in the same UK laboratories. Plasma glucose was measured by standard automated laboratory methods. Insulin was measured by an immunoenzymometric assay (Insulin EASIA; Biosource-Invitrogen, Merelbeke, Belgium). C-peptide was measured by an immunochemiluminometric assay (Siemens Medical Solutions Diagnostics, Newbury, UK). GIP and GLP-1 concentrations were measured after extraction from plasma with 70% ethanol. GIP was measured using RIA with C-terminally-directed antiserum R65 [13], which cross-reacts fully with human GIP and the primary metabolite GIP3–42, but not GIP 8000. Human GIP and ^{125}I -labelled human GIP (70 MBq/nmol) were used for standards and tracer. Plasma GLP-1 was measured against standards of synthetic GLP-1 7–36 amide using antiserum code no. 89390 [14] (specific for the amidated C-terminus of GLP-1 and therefore not reactive with GLP-1-containing peptides from the pancreas). The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9–36 amide, into which GLP-1 is rapidly converted [15]. For

both incretin hormone assays sensitivity was <1 pmol/l, intra-assay CV $<6\%$ at 20 pmol/l, and recovery of standard added to plasma before extraction about 100% when corrected for losses inherent in the plasma extraction procedure.

DNA from all study participants was sequenced for *GCK* mutations. PCR products were purified and sequenced using BigDye Terminator chemistry (Applied Biosystems, Warrington, UK). Reactions were analysed on an ABI Prism 3100 DNA Sequencer (Applied Biosystems) and sequences compared using Mutation Surveyor v2.51 software (SoftGenetics, State College, PA, USA).

Data analysis and mathematical modelling Incretin hormone secretion at five time-points during the OGTT, modelled components of beta cell function and any decline in beta cell function with age were each compared between heterozygous *GCK* mutation carriers and controls.

Empirical indices of beta cell function included calculation of the homeostasis model assessment of beta cell function index (HOMA(B)), from fasting insulin and glucose values through the computer model v2.2 [16, 17]. Other empirical indices of beta cell function were derived from OGTT values (glucose in mmol/l and insulin in pmol/l): insulinogenic index (IGI), calculated as (30 min insulin–basal insulin)/(30 min glucose–basal glucose), and the early insulin response (EIR) calculated using the formula (30 min insulin–basal insulin)/(30 min glucose) [18].

Mathematical modelling was used to obtain more sophisticated variables describing the beta cell insulin secretory process. The model [19, 20] describes the relationship between insulin secretion ($\text{pmol min}^{-1} \text{m}^{-2}$) and glucose concentration coupled with a model of C-peptide kinetics. Importantly it provides three main indices of beta cell function: beta cell sensitivity to glucose (glucose sensitivity), beta cell sensitivity to the rate of change of glucose (rate sensitivity) and a potentiation factor (expressing relative potentiation of insulin secretion from the beginning to the end of the OGTT). Potentiation is related to factors such as prolonged exposure to hyperglycaemia, non-glucose substrates, incretin hormones and neurotransmitters. The model also computed basal insulin secretion rate (BSR) and the total insulin secretion (TIS) during the OGTT. The model variables were estimated from the glucose and C-peptide concentrations by regularised least-squares and implemented using Matlab (The MathWorks, Natick, MA, USA) [21].

We also examined a number of surrogate measures of insulin sensitivity using fasting measures or formulas incorporating fasting and OGTT data (the homeostasis model assessment of insulin sensitivity [HOMA(S)], the quantitative insulin sensitivity check index [QUICKI] and the index of composite whole-body insulin sensitivity [ISComp]).

Statistical analysis Baseline means from participants with *GCK* mutations were compared with those without mutations using Student's *t* test. Distribution of sex amongst the two groups was compared using χ^2 tests. Mean hormone concentrations during five time-points of the OGTT were compared using repeated-measures analysis using SPSS v11.5 and are shown with 95% CIs. All other means were compared by ANCOVA using age and BMI as covariates and are presented with SEs. Data showing a skewed distribution were log transformed (\log_{10}). All tests were two-tailed and the significance level was 0.05.

Results

Of the 77 participants studied, 49 (64%) carried the *GCK* mutation. The baseline characteristics (Table 1) showed no significant difference other than glucose values, which were greater in *GCK* mutation carriers than non-carriers (Fig. 1a).

Mean GLP-1 (Fig. 1b) and GIP (Fig. 1c) concentrations during the 75 g OGTT were not different between *GCK* mutation carriers and non-carriers ($p=0.70$ and $p=0.32$, respectively). Peak GIP or GLP-1 (at 30 min following 75 g oral glucose) did not alter with age, sex, BMI or peak circulating plasma glucose.

Insulin (Fig. 1d) and C-peptide concentrations were similar between carriers and non-carriers ($p=0.14$ and $p=0.20$, respectively), but the modelled glucose/ISR dose–response curve was right-shifted (Fig. 2). A reduction in beta cell function in carriers vs non-carriers was found using all the empirical measures except IGI (Table 1). Of the modelled beta cell function variables, glucose sensitivity and potentiation factor were reduced in *GCK* mutation carriers, while BSR, TIS and rate sensitivity were unaltered (Table 1).

The glucose sensitivity was not significantly correlated with age in either group (carriers: $r^2=0.05$, $p=0.13$; controls $r^2=0.03$, $p=0.36$). There were no significant differences detected in any of the measures of insulin sensitivity.

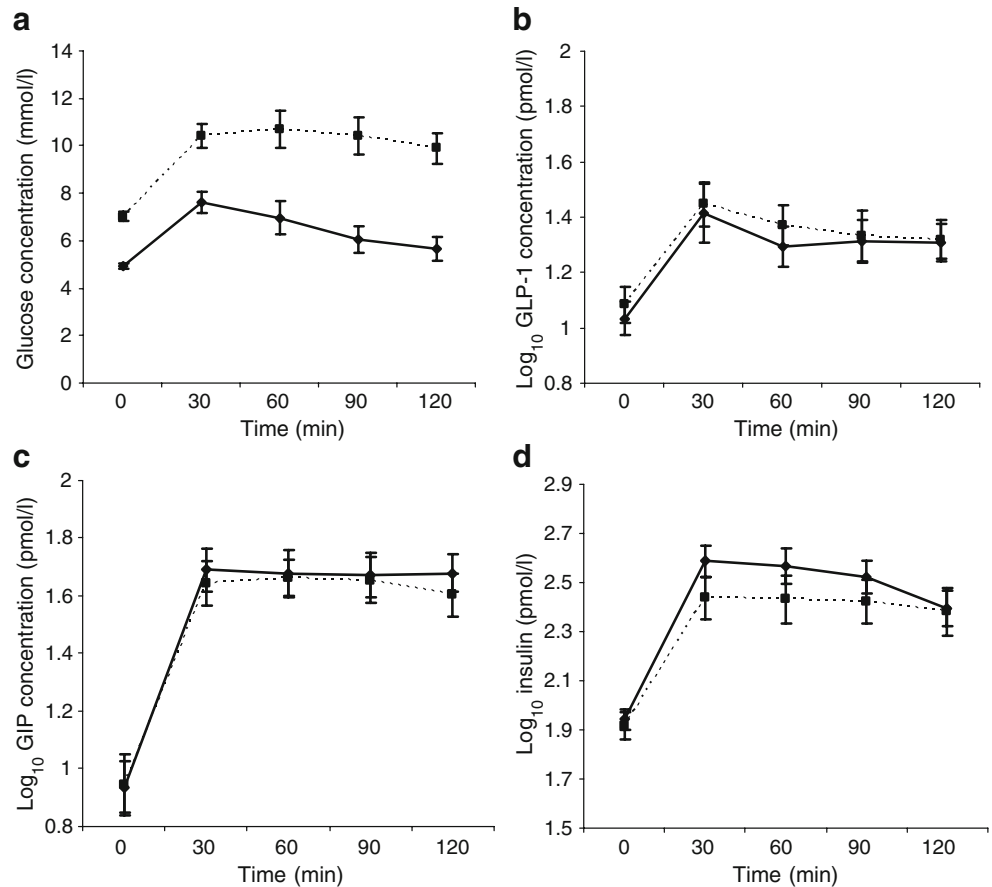
Discussion

Our results show clear effects of a heterozygous mutation in *GCK* on the beta cell in altering insulin secretion, but not in the gut in altering incretin hormone secretion. There was no difference in incretin hormone secretion among *GCK* mutation carriers and controls following identical gut exposure to a 75 g oral glucose load. All participants will have had a similar concentration of glucose in their gut lumen even though their plasma glucose levels differed according to *GCK* mutation status. Since gut glucose sensing is thought to be luminal and not systemic [22], this suggests that glucokinase is not acting as the main

Table 1 Empirical and model-based measures of beta cell function using OGTT data from *GCK* mutation carriers and non-carriers

Variable	Carriers (<i>n</i> =49)	Non-carriers (<i>n</i> =28)	<i>p</i> value
Baseline characteristics			
Sex (male/female)	14:35	11:17	0.33
Age (years)	40.1 (1.9)	34.2 (2.5)	0.06
BMI (kg/m ²)	25.6 (0.8)	27.1 (1.0)	0.27
WHR	0.86 (0.02)	0.86 (0.02)	0.88
Body fat (%)	28.0 (1.4)	28.1 (1.9)	0.96
Fasting glucose (mmol/l)	7.02 (0.07)	4.98 (0.10)	<0.001
120 min glucose (mmol/l)	9.89 (0.30)	5.67 (0.40)	<0.001
Empirical measures of beta cell function			
Log ₁₀ IGI (pmol/mmol)	1.69 (0.05)	1.83 (0.07)	0.12
Log ₁₀ EIR (pmol/mmol)	1.27 (0.04)	1.57 (0.06)	<0.001
Log ₁₀ HOMA(B)	1.86 (0.02)	2.17 (0.02)	<0.001
Log ₁₀ (AUC insulin/AUC glucose) (pmol/mmol)	-1.31 (0.04)	-1.05 (0.06)	<0.001
Modelled measures of beta cell function			
Log ₁₀ BSR (pmol min ⁻¹ m ⁻²)	2.06 (0.02)	2.04 (0.03)	0.54
TIS (nmol/m ²)	46.8 (2.0)	50.9 (2.7)	0.23
Glucose sensitivity (pmol min ⁻¹ m ⁻² [mmol/l] ⁻¹)	89 (11)	179 (14)	<0.001
Rate sensitivity (pmol m ⁻² [mmol/l] ⁻¹)	614 (123)	952 (165)	0.11
Potential factor mean time (min)	1.46 (0.17)	2.28 (0.23)	0.007
Empirical measures of insulin sensitivity			
HOMA(S)	60.6 (3.5)	57.7 (4.7)	0.63
QUICKI	0.38 (0.01)	0.39 (0.01)	0.83
ISIcomp	4.38 (0.31)	5.34 (0.41)	0.07

Fig. 1 OGTT results showing glucose (a), GLP-1 (b), GIP (c) and insulin (d) concentrations with time in *GCK* mutation carriers (dashed line) and non-mutation carrying controls (solid line); $p < 0.001$, $p = 0.32$, $p = 0.70$, $p = 0.14$, respectively. Vertical bars are SEs



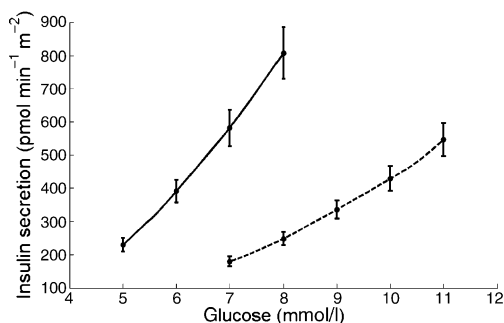


Fig. 2 Relationship between plasma glucose concentrations and ISRs determined by modelling from the OGTT in participants with *GCK* mutations (dashed line) and non-mutation carrying controls (solid line). Vertical bars are SEs

glucose sensor in the enteroendocrine cells that secrete GIP and GLP-1. This is an important result, as glucokinase has been shown to be the sensor of circulating glucose not only in the pancreatic beta cell [23], but also for the synthesis of glycogen in the liver [24] and in glucagon responses to hypoglycaemia [25].

Electrophysiological studies using immortalised L cell lines show these cells are electrically excitable and depolarise in response to a range of nutrients including glucose [8]. ATP generated from glucose metabolism in the enteroendocrine cells closes KATP channels and leads to membrane depolarisation and opening of the voltage-gated calcium channels, in a similar process to that in pancreatic beta cells and is necessary for stimulation of GLP-1 release. In addition, SGLT action (whereby luminal glucose is transported into the gut cell coupled to sodium entry, causing depolarisation from sodium influx at higher sugar concentrations) has also been demonstrated to precede GLP-1 secretion in GLUTag cells [2].

A recently proposed mechanism of incretin secretion is by sensing gut glucose through the taste cell pathways as used by the tongue [3]. The sweet taste receptor T1R2+T1R3 is expressed on the luminal membrane of gut enteroendocrine cells along with α -gustducin and other components known to function in taste transduction. Rodent and human enteroendocrine cell lines expressing these taste signalling elements have shown that enhanced GLP-1 and GIP secretion occurs with non-metabolisable artificial sweetener, and incretin hormone secretion is blocked by specific inhibitors of the sweet taste receptor or small interfering RNA for α -gustducin [26, 27]. Isolated small bowel and intestinal villi from α -gustducin-null mice showed markedly defective GLP-1 secretion in response to glucose [27].

The mathematical model we used to assess beta cell function based on five-sample OGTT data (with insulin and C-peptide measurements) enabled us to discern the specific glucose-sensing abnormality in *GCK* mutation carriers,

while all the empirical indices, except IGI, suggested a non-specific reduction in beta cell function. Since IGI reflects the increment in insulin relative to the increment in glucose, this was similar in both *GCK* mutation carriers and control participants, reflecting a purely glucose-sensing abnormality in the former. The rightward shift in the glucose/ISR dose–response curve from simple five-point OGTT-derived data is very similar to that obtained previously with much more complex and prolonged protocols involving graded i.v. glucose infusion in *GCK* mutation participants [12].

OGTT modelling suggested a reduction in potentiation factor in this group. This is in agreement with previous studies [28] that have shown that hyperglycaemic states are accompanied by a decrease in the potentiation factor, which is progressively reduced as glucose tolerance worsens. The mechanisms underlying this phenomenon are unknown; however, studies on the incretin effects in glucose intolerance and type 2 diabetes suggest that this defect is intrinsic to the beta cell rather than dependent on the secretion of incretin hormones [29]. Indeed, besides incretin hormone-mediated potentiation in insulin release, there is also glucose potentiation and neural modulation.

The glucose toxicity theory proposes that continual exposure to modest increases in blood glucose over a long period of time could have adverse effects on beta cell glucose sensitivity and hence function [30–32]. We found no difference in the deterioration in glucose sensitivity with age among *GCK* mutation carriers compared with controls. This is in keeping with the stable glycaemia seen on prolonged follow up of patients with heterozygous mutations [33] and the modest decline with age seen in cross-sectional studies of patients with *GCK* mutations [34]. This suggests that continuous exposure to the level of mild hyperglycaemia experienced by *GCK* mutation carriers has no significant progressive glucose toxic effect on the beta cell.

We acknowledge limitations of our study. First, we measured incretin hormones at five time-points only, so subtle differences in early incretin hormone release may not have been detected by our protocol. However, the trajectory of incretin hormone release as measured by more frequent sampling is captured reasonably well by the five time-points we chose to study. Second, we used only one 75 g oral dose of glucose. This was not a saturating dose of oral glucose in triggering maximal incretin hormone release [35], hence we cannot exclude an impact at higher levels of glucose. However, in other situations where glucokinase is the glucose sensor it operates at all concentrations of glucose and not just at maximal stimuli. Third, our study of 77 participants had 80% power to detect a mean difference of 0.14 in log GLP-1 and 0.13 in log GIP secretion, so we cannot exclude differences smaller than this.

In conclusion, there was no reduction in incretin hormone secretion in *GCK* mutation carriers; however,

they had reduced beta cell glucose sensing and potentiation of insulin secretion as modelled using data from a five-sample OGTT. This supports the belief that although glucokinase is the key pancreatic beta cell glucose sensor, it is not the main gut glucose sensor for the incretin hormones GIP and GLP-1.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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