HNF4α is a novel regulator of intestinal glucose-dependent insulinotropic polypeptide

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Mutations in the HNF4A gene cause MODY1 and are associated with an increased risk of Type 2 diabetes mellitus. On the other hand, incretins are hormones that potentiate reductions in blood glucose levels. Given the established role of incretin-based therapy to treat diabetes and metabolic disorders, we investigated a possible regulatory link between intestinal epithelial HNF4α and glucose-dependent insulinotropic polypeptide (GIP), an incretin that is specifically produced by gut enteroendocrine cells. Conditional deletion of HNF4α in the whole intestinal epithelium was achieved by crossing Villin-Cre and Hnf4αloxP/loxPC57BL/6 mouse models. GIP expression was measured by qPCR, immunofluorescence and ELISA. Gene transcription was assessed by luciferase and electrophoretic mobility shift assays. Metabolic parameters were analyzed by indirect calorimetry and dual-energy X-ray absorptiometry. HNF4α specific deletion in the intestine led to a reduction in GIP. HNF4α was able to positively control Gip transcriptional activity in collaboration with GATA-4 transcription factor. Glucose homeostasis and glucose-stimulated insulin secretion remained unchanged in HNF4α deficient mice. Changes in GIP production in these mice did not impact nutrition or energy metabolism under normal physiology but led to a reduction of bone area and mineral content, a well described physiological consequence of GIP deficiency. Our findings point to a novel regulatory role between intestinal HNF4α and GIP with possible functional impact on bone density.

Hepatocyte nuclear factor - 4 alpha (HNF4α) is a transcription factor that belongs to the steroid/thyroid hormone receptor superfamily originally identified as a liver-enriched transcription factor but also expressed in gastrointestinal epithelia, the pancreas and kidneys1–3. HNF4α supports the morphogenetic development of the visceral ectoderm as evidenced by deletion of Hnf4a leading to early mouse embryonic death4. This observation has led to the design of conditional deletion strategies for Hnf4a in order to timely define its precise role in various organs during development. For instance, early embryonic hepatic loss of HNF4α has been shown to severely impair hepatocyte differentiation and function leading to premature death5,6 whereas deletion in adulthood resulted in viability with important hepatic dysfunctions5,6. Similarly, conditional deletion of Hnf4a in the colonic epithelium at a period of time preceding the gut embryonic cytodifferentiation program disrupted colon morphogenesis7 whereas deletion occurring after this developmental transition only partially affected intestinal epithelial homeostasis8. It is therefore assumed that HNF4α acts as a morphogen during early embryonic development rather than being systematically involved in epithelia maintenance.

The pathological consequence of HNF4A mutations was first associated with a hereditary form of diabetes referred to as mature-onset diabetes of the young 1 (MODY1)9. Among the 13 monogenic forms of diabetes, MODY1 particularly evolved from neonatal hypoglycemia to impaired glucose-stimulated insulin secretion (GSIS) and hypoinsulinemia-induced hyperglycemia without being associated with β-cell autoimmunity or obesity phenotypes in humans10. In mouse models, the targeted loss of Hnf4a in pancreatic β-cells was not sufficient to cause hypoinsulinemia and there are conflicting reports as to whether HNF4α is crucial in sustaining GSIS in these murine models11,12. These observations suggest that other HNF4α-defective tissues could be involved to fully recapitulate MODY1 pathogenesis11,12.

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HNF4α controls metabolism in the liver where it regulates glycolytic enzymes and glucose transporters as well as lipid homeostasis. As a nuclear receptor, HNF4α harbors ligand-binding affinities for medium to long chain fatty acids and their metabolites that are capable of modulating its transcriptional activity. Given that MODY1 patients display lower circulating triglycerides as part of their pathological phenotype, the hepatic contribution of HNF4α to this metabolic disorder is suspected, although remains to be clarified. The intestinal epithelium represents another crucial tissue regulating whole organism metabolism. To this end, the enterocytes forming the intestinal barrier act as one of the first regulated steps of nutrient absorption and subsequent delivery. Previous observations support a functional role for HNF4α in interfering with these processes. Cultured intestinal epithelial cells made deficient in HNF4α were found less efficient in cellular lipid transport. Additionally, conditional deletion of Hnf4a in the murine intestine impacted enterocytic fatty acid uptake after lipid gavage. On the other hand, enteroendocrine cells from the intestinal epithelium produce a wide range of peptides mediating digestive rate, bone remodeling, appetite, GSIS, adipogenesis and global energy homeostasis control. Among these various peptides, incretins that comprise intestinal specific glucose-dependent insulinoactive polypeptide (GIP) actively contribute to GSIS by enhancing insulin secretion upon glucose sensing by pancreatic β-cells, and are also involved in a number of other biological activities including bone metabolism. The present study aimed to investigate whether intestinal epithelial HNF4α influences GIP regulation, glucose homeostasis and metabolism in mice. We report herein that conditional deletion of Hnf4a in the intestinal murine epithelium significantly reduces GIP production, impacts bone density but does not influence whole body energy metabolism under normal physiological conditions.

Materials and Methods

Animals and analytical procedures. C57BL/6J-Hnf4a<sup>loxP/loxP</sup> mice were first crossed with C57BL/6-12.4KbVilCre mice to generate 12.4KbVilCre/Hnf4a<sup>loxP/loxP</sup> mice which were subsequently bred with Hnf4a<sup>loxP/loxP</sup> mice to produce 12.4KbVilCre/Hnf4a<sup>Δ</sup> mice (HNF4α<sup>Δ</sup>) mutant mice and their controls. Mice were genotyped as previously described and treated in accordance with a protocol reviewed and approved by the Institutional Animal Research Review Committee of the Université de Sherbrooke (approval ID number 102–18) and in accordance with relevant guidelines and regulations. All experiments were carried out using male mice maintained on chow diet. Blood glucose values were determined from whole venous blood from fasted mice or during glucose tolerance tests as previously described. Glucose tolerance tests were performed per os (OGTT) or intraperitoneally (IPGTT) both with a 2 mg/g by weight glucose dose, while insulin tolerance test (ITT) was assessed with a 0.75 mU/g by weight dose. The following mouse serum hormone levels were measured using rat/mouse ELISA kits according to the manufacturer’s instructions: Total GIP (EMD Millipore, EZRMGIP-55K); GLP-1 (Crystal Chem, 81508) and Insulin (Crystal Chem, 90080). For metabolic analyses, mice were placed in metabolic cages as described previously. All groups were fed ad libitum throughout the duration of the study. Following a 5-day adaptation period, body weight (g), food intake (g), water intake (ml) and urine (ml) and feces (g) output were measured daily at the same hour. Fecal pellets were collected freshly and residual gross energy density was determined on dried samples using adiabatic bomb calorimetry (Parr Instruments, Moline, IL, USA). Body composition on post-mortem mice was measured by dual-energy X-ray absorptiometry (DEXA) using the PIXIMUS mouse densitometry apparatus (Lunar Corporation, Madison, WI, USA).

Indirect calorimetry. The Promethion High-Definition Room Calorimetry System was used for indirect calorimetry studies (GA3, Sable Systems, Las Vegas, NV). Data acquisition and instrument control were coordinated by MetaScreen v. 1.6.2 and the obtained raw data was processed using ExpeData v. 1.4.3 (Sable Systems, Las Vegas, NV) using an analysis script detailing all aspects of data transformation. A standard 12 h light/dark cycle (6:00–18:00) was maintained throughout the calorimetry studies. Prior to data collection, all animals were acclimated to cages for 3 days followed by 4 days of data acquisition. The derived Weir’s equations revised by the non-protein assumption were used to estimate mouse oxidative rates for carbohydrates (4.585 CO2–3.226 VO2 (mg/min/kg body weight)) and fat (1.695 VO2–1.701 CO2 (mg/min/kg body weight)).

RNA isolation and qRT-PCR. Total RNA from the jejunum and colon was isolated and qRT-PCR was performed as previously described. Target expression was quantitated relatively to TATA box binding protein (Tbp) gene expression. Primer sequences used for qPCR were as follows: Hnf4a sense: 5′-GTGCTGTCCTCTAGGCAATGA-3′; Hnf4a antisense: 5′-ACTCAGGCCCTTGCGATCT-3′; Gip sense: 5′-GGCTAGGGGACACAATCTAGG-3′; Gip antisense: 5′-GGATCGGAACTCAACCTCTTC-3′; Gcg sense: 5′-GTGCTGCTCCTAGGCAATGA-3′; Gcg antisense: 5′-GCTTGGCCCCTCTAGGCAATGA-3′; Tbp sense: 5′-GGGGAGCTGTGATGTGAAGT-3′; Tbp antisense: 5′-ACTCAGGCCCTTGCGATCT-3′.

Immunofluorescence. Mouse pancreas, duodenum, jejunum, ileum and colon biopsies were fixed in 4% paraformaldehyde overnight at 4°C, dehydrated, embedded in paraffin and cut in 5-μm sections. Immunofluorescence studies were performed as previously described. The following primary affinity-purified antibodies were used: goat anti-GIP (Santa Cruz, sc-23554; diluted 1/200), mouse anti-GLP-1 (Santa Cruz, sc-57166, diluted 1/200), mouse anti-insulin (Santa Cruz, sc-8033, diluted 1/50) and mouse anti-glucagon (Santa Cruz, sc-514592, diluted 1/300). Alexa 488 (Cell Signaling Technology; #4408; diluted 1/400) and Alexa 594 (Cell Signaling Technology; #8890; diluted 1/400) were used as secondary antibodies. Stained sections were analyzed using the NanoZoomer 2.0-RS (Hamamatsu Photonics, Japan) digital slide scanner, the LX2000 fluorescence module (Hamamatsu Photonics, Japan) and NDPView software.
Plasmid construction, cell culture and luciferase assays. The −192 to +39 region of the mouse Gip promoter was amplified by PCR from purified genomic DNA isolated from C57BL/6 mouse tail with the following primers: 5′-GCCCCAGATAACGTAGAGA-3′ and 5′-TCTTCCTCTCTATCTGTTG-3′. The amplicon was subcloned into the pGL3basic luciferase reporter vector (Promega, Madison, WI). Mutagenesis of the Gip promoter constructs for the HNF4α site (H1) and the GATA site 1 (G1) was performed with the QuickChange Lightning site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA) while mutagenesis of the GATA site 2 (G2) was performed with GenScript custom services (Piscataway, NJ). The respective integrities of subcloned promoter and mutagenesis products were all confirmed by sequence analysis. 293T cells were plated in 24-well plates and transfected with 200 ng of wild-type or mutated Gip promoter luciferase constructs, 5 ng of the phRL-CMV Renilla luciferase vector (Promega, Madison, WI), a combination of 100 ng of pBabepuro/HNF4α13 and 100 ng of pBabepuro/GATA-413 expression vectors with a constant total DNA amount of 800 ng per transfected well, and 1.6 μl of Lipofectamine 2000 for a total of 100 μl of OptiMEM (Life Technologies Inc, Burlington, ON). The medium was replaced after 4 h by fresh DMEM supplemented with 10% FBS. Luciferase and Renilla activities were determined 48 h after the transfection with the dual luciferase assay kit (Promega, Madison, WI). Each experiment was repeated three times in triplicate.

EMSA. Electrophoretic mobility shift assays (EMSA) were performed as described previously32. The reactions were performed with 5 μg of nuclear protein extracts from 293T cells transfected or not with pBabepuro/GATA4 or pBabepuro/HNF4α1 expression vectors. For the supershift analysis, 200 ng of HNF4α-C-19 (sc-6556 X) antibody, 200 ng of GATA4-C-20 (sc-1237 X) or 200 ng of irrelevant HNF1α-C-19 (sc-6547 X) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were added and the binding reactions were continued for 10 minutes at room temperature. Retarded complexes were then separated on 5% polyacrylamide gel at 4°C during 4 hours, dried for 1 hour at 80°C and exposed on autography film. The DNA probes consisted of double-stranded oligonucleotides of individual binding sites for both HNF4α (H1) and GATA4 (G1 and G2) within the promoter region of the Gip gene (MatInspector software tool, http://www.genomatix.de)33. Positive controls for GATA4 and HNF4α binding were used from the Sis gene promoter34 and APOC3 gene binding35.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 7 software. Figures 1B–D, 3B and 4E were analyzed using the Mann-Whitney test while 2-way ANOVA tests were used to analyze Figs 1A,E,F, 2B,E, 3C, 4A–D,F,G, 5 and 6. 2-way ANOVA tests were corrected for multiple comparisons using the Holm-Sidak method. Differences were considered significant with a P value of <0.05. The data are presented as mean ± standard error of the mean; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Results

Loss of intestinal HNF4α negatively impacts incretins production from the gut. Mice were conditionally deleted for Hnf4α in the intestinal epithelium as previously described30,31. Total RNA isolated from the jejunal and colon of 1 week-old Hnf4αΔIEC and control mice were used to confirm that Hnf4α gene transcript expression was decreased in the intestinal epithelium of Hnf4αΔIEC mice as determined by qRT-PCR (Fig. 1A). In order to evaluate a possible regulatory link between HNF4α and gut specific incretins, GIP and GLP-1 gene transcript expression was measured from the jejunal and the colon, respectively. A significant reduction was observed for jejunal Gip gene transcript (46%, P < 0.01) and colonic Gcg gene transcript (37%, P < 0.01) of 1 week-old Hnf4αΔIEC mice (Fig. 1B). This reduction in Gip and Gcg transcripts was also maintained in adult Hnf4αΔIEC mice (data not shown). To assess whether modulation at the gene transcript level impacted mature incretins production, ELISA essays were performed on intestinal tissues. GIP peptide concentration was reduced by more than 61% (P < 0.001) in the jejunal of fasted Hnf4αΔIEC mice (Fig. 1C) while GLP-1 peptide concentration dropped by 87% (P < 0.01) in the colon of these mice (Fig. 1D). Immunoﬂuorescence experiments were next performed to assess whether changes observed in incretin production was associated with variations in K (GIP) and L (GLP-1) enteroendocrine cell populations. As expected, the relative number of GIP+ cells was most abundant in the proximal intestine and gradually decreased toward the distal part while GLP-1+ cells were more abundant in the distal intestine as previously reported elsewhere35. Hnf4αΔIEC mice displayed non-significant changes in the relative number of GIP+ cells along the intestinal segments (Fig. 1E). Intriguingly, loss of intestinal HNF4α led to a reduction in the number of GLP-1+ cells in the colon (62%, P < 0.01) without affecting their number in both the jejunum and the ileum (Fig. 1F). Representative immunostaining results are shown for GIP+ cells within the duodenum (Fig. 1G) and GLP-1+ cells within the colon (Fig. 1H). Collectively, these observations suggest that HNF4α positively impacts Gip gene transcript and peptide expression, while mediating GLP-1 expression in a complex regional manner along the anterior-posterior axis of the gut epithelium.

HNF4α regulates Gip promoter activity. Since the loss of HNF4α was found to correlate well with Gip gene transcript expression without majorly affecting Gip + cell production throughout the small intestine, Gip promoter was further screened for potential HNF4α DNA binding sites. A 193 bp region upstream of the transcription start site (TSS) of the murine Gip gene was analyzed since it was previously reported to represent the minimal promoter region required to direct specific expression in endocrine cells26. This analysis predicted one HNF4α binding site (H1) flanked by 2 GATA DNA binding sites (G1 and G2) (Fig. 2A). Interestingly, comparison of the GIP promoter among different species including human displayed a relatively well conserved distribution pattern of these GATA/HNF4α predicted binding sites (Supplementary Fig. 1). Co-transfection experiments with increasing concentrations of HNF4α did not impact −192 to +39 Gip promoter activity (data not shown). Since GATA-4 has been shown to regulate GIP expression27 and to physically interact with HNF4α28, cooperative interaction of both HNF4α and GATA-4 was tested on Gip promoter activity. GATA-4 alone was able to induce a greater than 7.3-fold increase (P < 0.0001) in Gip promoter activity while HNF4α alone had no effect (Fig. 2B).
However, the combination of GATA4 and HNF4α led to a 11.3-fold ($P < 0.0001$) synergistic induction of the $-192$ to $+39$ Gip promoter construct (Fig. 2B). Both GATA-4 and HNF4α expression vector did not influence pGL3basic empty reporter activity (Fig. 2B). To further determine whether the predicted HNF4α and GATA-4
DNA binding sites of the *Gip* promoter were functional, EMSA was performed with double stranded 32P-labelled probes corresponding to these specific sites. Nuclear extracts isolated from 293 T cells transfected with an HNF4α expression vector were able to generate a complex with both the *Apoc3* HNF4α binding site used as a positive control (lane 1, Fig. 2C) and the *Gip* H1 site (lane 5, Fig. 2C) as opposed to empty nuclear extracts isolated from non-transfected 293 T cells (lanes 4 and 8, Fig. 2C). This complex was specific for HNF4α binding since inclusion of HNF4α antibodies led to the formation of supershifted complexes (lanes 2 and 6, Fig. 2C) as opposed to the use of irrelevant antibodies (lanes 3 and 7, Fig. 2C). A similar approach was also used with nuclear extracts isolated from 293 T cells transfected or not with a GATA-4 expression vector. A GATA-4 complex was observed for the *Sis* GATA binding site used as a positive control (lane 1, Fig. 2D), as well as for the *Gip* G1 (lane 5, Fig. 2D) and *Gip* G2 (lane 9, Fig. 2D) sites when GATA-4 positive nuclear extracts were used as opposed to empty 293 T nuclear extracts (lanes 4, 8 and 12, Fig. 2D). Again, this complex was specific for GATA-4 since the use of GATA-4 antibodies produced supershifted complexes (lanes 2, 6 and 10, Fig. 2D) as opposed to the use of irrelevant antibodies (lanes 3, 7 and 11, Fig. 2D). Mutagenesis of H1 (HNF4α), G1 (GATA) and G2 (GATA) sites was next achieved to monitor the importance of these sites for synergistic activation of the *Gip* promoter from both HNF4α and GATA-4. While mutagenesis of either G2 alone or G1 alone did not significantly impact the combined GATA-4 and HNF4α induction of *Gip* transcription, mutagenesis of H1 in the presence of both GATA-4 and HNF4α led to a 63% reduction in synergistic activation of the *Gip* promoter (P < 0.0001) (Fig. 2E). Mutagenesis of all G1, G2 and H1 sites reduced the GATA-4 and HNF4α synergistic effect on *Gip* promoter.
transcription by more than 86% ($P < 0.0001$) (Fig. 2E). Collectively, these observations support that the interaction of HNF4α with its binding site is of functional importance to positively regulate Gip promoter activity.

Loss of intestinal HNF4α does not influence glucose homeostasis or glucose-stimulated insulin secretion after oral or intraperitoneal glucose challenges. Given that incretins support pancreatic functions, we next monitored whether loss of intestinal HNF4α and associated reduction of intestinal GIP and GLP-1 production influenced pancreatic islets features. Both control and HNF4αΔIEC pancreatic islets displayed typical structures with inner insulin-expressing cells (β-cells) surrounded by glucagon-expressing cells (α-cells) (Fig. 3A). When compared to control mice, HNF4αΔIEC mice did not show significant change in islets size (Fig. 3B) as well as in β-cell and α-cell distribution (Fig. 3C). We then investigated whether the observed changes in gut incretins expression could impact circulating levels of both GIP and GLP-1 as well as glycemic parameters in HNF4αΔIEC mice following an oral glucose tolerance test (OGTT). Fasting circulating levels of GIP in HNF4αΔIEC mice were reduced by 52% when compared to control mice ($P < 0.05$) (Fig. 4A). OGTT increased GIP levels reaching a peak at 10 min and then decreasing at 30 min in both control and HNF4αΔIEC mice (Fig. 4A). However, the magnitude of the GIP response remained weaker in HNF4αΔIEC mice with a 32% reduction ($P < 0.05$) at 10 min and 55% reduction ($P < 0.01$) at 30 min in circulating GIP levels compared to controls. In contrast to GIP, fasting circulating levels of GLP-1 were not affected in HNF4αΔIEC mice (Fig. 4B). Insulin release (Fig. 4C) and blood glucose concentration (Fig. 4D) remained unchanged between HNF4αΔIEC and control mice during OGTT, consistent with a similar insulinogenic index in these mice (Fig. 4E). In order to gain further insights into glycemic control in these mice, glucose and insulin tolerance tests were achieved intraperitoneally. Both glucose tolerance (Fig. 4F) and insulin tolerance (Fig. 4G) remained similar between HNF4αΔIEC and control mice. These data indicate a defective glucose-stimulated GIP response in HNF4αΔIEC mice that was not counteracted by GLP-1 and without influencing GSIS.

Loss of intestinal HNF4α and coincidental GIP down-regulation do not impact nutrition and energy metabolism but negatively influence bone density. To gain further insight into the physiological impact of reduced GIP production in HNF4αΔIEC mice, metabolic parameters were next assessed.

Figure 3. Pancreatic islet structures of HNF4αΔIEC mice. (A) Representative immunofluorescence of insulin and glucagon on two distinct islets of Langerhans sections obtained from adult control and HNF4αΔIEC mice. Computed image analysis using CellProfiler (3.1.5) was designed to quantify labeled cells. Representative image analysis obtained from HNF4αΔIEC islet sections (bottom panel) highlights positive detected cells (colored nuclei), overlapping immunofluorescence (white diffuse staining) and non-labeled islet cells (empty and white circled nuclei). (B) Pancreatic islets area measured in adult control (black column) and HNF4αΔIEC (white column) mice (n = 4–5). (C) Relative population of cells positive for insulin (green columns) or glucagon (red columns) signals in pancreatic islets of adult control and HNF4αΔIEC mice (n = 3–5).
HNF4αΔIEC mice showed similar weight growth curves when compared to control mice (Fig. 5A), consistent with similar food intakes from both groups (Fig. 5B). Gross intestinal absorption ability remained similar in 4-month-old and 7-month-old HNF4αΔIEC and control mice as determined by total residual fecal caloric concentrations (Fig. 5C). Sleep and daily rhythms were also similar between HNF4αΔIEC and control mice (Fig. 5D) with no significant change in energy homeostasis as determined by oxygen consumption (Fig. 5E), respiratory exchange ratio (RER) (Fig. 5F) and energy expenditure (EE) (Fig. 5G). Oxidative rates fluctuations during light and dark phases for both carbohydrate (Fig. 5H) and fat (Fig. 5I) remained also similar between control and HNF4αΔIEC mice. Because incretins can regulate adipogenesis, we also assessed whether adipose density was affected in HNF4αΔIEC mice. Analysis of the white adipocyte tissue showed a similar frequency distribution of adipocyte sizes when compared between HNF4αΔIEC and control mice (Fig. 6A,B). DXA body composition analysis of young adults (4-month-old) and aging (7-month-old) HNF4αΔIEC and control mice also showed similar lean and fat mass among the groups (Fig. 6C). However, DXA analysis revealed a significant reduction in bone area values (8.3% at 7 months, \( P < 0.01 \); Fig. 6D), in bone mineral content (9.8% at 4 months, \( P < 0.05 \); 12.9% at 7 months, \( P < 0.001 \); Fig. 6E) and in bone mineral density values (5.8% at 4 months, \( P < 0.05 \); 6% at 7 months, \( P < 0.05 \); Fig. 6F) in HNF4αΔIEC mice when compared to controls. Altogether, these data support that HNF4αΔIEC mice retain normal physiology under standard conditions except for altered bone density, a well-described osteotrophic effects of GIP deficiency in vivo.

Discussion

Incretins have long been recognized to play a central role in controlling the nutrition metabolism axis. Numerous reports have linked incretins to a number of metabolic features including fat metabolism and obesity,\(^{39,40}\), bone metabolism,\(^{41}\) and diabetes.\(^{42}\) To date, only a small number of transcription factors have been reported to regulate GIP synthesis. GATA-4 regulate GIP expression in cell lines and Forkhead box protein O1 (FoxO1) as well as LEF1/β-catenin transcriptional complex mediate glucose and insulin-positive GIP regulation.\(^{43}\) The regulatory factor X6 (Rfx6) acts as a positive regulator of GIP and coincidently, RFX6 haploinsufficiency was found to be associated with a reduction of GIP in MODY.\(^{45}\) The present findings further identify an additional disease-relevant regulatory connection between the transcription factor HNF4α, for which mutations cause
Figure 5. Nutrition behaviors and energy metabolism in HNF4αΔIEC mice. (A) Weight curves for adult control (black squares) and HNF4αΔIEC (blue squares) mice (n = 4–13). (B) Cumulative food intake was monitored in metabolic cages during 6 consecutive days for both adult control (black squares) and HNF4αΔIEC (blue squares) mice (n = 5–6). (C) Calorie density determined from fecal samples collected from 4-month-old and 7-month-old control (black columns) and HNF4αΔIEC (white columns) mice (n = 3–5). (D) Sleeping time monitored in metabolic cages and calculated as the mean of 3 consecutive days for both adult control (black columns) and HNF4αΔIEC (white columns) mice (n = 7). Oxygen consumption (E), respiratory exchange ratio (RER) (F), and energy expenditure (EE) (G) measurements acquired for 3 consecutive days for both adult control (black circles) and HNF4αΔIEC (blue circles) mice (n = 7) with corresponding area under the curve (AUC). Estimated oxidative rates for carbohydrates (H) and fat (I) with AUC calculated for 3 consecutive days for both adult control (black circles) and HNF4αΔIEC (blue circles) mice (n = 7).
MODY1, and the intestinal specific regulation of GIP. Since MODY1 (HNF4A) mutations are somatic, concomitant alterations occurring in other tissues expressing HNF4α such as the intestinal epithelium are thus likely to be functionally involved during the onset of this disease. In support of this, patients with MODY1 showed a correlation between the total of insulin secretion during a test meal and GIP secretion. In addition, a recent case report described an HNF4A mutation associated with an impaired incretin response during the progression of the MODY phenotype. Our results open up for a more thorough investigation of this possible regulatory link in the context of MODY1 and other metabolic diseases.

Our results demonstrate that the loss of intestinal epithelial HNF4α impacts glucose-stimulated GIP production. Although a reduction of colonic GLP-1 producing cells was observed exclusively in the colon of HNF4αΔIEC mice, this observation did not significantly impact GLP-1 circulating levels under these conditions. It is possible that other GLP-1 producing sources may contribute to maintain homeostatic circulating GLP-1 levels in HNF4αΔIEC mice. HNF4α possible implication in the regulation of GLP-1 appears complex and will require further investigations. However, no significant difference in glucose tolerance was observed in HNF4αΔIEC mice, an observation reminiscent of the acute loss of K (GIP+) enteroendocrine cells in transgenic mice. Glucose homeostasis is complex and involves several hormones and factors produced by various tissues. The impact of our findings on glucose homeostasis will have to be measured in multi-tissues knockout models. However, our model based on the loss of HNF4α in the intestinal epithelium recapitulates the findings made in various mouse models impaired for GIP signaling where bone metabolism was the most consistent physiological impairments observed in these mice.

In conclusion, the present study allowed identifying a novel positive regulatory link between HNF4α (MODY1) and GIP incretin in the intestine and paves the way for further studies as to whether this molecular relationship may be therapeutically exploited in metabolism disorders including diabetes.
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Supplementary information

Additional Information

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