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Control of liver glucokinase activity: A potential new target for incretin hormones?

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ABSTRACT

We tested the exendin-4 and des-fluoro-sitagliptin effects on fructose-induced increase in liver glucokinase activity in rats with impaired glucose tolerance and the exendin-4 effect on glucokinase activity in HepG2 cells incubated with fructose in the presence/absence of exendin-9-39. After 3 weeks of in vivo fructose administration we measured: (1) serum glucose, insulin and triglyceride levels; (2) liver and HepG2 cells glucokinase activity and (3) liver glucokinase and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase mRNA and protein levels. Fructose fed rats had: hypertriglyceridemia, hyperinsulinemia and high liver glucokinase activity (mainly located in the cytosolic fraction) together with higher glucokinase and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase mRNA and protein concentrations compared to control rats. Co-administration of either exendin-4 or des-fluoro-sitagliptin prevented serum and liver changes except glucokinase protein expression. Exendin-4 also prevented fructose-induced increase in glucokinase activity in cultured HepG2 cells, effect blunted by co-incubation with exendin-9-36. In conclusion exendin-4/des-fluro-sitagliptin prevented fructose-induced effect on glucokinase activity, mainly affecting enzyme activity modulators. Exendin 9-39 blunted in vitro protective exendin-4 effect on glucokinase activity, thus suggesting a direct effect of the later on hepatocytes through GLP-1 receptor. Alterations of glucokinase activity modulators could play a role in the pathogenesis of liver dysfunction, becoming a potential new treatment target for GLP-1 receptor agonists.

1. Introduction

Incretin hormones (glucagon-like peptide-1, GLP-1, and glucose-dependent insulinotropic polypeptide, GIP) exert multiple biological effects such as enhancement of glucose-induced insulin secretion, affect glucagon and somatostatin secretion, increment of β-cell mass, delay of gastric emptying and appetite inhibition [6]. Regarding their glucagon effect, while GLP-1 inhibits GIP stimulates its secretion [7]. The significant decrease of all these pleiotropic effects present in people with Type 2 diabetes (T2DM), has been mainly ascribed to a glucotoxic-induced down regulation of incretin-receptors rather than to a decrease in their circulating levels [6,21,30]. Currently, many of these people are treated with either GLP-1 and its analogs, or specific inhibitors of their degrading enzyme dipeptidyl peptidase IV (DPP-IV) [6,25,29].

Administration of a fructose-rich diet to normal rats induces insulin resistance and impaired glucose tolerance or diabetes, depending on treatment duration [23]. We have shown that development of these abnormalities as well as fat deposit in liver, are effectively prevented by co-administration of either exendin-4 or sitagliptin [24]. Supporting the latter effect, other authors also reported incretin effects on liver dysfunction in people with T2DM [20] and on glucose metabolism as well as on glucokinase activity in experimental diabetes [9,19]. Additionally, we previously showed that in fructose fed rats liver glucokinase activity is greatly increased due to the combination of enzyme translocation from nucleus to cytosol and its interaction with an increased amount of PFK2, a cytosolic positive modulator of enzyme activity [16]. The effect on glucokinase could be independent of insulin action and mediated through GLP-1 receptor [10].

Abbreviations: T2DM, type 2 diabetes; GLP-1, glucagon-like peptide-1; DPP-IV, dipeptidyl peptidase IV; des-F-sitagliptin, des-fluoro-sitagliptin; PFK, 26-phosphofructo-2-kinase/fructose-2,6-biphosphatase; CF, cytosolic fraction; DNF, nuclear fraction; qPCR, real-time PCR; GIP, gastric inhibitory peptide.

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Since little is known about the mechanism by which incretin affects liver glucokinase activity and the presence of GLP-1 receptor in hepatocytes is controversial [18,31], we decided to evaluate in vivo effects of exendin-4 and des-fluoro-sitagliptin (des-F-sitagliptin) on fructose-induced changes in liver glucokinase and mechanisms involved in its activation. In addition, we used HepG2 cells to test in vitro whether the incretin effect on fructose-induced changes in glucokinase activity depends on its general metabolic effect or on a direct effect on the liver cell acting either through or independently of GLP-1 receptor.

2. Materials and methods

2.1. Chemicals and drugs

Reagents of the purest available grade and β-actin antibody were obtained from Sigma Chemical Co. (St., Louis, MO, USA). Des-F-sitagliptin was kindly provided by Merck, Sharp and Dohme (Argentina). Glucokinase antibody (sheep anti-GST-glucokinase fusion protein antibody) was kindly provided by Dr. Mark Magnusson (Vanderbilt University, USA). This antibody and another from Santa Cruz Biotechnology Inc. (GCK N-19: sc:1980) were used to check the presence of glucokinase protein in HepG2 cells. PFK-2 polyclonal antibody (IgY-FBPase-2) was kindly provided by Prof. Sigurd Lenzen (Medizinische Hochschule, Hannover, Germany).

2.1.1. "In vivo" experiments

Normal male Wistar rats (180–200 g) were divided into two groups: animals fed a standard commercial diet (control, C) and the same diet plus 10% fructose (w/v) in drinking water for 3 weeks (F). C and F animals were randomly divided into three subgroups (10 animals each): untreated (C and F), treated with des-F-sitagliptin (115.2 mg/day/rat, premixed with the milled pellet at 0.6% [w/w]) (CS and FS) and treated with exendin-4 (0.35 nmol/kg body weight/ip twice a day) (CE and FE). We have previously shown that these doses exert significant effects in our model (Maiztegui et al., Ref. [24]).

All animals were housed in a room with controlled temperature (25 °C) and 12 h light/dark cycles. Water and food intake were measured daily, whereas individual body weight was recorded once a week.

Twenty-one days after this treatment, blood samples from 4-h fasted animals were drawn from the retroorbital plexus under light halothane anesthesia and collected into heparinized tubes to measure blood glucose, serum triglyceride and immunoreactive insulin levels. Afterwards, the animals were killed by decapitation and the same portion of liver (median lobe) was removed to perform all assays.

The protocols and procedures for the care and use of laboratory animals were reviewed by the Institutional Animal Care and Use Committee (IACUC) of the Facultad de Ciencias Médicas, Universidad Nacional de La Plata. Animal experiments and handling were performed according to the "Guide for the Care and Use of Laboratory Animals" (1996, National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055 USA).

2.1.2. "In vitro" studies

HepG2 cells were obtained from American Type Culture Collection (ATCC HB-8065) and held in 95 cm^2 flasks in nitrocellulose-filtered (0.22 μ pore size) Eagle's minimal essential medium with 5.5 mM glucose (MEM) plus 100 μg/ml streptomycin supplemented with 10% fetal-bovine serum. Cultures were then harvested with trypsin (0.25% w/v) in phosphate-buffered saline (PBS: NaCl 137 mM, KCl 2.7 mM, Na_2HPO_4, 10.0 mM, KH_2PO_4 2.0; pH 7.4) and grown at 37 °C in a humidified atmosphere with 5% (v/v) CO_2 in air. The cultured cells were then incubated in serum-containing MEM until they reached the logarithmic growth phase and then washed and incubated for 72 h under the different experimental conditions: (a) control medium (C), (b) medium supplemented with 2 mM F, (c) 2 mM F plus 1 nM exendin-4 (FE4), (d) F plus 200 nM exendin-9-36 (FE9) and (e) F plus exendin-4 and exendin-9 (FE4/9).

2.2. Serum measurements

We measured the concentration of glucose (glucose-oxidase GOD-PAP method, Roche Diagnostics, Mannheim, Germany), triglyceride (enzymatic TG color assay GPO/PAP AA, Wiener, Buenos Aires, Argentina), and immunoreactive insulin (radioimmunoassay using an antibody against rat insulin, rat insulin standard [Linco Research Inc., IN, USA], and highly-purified porcine insulin labeled with ^{125}I).

2.3. Glucokinase activity assay

The liver portion removed from each animal was immediately homogenized in a hand-held homogenizer, suspended in ice cold phosphate saline buffer containing PMSF 0.1 mM, benzamidine 0.1 mM, DTT 2 mM, aprotinin 4 μg/ml and sucrose 0.3 M, adjusted to pH 7.4. Aliquots of these homogenates were centrifuged at different speed to isolate the nuclear and the cytosolic fractions (CF and DNF). Detailed description of the technique has been previously reported [26]. Glucokinase activity was finally measured in aliquots of both liver CF and DNF. The CF/DNF glucokinase activity ratio was also calculated.

For HepG2 cell, a pellet of 3 mg protein was re-suspended and disrupted by sonication in 50 μl of the same buffer used for liver homogenization and the product stored at −80 °C until enzyme activity measurement.

Rates of glucose phosphorylation in the 100,000 g soluble CF and in the DNF as well as in HepG2 samples were measured at 37 °C, pH 7.4 by recording the increase in absorbance at 340 nm in a well-established enzyme-coupled photometric assay [26,38] containing glucose-6-phosphate dehydrogenase, ATP and NADP. For each assay, five different experiments were done in triplicate. glucokinase activity was ascertained by subtracting activity measured at 1 mM glucose (hexokinase) from that measured at 100 mM glucose according to reported procedure [26,38]. Enzyme activities were expressed as mU per milligram of protein, one unit being defined as 1 μmol glucose-6-phosphate formed from glucose and ATP per minute at 37 °C.

2.4. Total RNA

Total liver RNA from control and treated rats was isolated using TRIzol Reagent (Gibco-BRL, Rockville, MD, USA). The integrity and purity of RNA isolated was checked by running it on 1% agarose-formaldehyde gel electrophoresis and by measuring the 260/280 nm absorbance ratio. DNA contamination was avoided by using DNase I digestion (Gibco-BRL). Reverse transcription-PCR was performed using SuperScript III (Gibco-BRL) and total RNA (50 ng) from FRD and C liver as a template.

2.5. Analysis of gene expression by real-time PCR (qPCR)

qPCR was performed with a Mini Opticon Real-Time PCR Detector Separate MJR (BioRad), using SYBR Green I as a fluorescent dye. 10 ng of cDNA were amplified in a 25 μl qPCR reaction containing 0.6 μM of each primer, 3 mM $MgCl_2$, 0.3 mM dNTPs, and 0.2 μl Platinum Taq DNA polymerase 6 U/μl (Invitrogen). Samples were first denatured at 94 °C for 3 min followed by 40 PCR cycles. Each cycle comprised a melting step at 94 °C for 30 s, an annealing step at 63 °C for 45 s, and an extension step at 72 °C for 30 s, followed

by a final extension at 72 °C for 10 min. The optimal parameters for PCR reactions were empirically defined. PCR amplification was performed in triplicate. The following oligonucleotide primers were used: *β-actin* gene (GenBank® accession no. NM_019130), Forward: 5′-AGAGGGAAATCGTGCGTGAC-3′ and reverse: 5′-CGATAGTGATGACCTGACCGT-3′; *glucokinase* gene (GenBank® accession no. NM_012565), forward: 5′-GTGTACAAGCTGCACCCGA-3′ and Reverse: 5′-CAGCATGCAAGCCTTCTTG-3′; *PFK2 liver isoform* gene (GenBank® accession no. Y00702), forward: 5′-CGATCTATCTACCTATGCCGCCAT-3′ and reverse: 5′-ACACCCGCATCAATCTCATTCA-3′.

All amplicons were designed in a size range of 90–50 bp. β-actin was used as housekeeping gene. SYBR Green fluorescence emission was determined after each cycle. The purity and specificity of amplified PCR products was verified by melting curves generated at the end of each PCR. Product length and PCR specificity were further checked by 2% (w/v) agarose gel electrophoresis and ethidium bromide staining. Data are expressed as relative gene expression after normalisation to the β-actin housekeeping gene using *Qgene96* and *LineRegPCR* software as described elsewhere [16].

2.6. Western blot analysis

Immunodetection of glucokinase, PFK2 and β-actin was run on the liver cell-cytosolic fraction. Protein concentration was quantified by Bio-Rad protein assay [3]. Thereafter, dithiothreitol and bromophenol blue were added to a final concentration of 100 mM and 0.1%, respectively. Aliquots of CF containing 20 μg for glucokinase and 100 μg for PFK2 of whole protein were placed in reducing 10% SDS-PAGE and electroblotted to polyvinylidene difluoride membranes. β-Actin density was used to normalize protein content: the target protein relative content was divided by the relative β-actin protein level in each group. Nonspecific binding sites of membranes were blocked by overnight incubation with non-fat dry milk at 4 °C. Enzyme identification and quantification were performed by using specific primary antibodies against glucokinase (final dilution of 1:2000) for 90 min, PFK2 antibody (final dilution of 1:10000) for 16 h and β-actin antibody (final dilution of 1:10000) for 60 min [26]. After the incubation period, membranes were rinsed in TBS and further incubated for 1 h with the corresponding secondary antibody: anti-sheep IgG streptavidin-peroxidase conjugate or anti-chicken IgY peroxidase-labelled, respectively, for glucokinase and PFK2 and biotinylated anti-mouse IgG for β-actin. Diaminobenzidine (DAB, Sigma Co.) was used for colour development. The bands were quantified by densitometry using Gel-Pro Analyser software.

In HepG2 cells, the presence of glucokinase was assessed by Western blot by using the protocol described above with both the Dr. Magnusson antibody and the glucokinase antibody from Santa Cruz Biotechnology. The latter antibody was diluted 1:200 and incubated overnight while the corresponding secondary antibody (anti-goat IgG) was diluted 1:20000 and incubated for 60 min. This second glucokinase antibody was employed because the provider uses HepG2 cells to demonstrate its specificity and thereby presence of the enzyme in these cells.

2.7. Statistical analysis

Statistical analysis was performed by ANOVA followed by Dunnett's test for multiple comparisons using the Prism analysis program (GraphPad). Bartlettís test was used to assess variance homogeneity. Results are expressed as mean ± SEM for the indicated number of observations and differences were considered significant when $P < 0.05$.

Table 1
Changes in body weight, food consumption and water intake.

	Body weight (g)			Caloric intake (Kcal/day/rat)
	Initial	Final	Δg	
C	205 ± 5.9	276 ± 11.2	71 ± 4.0	58.8 ± 0.7
CE	210 ± 2.9	256 ± 4.1	46 ± 5.1*	56.8 ± 7.2
CS	212 ± 5.2	295 ± 5.6	83 ± 4.9	52.5 ± 2.8
F	209 ± 7.6	279 ± 9.7	70 ± 3.9	61.1 ± 3.3
FE	202 ± 3.3	251 ± 5.3	49 ± 2.2**	48.9 ± 0.9
FS	210 ± 2.3	280 ± 2.5	70 ± 1.4	53.1 ± 1.9

Values are means ± SEM ($n = 20$). Body weight (Δg): *CE (control/exendin-4) vs. C (control), $P < 0.01$. **FE (fructose/exendin-4) vs. F (fructose), $P < 0.01$. CS and FS: control and fructose/des-F-sitagliptin.

3. Results

3.1. "In vivo" studies

3.1.1. Water intake and individual body weight

F animals drank a larger volume of water than C ones, while the ingested amount of solid food was significantly larger in the latter group. This fact resulted in a different percentage of daily intake of nutrients by F vs. C rats (carbohydrates:protein:lipids 59:32:9 vs. 45:43:12, respectively), with a comparable caloric intake (Table 1). Neither exendin-4 nor sitagliptin affected significantly the caloric intake in C and F animals (Table 1).

According to this isocaloric food intake, a similar increase in body weight was recorded in animals of all the experimental groups, except those treated with exendin-4 which showed a lower body weight increase (*C*, Δ 71 ± 3.8 g vs. CE, Δ 46 ± 5.1 g and F, Δ 70 ± 3.9 g vs. FE Δ 49 ± 2.2 g, $P < 0.05$) (Table 1).

3.1.2. Blood measurements

Comparable fasting blood glucose levels were measured in animals from all the experimental groups; conversely, triglyceride and insulin levels were significantly higher in F rats (Fig. 1). Neither exendin-4 nor des-F-sitagliptin co-administration affected these later levels in C animals but effectively prevented their increase in F rats.

3.1.3. Liver glucokinase activity

In the liver, glucokinase activity is partly regulated by interaction between the enzyme and the glucokinase regulatory protein, which decreases its affinity for glucose [42] and leads to its nuclear compartmentalization [36]. Thus, the cytosolic fraction represents the only active form of the enzyme. In our case, glucokinase activity was significantly higher in F rats, being the largest percentage of the enzyme located at the CF fraction (Fig. 2A). Conversely, in *C* rats glucokinase activity was mainly located at DNF. This uneven cellular distribution pattern of glucokinase activity can be easily seen in Fig. 2B.

While neither exendin-4 nor des-F-sitagliptin co-administration affected this enzyme distribution pattern in C rats, it did so significantly in F rats, bringing the percentage to values comparable to those measured in C rats as shown by CF/DNF activity ratio (*C*, 0.6 ± 0.05; CE, 0.49 ± 0.04; CS, 0.42 ± 0.06; *F*, 1.39 ± 0.12; FE, 0.36 ± 0.05; FS, 0.5 ± 0.04, C vs. F and F vs. FE and FS, $P < 0.05$. Thus, the main location of glucokinase in the cytosol fraction recorded in rats fed with fructose might be responsible – at least partially – for the higher glucokinase activity measured in these rats; this effect was prevented by co-administration of either exendin-4 or des-F-sitagliptin to F rats.

3.1.4. Analysis of gene expression by qPCR

Glucokinase mRNA level in the liver was significantly higher in F rats. Co-administration of either exendin-4 or des-F-sitagliptin

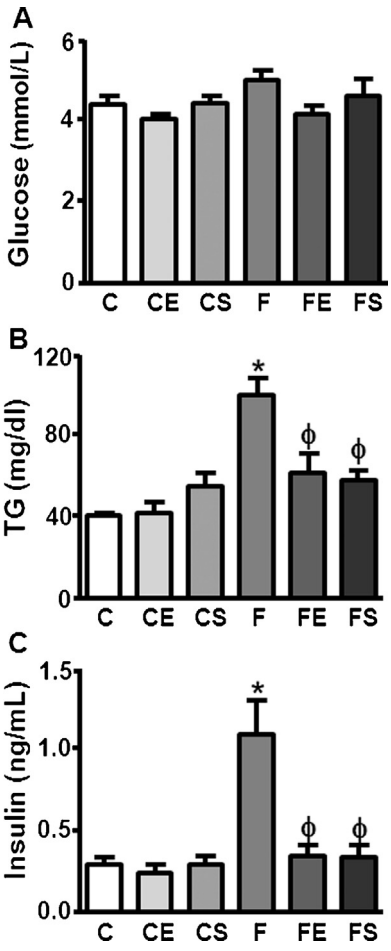

Fig. 1. (A) Blood glucose (mmol/l), (B) triglyceride (TG) (mmol/l) and (C) insulin (ng/ml) levels. Values represent the mean ± S.E.M. ($n = 20$). *F compared to C, $P < 0.001$; Φ FE and FS vs. F. $P < 0.05$. C = control; F = fructose; CE and CS = control exendin-4 and des-F-sitagliptin; FE and FS = fructose exendin-4 and des-F-sitagliptin.

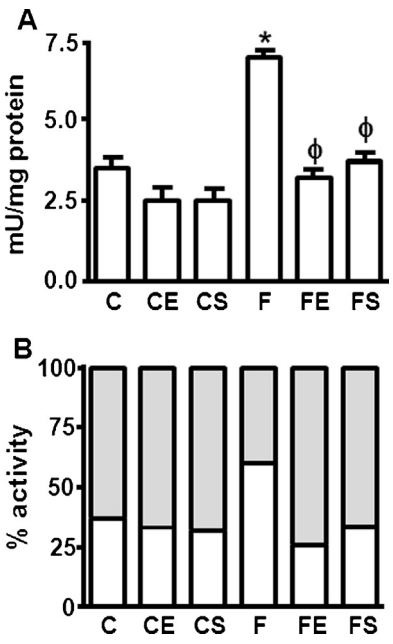

Fig. 2. Liver glucokinase activity. Rates of glucose phosphorylation in soluble CF (white columns) and in DNF (gray columns) were measured recording the increase in absorbance at 340 nm in an enzyme-coupled photometric assay. (A) CF activity. Values represent the mean ± S.E.M. ($n = 6$). *F compared C, $P < 0.001$; Φ FE and FS compared to F, $P < 0.001$. (B) Represent CF/DNF glucokinase activities as percentage. C = control; F = fructose; CE and CS = control exendin-4 and des-F-sitagliptin; FE and FS = fructose exendin-4 and des-F-sitagliptin.

prevented this increase but did not modify the values measured in C rats (Fig. 3A).

Similar behaviour was observed with *PFK2*-mRNA level: it was higher in F rats and both exendin-4 and des-F-sitagliptin prevented that increment. However, the effect was statistically significant only with the second compound (Fig. 3B).

3.1.5. *Western blot analysis*

Fructose administration induced a significant increase in glucokinase and PFK2 protein concentration in liver homogenates (Fig. 3C, D, respectively). Neither exendin-4 nor des-F-sitagliptin co-administration to F rats modified glucokinase protein concentration, though they induced a significant decrease in that of PFK2.

3.2. *"In vitro" studies*

3.2.1. *Glucokinase activity*

The presence of glucokinase in HepG2 cells was assessed by Western blot using two different antibodies (see material and methods); in both cases we detected a clear single band (Fig. 4A,B).

Glucokinase activity increased significantly in HepG2 cells cultured for 72 h in a medium rich in fructose (Fig. 4C). This increase was fully prevented by co-incubation with exendin-4, but not with exendin-9. The simultaneous addition of these two compounds to the culture medium (last column) blunted the preventive effect of the former on fructose-induced increase in glucokinase activity. Exendin-4 had no effect on HepG2 cells cultured in control medium.

4. Discussion

The present results confirm previous data published by our group: rats fed with F for 3 weeks developed a significant increase in serum triglyceride levels and an insulin resistance state (demonstrated by hyperinsulinemia with normoglycemia, high insulin:glucose molar ratio and Homeostasis Model of Assessment of Insulin Resistance index) [16,17,24]. Sitagliptin co-administration as well as exendin-4 injection administered to F rats during the 3-week period prevented the development of all these changes [24]. Supporting our previous report, we currently recorded a significant increase in liver glucokinase activity in F—fed rats [16]. This effect results from a combination of an increase in its mRNA and protein concentration, a larger cellular enzyme location in the cytosol, and the interaction of glucokinase with an increased amount of PFK2, a cytosolic positive modulator of the enzyme activity [16]. Thus, the increased activity recorded would result from a raised concentration of glucokinase modulators rather than from a single increase in the enzyme protein concentration. We could further assume that this increased in glucokinase activity must represent part of the overall metabolic adaptive reaction to fructose overload. In this regard, comparable increase in liver glucokinase activity in response to fructose has been reported by other authors both in dogs and humans [27,32,44].

Whereas liver glucokinase activity was increased in our F rats with impaired glucose tolerance, a lower enzyme activity has been reported in obese and diabetic db/db mice [9,10]. In these mice, exendin-4 administration raised decreased glucokinase protein concentration and activity levels to normal levels but, as in our case, did not affect normal enzyme activity in lean control mice [10]. These divergent results may represent an apparent rather than a real contradiction. In fact, in both cases exendin-4 admin-

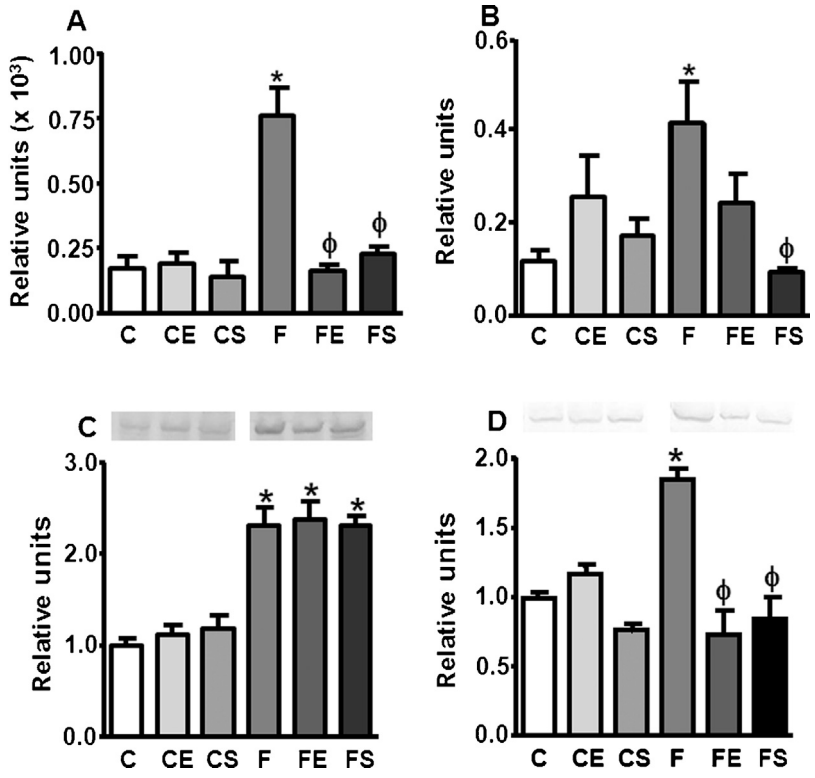

Fig. 3. Glucokinase and PFK2 gene expression and protein level in liver homogenates. Values represent mean ± S.E.M. of the relative gene expression and Western blots of 3 different experiments run in triplicate. Relative GK gene expression (A) *C compared to F, $P < 0.01$ and PFK2 relative gene expression (B) *C compared to F, $P < 0.02$. Glucokinase (C) and PFK2 (D) relative protein expression in liver. Representative blots show bands corresponding to glucokinase (C) or PFK2 (D) proteins in C, CE, CS, F, FE, FS respectively. Band intensities represent mean ± S.E.M. ($n = 6$). *F, FE, FS compared to C, $P < 0.001$ and Φ FE, FS compared to F, $P < 0.05$. C = control; F = fructose; CE and CS = control exendin-4 and des-F-sitagliptin; FE and FS = fructose exendin-4 and des-F-sitagliptin.

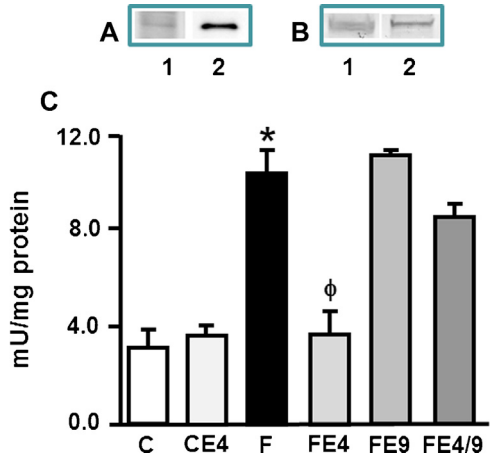

Fig. 4. Glucokinase activity en HepG2 cells. (A) Western blot of HepG2 cells (1) and RINm5F overexpression glucokinase (2) used as positive control with anti glucokinase-antibody from Santa Cruz. (B) Western blot of HepG2 cells (1) and liver (2) used as positive control with anti-glucokinase antibody provided by Dr. Mark Magnusson (Vanderbilt University, USA). (C) Glucokinase activity in HepG2 cells, *F vs. C, $P < 0.001$; Φ FE compared to, $P < 0.001$. C = control; CE4 = control exendin-4; F = fructose; FE4, FE9 and FE4/9, fructose and exendin-4, exendin-9 or both respectively.

istration switched abnormal glucokinase values (either high or low) to normal ones, thus contributing to improvement of glucose homeostasis. We could thus assume that the exendin-4 effect on glucokinase depended on the metabolic state of the animals, adjusting activity values to those recorded in control animals. In the present work, development of all the above metabolic and endocrine changes was prevented by co-administration of either des-F-sitagliptin or exendin-4 to F rats. It is important to mention that our results show a down-regulation of GK mRNA expression in FE and FS compared to F animals even though no changes in GK protein level were recorded in the same animals; thus, the drug unevenly affects levels of mRNA and protein. Although we do not know the time-course of these effects, we could hypothesize that down-regulation of mRNA might occur at an earlier stage of the treatment course than protein down-regulation. Our current experimental design does not allow us to ascertain the underlying mechanism to explain this uneven effect of exendin-4 and des-F-sitagliptin on GK gene and protein expression.

Although most of the incretin effects on glucose metabolism can be secondary to their action on insulin/glucagon secretion, some evidence strongly suggests that they could also act directly on liver glucose utilization and production [4,13,22,27,34,44]. Supporting this concept, Ayala et al. [1] using a model with controlled circulating insulin levels have recently demonstrated that GLP-1 receptor participates directly in regulation of hepatic production and muscle uptake of glucose. Using the hyperinsulinemic-euglycemic clamp model, other authors have also demonstrated that acute infusion of des-F-sitagliptin induces insulin-mediated suppression of endogenous glucose production, mainly at hepatic level, with no differences in glucose disposal rate [12].

The effect of GLP-1 and GIP occurred via activation of specific G-protein coupled receptors for both compounds (GLP-1 receptor and GIP receptor) expressed in most body tissues [4,22]. Although the effect of incretins at hepatic level has been clearly shown, whether these effects occur through or independently of GLP-1 receptor is still a matter of discussion [39–41]. While it has been shown that radiolabeled GLP-1 binds to hepatic membranes [43] and GLP-1 receptor has recently been identified in human and rodent hepatocytes [18,37], the latter results have been seriously challenged

[31]. On the other hand, it has been postulated that GLP-1 receptor activation could directly reduce liver steatosis [11,28] probably acting via insulin- [18] and AMPK-signaling pathway [2]. Additionally, Svegliati-Baroni et al. [37] also demonstrated that GLP-1 receptors in hepatocytes were reduced in patient with NASH. Our current in vitro results obtained using HepG2 cells, suggest that exendin-4 exerts its preventive effect on F-induced glucokinase increased activity, by acting directly on liver cells, interacting mainly with GLP-1 receptor. However, we cannot rule out the participation of other mechanisms in this effect. In this regard, it has been reported that exendin-9 could inhibit insulin secretion even in the absence of high levels of circulating GLP-1 [8,35], thereby suggesting that exendin-9 is an inverse agonist of the GLP-1 receptor [5]. This concept implies tonic regulation via ligand-independent activity of the GLP-1 receptor inhibited by exendin-9 [5]. This phenomenon, however, was not observed in our model since GK activity level reported in HepG2 cells cultured in presence of fructose was not modified by co-incubation with exendin-9 alone.

The beneficial effect of DPP-4 inhibitors and GLP-1 receptor agonists on glucose metabolism and β-cell mass/function in different models of T2DM has been largely and consistently documented [14,15,29,33]. However, their effect on liver carbohydrate metabolism, particularly glucokinase activity, in an animal model with characteristics that resemble those recorded in human prediabetes is not conclusive. Our data is the first demonstration that the preventive effect of both exendin-4 and des-F-sitagliptin on fructose-induced high liver glucokinase activity, could be partly due to their direct effect on liver cells. Since exendin-9 blocks the exendin-4 preventive effect in HepG2 cells, the latter effect may be mediated through exendin-4 interaction with liver GLP-1 receptor. This assumption is supported by the current demonstration of the presence of glucokinase in these cells and the blocking effect of exendin-9 on that of exendin-4 on glucokinase activity (Fig. 4C).

5. Conclusions

Our results strongly suggest that liver glucokinase activity modulators could be a new incretin target that contributes to produce their beneficial effect on glucose metabolism in the liver of animals with impaired glucose tolerance. This knowledge could help to design effective strategies to potentially prevent the transition from impaired glucose tolerance to overt type 2 diabetes.

Authors contribution

María L. Massa, Flavio Francini and Juan J. Gagliardino conceived and designed the study, performed the statistical analysis and drafted the manuscript. Mónica P. Polo performed the HepG2 cell cultures. María C. Castro and María L. Massa carried out Western blot analysis and metabolic determinations (glucokinase activity). María Cecilia Castro, Flavio Francini and Hernán Villagarcía performed total RNA isolation and gene expression measurements, handled the experimental animals and carried out the biochemical determinations. All authors have read and approved the final manucript.

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