Reduced Insulin Secretion in Protein Malnourished Mice Is Associated with Multiple Changes in the β-Cell Stimulus-Secretion Coupling

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The mechanism by which protein malnutrition impairs glucose-stimulated insulin secretion in the pancreatic β-cell is not completely known but may be related to alterations in the signaling events involved in insulin release. Here, we aimed to study the stimulus-secretion coupling of β-cells from mice fed with low-protein (LP) diet or normal-protein (NP) diet for 8 wk after weaning. Patch-clamp measurements in isolated cells showed that β-cells from LP mice had a resting membrane potential that was more hyperpolarized than controls. Additionally, depolarization and generation of action potentials in response to stimulatory glucose concentrations were also impaired in β-cells of LP mice. All these alterations in the LP group were most likely attributed to higher ATP-dependent K^+ (K_{ATP}) channel activity in resting conditions and lower efficiency of glucose to induce the closure of these channels. Moreover, a Western blot analysis revealed higher protein levels of the sulphonylurea receptor of the K_{ATP} channel in islets of LP mice. Because β-cell Ca^{2+} signals depend on electrical activity, intracellular Ca^{2+} oscillations were measured by fluorescence microscopy in intact islets, indicating a lower response to glucose in the LP group. Finally, cell-to-cell synchrony of Ca^{2+} signals was analyzed by confocal microscopy. Islets from LP mice exhibited a decreased level of coupling among β-cells, which was probably due to the low expression levels of connexin 36. Therefore, low-protein diet leads to several alterations in the stimulus-secretion coupling of pancreatic β-cells that might explain the diminished insulin secretion in response to glucose in this malnutrition state. **(*Endocrinology* 151: 3543–3554, 2010)**

Diabetes is a multifactorial disease that is closely related to nutrition. The incidence of type 2 diabetes mellitus (T2DM) is growing with millions of people diagnosed with this profound metabolic disorder every year. In developed countries, the most common risk factors for the development of T2DM are family history of T2DM and a lifestyle that includes inactivity and overnutrition that lead to obesity (1). On the other hand, in developing countries, malnutrition is also an important risk factor for the susceptibility of multiple disorders including endocrine pancreas dysfunction and diabetes, which particularly affects children during their first year of life (2–4). Early malnutrition promotes adaptive metabolic changes to permit survival in critical conditions (5). One of the main organs affected by malnutrition is the endocrine pancreas which undergoes several structural and functional adap-

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Abbreviations: AUC, Area under the curve; GSIS, glucose-stimulated insulin secretion; LP, low protein; NP, normal protein; T2DM, type 2 diabetes mellitus.

tations to maintain glucose homeostasis (6). Among the structural alterations, a marked reduction of the absolute β-cell mass and diminished number of β-cells per islet are observed in endocrine pancreas of malnourished rodent models (7, 8). Additionally, several functional changes have been reported, which include alterations in different signal transduction pathways and failures in glucose-stimulated insulin secretion (GSIS) (9). Depending on the type and intensity of the nutritional deprivation, and particularly if this nutrient deficiency occurs during intrauterine or postnatal periods, there is a progression of the β-cell dysfunction leading to insulinopenia (10). Epidemiological studies have also pointed to the relationship between low weight at birth, which is frequently associated to malnutrition states (11), with higher prevalence of obesity and diabetes in the adult life. Thus, it is necessary to have a better knowledge of the functional adaptations and changes that the endocrine pancreas undergoes during nutritional deprivation states.

We have previously shown that rats submitted to low-protein (LP) diet for 8 wk after weaning exhibit reduced body weight gain, hypoalbuminemia, elevated hepatic glycogen content, and reduced plasma insulin levels (9, 12–14). Islets from these rats are less responsive to glucose and do not exhibit the characteristic insulin secretory biphasic pattern (6, 13–15). Despite these abnormalities in insulin secretion, these animals maintain a normoglycemic state, which has been attributed to enhanced peripheral insulin sensitivity (9, 15). To date, the mechanisms that lead to impaired GSIS during protein malnutrition are not completely known. Given that alterations in the signaling events involved in insulin secretion may be related to the altered GSIS observed in protein-deficiency diets (6), in the present study we explored the stimulus-secretion coupling of β-cells from mice fed with LP diet or normal-protein (NP) diet. Electrophysiology measurements in isolated β-cells revealed several differences between the NP and LP group in terms of the resting membrane potential and the ability of glucose to induce depolarization and action potentials. Analysis of the K_{ATP} channel open probability indicated a higher activity in resting conditions and lower competence of glucose to inhibit these channels in the LP group. The ability of glucose to induce intracellular Ca^{2+} signals was also reduced in β-cells from LP mice. Finally, islets from LP mice exhibited a decreased level of cell-to-cell synchrony in the intracellular Ca^{2+} signals, which was likely related to the low expression levels of connexin 36. Thus, protein-deficient diets lead to several alterations in the signaling events involved in GSIS in the β-cell that might explain the reduced insulin secretion in this nutritional state.

Materials and Methods

Animals and diets

All protocols were approved by our Animal Care Committee according to national regulations. Experiments were performed with Swiss albino OF1 mice that were distributed in two groups according to the isocaloric diets: NP diet (17% protein) or LP diet (6% protein). The composition of these diets is described in the Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). A further description can be found elsewhere (12, 16, 17). Three days before the end of lactation period, a group of mothers was fed with the LP diet before the separation from their pups. After weaning, 21-d-old male pups were fed for 8 wk with either the NP or the LP diet. Thus, all the experiments and analysis in mice were performed at 11 wk old.

Plasma levels of insulin, glucagon, protein, and albumin

Blood samples from fasted animals were collected for biochemical analysis, and then total plasma protein (18) and total plasma albumin (19) levels were determined. Plasma insulin and glucagon levels were analyzed by RIA as previously described (20).

Glucose and insulin tolerance test

For ip glucose tolerance test, a glucose load of 2 g/kg body weight was administered in overnight fasted mice by ip injection, as previously described (9, 21), and blood samples were collected at 15, 30, 60, 120, and 180 min for measuring plasma glucose. For ip insulin tolerance test, a separated group of fed mice were injected with 0.75 U/Kg body weight of human recombinant insulin (Biohulin R, Biobrás, Brazil). Blood samples were collected before insulin injection and at 15, 30, 60, and 90 min for glucose analysis, as previously shown (21).

Islet isolation and cell culture

Adult mice from NP or LP groups were killed at 11 wk old by cervical dislocation, and islets were then isolated by collagenase digestion (22). In some experiments, isolated islets were dispersed into single cells by trypsin enzymatic digestion and then cultured overnight at 37 C in RPMI 1640 (Sigma, Madrid, Spain) supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 11 mM D-glucose (22). Except when indicated, all experiments were done at 37 C.

Ca^{2+} signaling measurements by conventional fluorescence microscopy and confocal microscopy

For Ca^{2+} experiments, isolated islets were loaded with either Fura-2 or Fluo-4 (5 μM) for 1 h at room temperature (22). Islets were placed on a perfusion chamber mounted on the microscope stage and perfused at a rate of 1.5 ml/min with a modified Ringer solution containing the following (in mM): 120 NaCl, 5 KCl, 25 $NaHCO_3$, 1.1 $MgCl_2$, and 2.5 $CaCl_2$ (pH 7.4), gassed with 95% O_2 and 5% CO_2. Ca^{2+} signals in whole islets were recorded using an inverted epifluorescence microscope (Zeiss, Axiovert 200) and a Hamamatsu Digital Camera C4742-95 (Hamamatsu Photonics, Barcelona, Spain). Images were acquired every 3 s using a dual filter wheel (Sutter Instrument Co., Novato, CA) equipped with 340- and 380-nm band-pass filters (Omega Optics, Madrid, Spain). Data were acquired using ORCA software

from Hamamatsu (Hamamatsu Photonics, Barcelona, Spain). Fluorescence records were expressed as the ratio of fluorescence (R) at 340 nm and 380 nm (F340/F380), and the increment of fluorescence (ΔR) was obtained by subtracting the mean basal fluorescence levels in the absence of stimulus from the fluorescence value during the stimulus. As previously described (23, 24), when the stimuli induced a single Ca^{2+} transient, such as tolbutamide and K^+, we analyzed the maximal amplitude of this transient (ΔR) as an indicator of the magnitude of the Ca^{2+} signal. In the case of glucose, because this stimulus induces Ca^{2+} oscillations, we analyzed the area under the curve (AUC) of the whole period of stimulation using Origin software (Origin, Originlab Corporation, Northampton, MA), and the results were expressed in ΔR/min. This parameter is an indicator of the global Ca^{2+} increase in the cells (23, 24). For the analysis of synchrony, Ca^{2+} signals were monitored in individual cells using a Zeiss LSM 510 laser confocal microscope. The configuration of the system was set to excite the Ca^{2+} probe at 488 nm and collect the emission with a band-pass filter at 505–530 nm from an optical section of 8 μm (22). As previously reported, individual cells loaded with Fluo-4 were easily identified at the periphery of the islet (22). Fluorescence records were represented as the percentage of $\Delta F/F_0$ where F_0 is the fluorescence signal at the beginning of a record and ΔF is $F\text{-}F_0$. The oscillatory Ca^{2+} signal of four to seven individual β-cells were analyzed in different islets. A synchronized cell within an islet was considered when the analyzed cell exhibited coupling of its Ca^{2+} oscillations with the rest of cells (22, 25). Nonsynchronized cells were easily identified, because their oscillations were out-of-phase with the rest of Ca^{2+} signals (25).

Insulin secretion

Groups of five islets were first incubated for 45 min at 37 C in Krebs-bicarbonate buffer containing glucose 5.6 mM equilibrated with a mixture of 95% O_2-5% CO_2 (pH 7.4). The incubation medium contained in mM: 115 NaCl, 5 KCl, 24 $NaHCO_3$, 1 $CaCl_2$, 1 $MgCl_2$, and albumin (3 g/liter; BSA). The solution was then replaced with fresh buffer containing 3 mM, 11 mM, 22 mM glucose or 500 μM tolbutamide and incubated for 1 h. Insulin was measured by RIA.

Patch-clamp recordings

Isolated cells were cultured overnight as previously described (26). K_{ATP} channel activity and membrane potential were recorded using standard patch clamp recording procedures. Currents and membrane potential were recorded using an Axopatch 200B patch-clamp amplifier (Axon Instruments Co., Sunnyvale, CA). Patch pipettes were pulled from borosilicate capillaries (Sutter Instruments Co.) using a flaming/brown micropipette puller P-97 (Sutter Instruments Co.) with resistance between 3–5 MΩ when filled with the pipettes solutions as specified below. Bath solution contained the following (in mM): 5 KCl, 135 NaCl, 2.5 $CaCl_2$, 10 HEPES, and 1.1 $MgCl_2$ (pH 7.4), supplemented with glucose as indicated. In cell-attached experiments the pipette solution contained the following (in mM): 140 KCl, 1 $MgCl_2$, 10 HEPES, and 1 EGTA (pH 7.2). The pipette potential was held at 0 mV throughout recording. K_{ATP} channel activity was quantified by digitizing 60-s sections of the current record, filtered at 1 kHz, sampled at 10 kHz by a Digidata 1322A (Axon Instruments Co.), and calculating the mean NP_o during the sweep. Channel activity was defined as the product of N, the number of functional channels, and P_o, the open-state probability. P_o was determined by dividing the total time channels spent in the open state by the total sample time. In the experiments using whole-cell configuration, the pipettes were filled with the following (in mM): 76 K_2SO_4, 10 NaCl, 10 KCl, 1 $MgCl_2$ and 5 HEPES. Experiments were carried out at room temperature (20–24 C).

Real-time PCR

Total RNA was isolated using RNeasy Plus Mini Kit (Qiagen). Extracted RNA was quantified by OD260/280 measurement. Extracted RNA was used to generate cDNA using Expand Reverse Transcriptase M-MuLV (Roche, Mannheim, Germany). Quantitative PCR assays were performed using LightCycler (Roche). Reactions were carried out in a final volume of 10 μl, containing 200 nM of each primer, 100 nM of endogenous control primer, 1 μl of cDNA, and 1× LightCycler FastStart DNA Master^{PLUS} SYBR Green I (Roche). Samples were subjected to the following thermal cycler conditions: 10 min at 95 C, 40 cycles (10 sec at 95 C/7 sec at 60 C/12 sec at 72 C), and melting curve since 60 to 95 C with a slope of 0.1 C/sec. Actin was used as the endogenous control for quantification. The resulting values were expressed as relative expression respect to control levels ($2^{-\Delta\Delta ct}$) (27). Primers are described in Supplemental Table 2.

Western blot analysis

Isolated islets were resuspended in 200 μl of Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA). Islets extracts were subjected to SDS-PAGE (10% gels). Prestained SDS-PAGE standards were included for molecular mass estimation. The transfer to PVDF membranes was performed at 125 mA for 90 min in a buffer with 2.5 mM Tris base, 9 mM glycine, 20% methanol. After membranes were blocked with 2% nonfat dry milk, and they were incubated with anti-Kir6.2 antibody (1:200; Alomone Labs), anti-Sur1(C-16) antibody (1:500; Santa Cruz Biotechnology, Santa Cruz, CA), or anti-Actin antibody (1:1000; Sigma-Aldrich), before being incubated with appropriate HRP-conjugated antibodies. Protein bands were revealed by using the ECL chemiluminescence Reagents kit (Amersham Biosciences, Barcelona, Spain). Intensity of the bands was quantified using Scion image software (Frederick, MD).

Statistical analysis

Some data are shown as mean ± SE. Student's t test or one-way ANOVA were performed as appropriate with a level of significance $P < 0.05$.

Results

Glucose homeostasis in mice fed with low-protein diet

Mice that were subjected to LP diet for 8 wk had lower body weight compared with mice under NP diet (37.35 ± 1.13 and 45.58 ± 1.06 g, respectively; n = 12; $P < 0.001$) (Table 1). Additionally, LP mice had decreased plasma levels of total protein compared with NP mice as well as total plasma albumin (Table 1). All these parameters are in agreement with previous findings in rodents subjected

TABLE 1. Body weight and plasma parameters in the fasted state of NP and LP mice

	NP	LP	n
Body weight (g)	45.58 ± 1.06	37.35 ± 1.13[b]	12
Total plasma protein (g/dl)	4.06 ± 0.16	3.53 ± 0.09[a]	7
Total plasma albumin (g/dl)	2.95 ± 0.08	2.56 ± 0.16[a]	7

Statistical significance *vs.* control (NP): [a] $P < 0.05$; [b] $P < 0.001$.
NP, Normal protein; LP, low protein.

to LP diet and indicated that the protocol produced several features of a protein-deficient nutritional status (6).

We have previously demonstrated that rats fed with LP diet are normoglycemic despite a remarkable impairment in GSIS (9). In these conditions, normal glucose levels are attained due to enhanced peripheral insulin sensitivity in these malnourished animals (9). To confirm whether mice fed with LP diet have the same metabolic changes as malnourished rats, we analyzed several *in vivo* metabolic parameters. Fasting blood glucose concentrations obtained before the glucose tolerance test were similar in the two groups (Fig. 1A). However, fasting plasma insulin levels were significantly lower in the LP compared with the NP group (Fig. 1B). In contrast, plasma glucagon levels were similar in both groups (Fig. 1C), suggesting that islet functional alterations in LP mice were probably occurring only in β-cells. To check peripheral insulin sensitivity and glucose tolerance, we performed an ip insulin and glucose tolerance tests. After the insulin load, the glucose disappearance rate was significantly higher in LP compared with NP mice (Fig. 1D). The augmented insulin sensitivity in LP mice was associated with increased glucose tolerance as judged by ip glucose tolerance test experiments (Fig. 1E). Despite lower plasma insulin levels observed at the beginning of the experiment, LP mice had augmented glucose tolerance compared with NP mice (Fig. 1E). These results indicate that mice fed with a protein-deficient diet have lower plasma insulin levels but higher insulin sensitivity in peripheral tissues, in agreement with previous findings in malnourished rats (6, 9, 15).

When the endocrine pancreas function was analyzed, we observed that insulin secretion from isolated islets in the presence of 3 mM glucose was similar in both dietary groups (Fig. 1F). However, when islets were challenged with stimulatory glucose concentrations (11 and 22 mM), insulin release in LP mice was lower than that in NP mice (Fig. 1F). These results are consistent with previous findings in malnourished rats (9). Given that the mechanisms involved in this altered GSIS in the LP islets have not been studied in detail, the next experiments were designed to analyze the main signaling events involved in β-cell insulin secretion.

Electrical activity in isolated β-cells from mice under protein-deficient diet

We studied the electrical activity in isolated β-cells using patch-clamp in the whole-cell configuration. In the

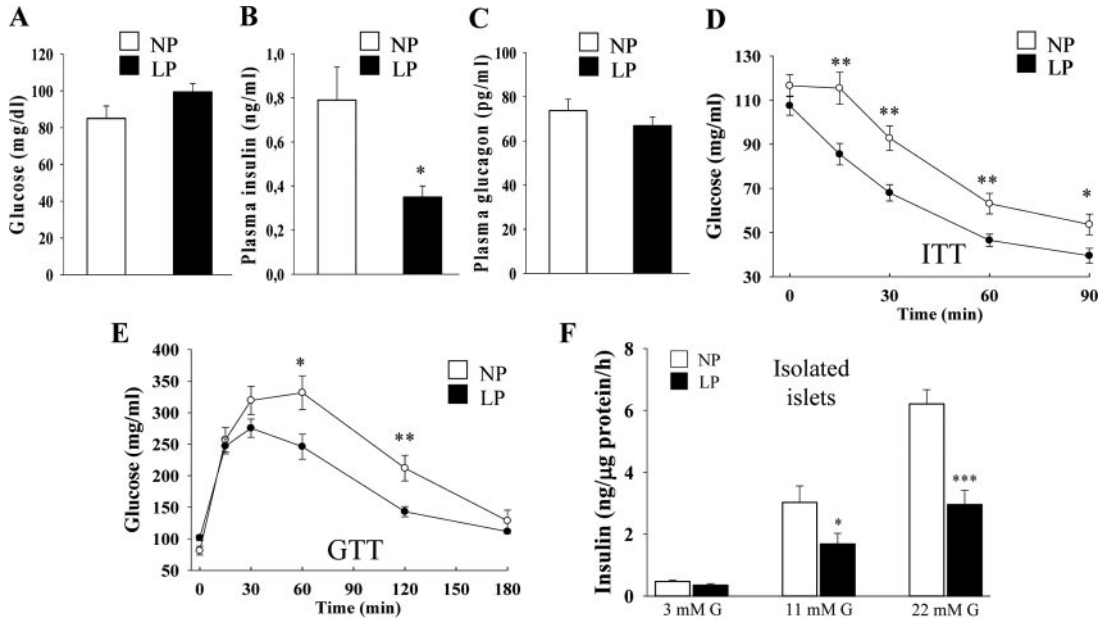


FIG. 1. Insulin release in response to glucose is reduced in islets from LP mice. A–C, Blood glucose concentrations (n = 8) as well as plasma insulin and glucagon levels (n = 8, n = 9, respectively) were examined in LP and NP mice in the fasted state. D and E, Insulin tolerance test (ITT) and glucose tolerance test (GTT) in LP and NP mice (n = 8, n = 8; respectively). F, Insulin release in response to 3, 11, and 22 mM glucose was measured in isolated islets from both groups (n = 8). Insulin levels at 11 and 22 mM glucose were significantly higher compared with 3 mM glucose in both groups ($P < 0.01$). G, glucose. Statistically significant comparing LP values with its respective control in NP: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 2. Characteristics of the electrical activity in β-cells of NP and LP mice

	0 mM G		8 mM G		11 mM G		500 μM Tolbutamide	
	NP	LP	NP	LP	NP	LP	NP	LP
V_m (mV)	−64.67 ± 1.07	−70.61 ± 1.14 [a]	−46.3 ± 1.68	−63.18 ± 1.15[b]	−40.49 ± 1.89	−53.61 ± 4.22[c]	−42.62 ± 3.12	−39.13 ± 1.50
ΔV_m (mV)			18.37 ± 0.98	7.43 ± 2.25[a]	24.18 ± 3.10	16.39 ± 9.93	22.05 ± 3.79	31.48 ± 1.54[c]
Elapsed time (min)			1.38 ± 0.153	7 ± 1.52[c]	1.35 ± 0.25	1.25 ± 0.13	0.22 ± 0.03	0.30 ± 0.018[c]
n	7	5	4	5	3	3	4	5

The membrane potential (V_m), the increase in membrane potential (ΔV_m), and the elapsed time after the application of stimuli was calculated from the experiments shown in Fig. 2. Statistical significance *vs.* control (NP): [a] $P < 0.01$; [b] $P < 0.001$; [c] $P < 0.05$. The V_m obtained with the three stimuli was found significant compared with 0 mM glucose in both groups. For the sake of clarity, statistical symbols were not included. G, Glucose; NP, normal protein; LP, low protein.

FIG. 2. Glucose-stimulated changes in membrane potential and electrical activity are impaired in β-cells from LP mice. Membrane potential (Vm) was measured in isolated β-cells from islets of NP and LP mice using patch-clamp in the whole-cell configuration. A, Representative recording in a β-cell of a NP mouse. Glucose at 8 and 11 mM as well as 500 μM tolbutamide induced depolarization and electrical activity in NP mice. B, The extended trace of a period indicated in A (*) illustrates the typical action potentials of the β-cell. C, Representative recording in a β-cell of a LP mouse. Although 8 mM glucose induced a small depolarization, it was not sufficient to trigger electrical activity. Glucose at 11 mM and tolbutamide (500 μM) produced depolarization and action potentials. D, The extended trace of a period indicated in C (*) illustrates the typical action potentials in this β-cell. The number of experiments is indicated in Table 2. G, glucose; Tolb, tolbutamide.

absence of glucose, the resting membrane potential of NP β-cells was −64.67 ± 1.07 mV, whereas this parameter was −70.61 ± 1.14 mV in β-cells from LP mice ($P < 0.01$) (Table 2). Thus, β-cells from LP mice are more hyperpolarized in resting conditions. Figure 2A shows a typical recording of the β-cell electrical activity in response to glucose and the K_{ATP} channel blocker tolbutamide in NP mice. In the presence of 8 mM and 11 mM glucose, the totality of control β-cells from NP mice generated action potentials that originated from −46.3 ± 1.68 and −40.49 ± 1.89 mV, respectively (Fig. 2, A and B; Table 2). However, in β-cells from LP mice, 8 mM glucose induced a small depolarization that was not sufficient to trigger action potentials in 60% of cases (Fig. 2C; Table 2). Additionally, the analysis of the elapsed time from the addition of 8 mM glucose to the initiation of the depolarization indicated a 5-fold higher delay (~338 sec) in β-cells from LP compared with NP mice (Table 2). The alterations in the electrical response of LP β-cells were also evident in the presence of 11 mM glucose. The membrane potential attained with 11 mM glucose was also lower in the LP group than that obtained in control cells (Table 2). When action potentials took place (Fig. 2, B and D), no differences were found in the amplitude of the spikes between both groups. The depolarization by closure of K_{ATP} channels with tol-

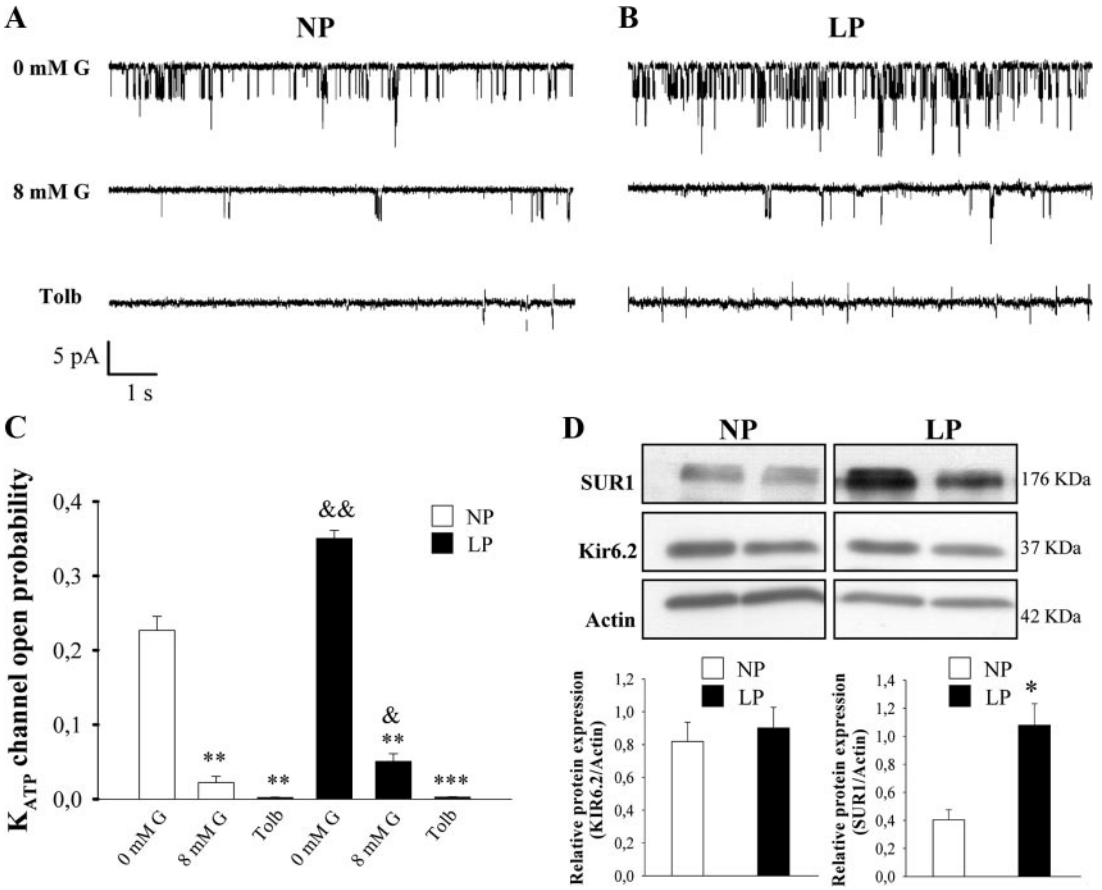


FIG. 3. Lower response of the K_{ATP} channel to glucose in β-cells from LP mice. A and B, K_{ATP} channel activity was measured in cell-attached configuration in isolated β-cells. These traces represent the activity of the channel in 0 and 8 mM glucose as well as 500 μM tolbutamide in a β-cell from a NP (A) and from a LP (B) mouse (n = 5, n = 4, respectively). Glucose at 8 mM significantly inhibited these channels. Tolbutamide further decreased K_{ATP} channel activity. Action currents were evident in this condition. C, Analysis of K_{ATP} channel open probability. A total of 5, 11, and 4 experiments for 0 mM glucose, 8 mM glucose, and tolbutamide, respectively, were analyzed in NP mice while 4, 7, and 4 experiments, respectively, were analyzed in LP mice. D, Western blot analysis of SUR1 and Kir6.2 in islet lysates from both groups (n = 3). A duplicate sample for each group is shown. The relative quantification indicates higher levels of SUR1 protein in LP mice. G, glucose. *, Statistically significant comparing each value with its control (0 mM glucose). &, Statistically significant comparing LP values with its respective control in NP. * and &, $P < 0.05$; ** and &&, $P < 0.01$; ***, $P < 0.001$.

butamide led to similar membrane potential levels and triggered normal electrical activity in both groups (Fig. 2, A and C; Table 2), indicating that cells were responsive during these experiments and that failures in the K_{ATP} channel were unlikely involved in the lower effect of glucose in the LP group. Because LP β-cells were more hyperpolarized in resting conditions, the increase in membrane potential with tolbutamide (ΔVm) was higher in these cells compared with controls, given that tolbutamide depolarized to the same level in both groups (Table 2). Although a small difference in the time delay of the response to tolbutamide (~5 sec) was found between both groups (Table 2), it may be attributable to small differences in each experiment related to the perfusion rate. Therefore, these results show for the first time that diet protein deficiency can lead to alterations in the typical electrical activity of the pancreatic β-cells in response to glucose.

K_{ATP} channel activity in LP mice

The K_{ATP} channel has a central function in GSIS, transducing the changes in extracellular glucose levels to the depolarization of the β-cell membrane potential, generation of action potentials, and voltage-dependent Ca^{2+} influx (28). Thus, we measured K_{ATP} channel activity in isolated β-cells from LP and NP mice. Figure 3, A and B, shows the basal K_{ATP} channel activity in the absence of glucose in isolated β-cells from NP and LP mice. As expected, increasing the glucose concentration to 8 mM decreased β-cell K_{ATP} channel activity in both groups, with a further inhibition in the presence of tolbutamide (Fig. 3, A and B). When the open probability of the K_{ATP} channel was analyzed, several differences were found between both groups (Fig. 3C). In β-cells from LP mice, basal K_{ATP} channel activity in the absence of glucose was higher ($P < 0.01$) than β-cells from NP mice. This result may be related to the more hyperpolarized state found in β-cells from LP

mice in resting conditions (Table 2), because the K_{ATP} channel is also responsible for the resting membrane potential in the β-cell. Interestingly, 8 mM glucose was not capable of decreasing the K_{ATP} channel activity in LP β-cells to the same extent as in β-cells from NP mice (Fig. 3C). Actually, the β-cell K_{ATP} channel activity in the presence of 8 mM glucose was 2-fold higher ($P < 0.05$) in LP mice (Fig. 3C). Given that glucose-induced depolarization in the β-cell depends on a narrow window of K_{ATP} channel activity (29, 30), the lower ability of glucose to inhibit these channels may explain the reduced effect of glucose on the membrane potential of LP β-cells (Fig. 2). Consistent with the experiments illustrated in Fig. 2, tolbutamide decreased the activity of K_{ATP} channels to the same level in both groups (Fig. 3C), indicating that these channels were operative. Thus, these results further suggest that metabolic changes rather than failures in K_{ATP} channel function are involved in the impaired GSIS found in LP mice.

We also checked the expression of the two K_{ATP} channel subunits by real-time PCR. No differences were found with the relative mRNA levels in both groups (Supplemental Fig. 1A). A Western blot analysis also revealed similar levels of Kir6.2 (inwardly rectifying potassium channel 6.2) protein content in both groups. However, the protein levels of SUR1 (sulfonylurea receptor 1) were increased 2.77-fold in islets from LP compared with NP mice (Fig. 3D), indicating that SUR1 regulation in LP islets might occur during posttranscriptional states. To further analyze the SUR1 protein increase in LP islets, we performed an immunostaining with isolated islet-cells (Supplemental Fig. 1B). Although we found a trend to increase in the SUR1 labeling intensity of β-cells from LP mice ($P = 0.054$), this increase was more evident in non-β-cells, suggesting that the augmented SUR1 levels found in LP islets (Fig. 3D) were mainly due to non-β-cells. Further experiments should be performed to determine whether the upregulation of this subunit results in a higher density and/or K_{ATP} channel function in β-cells and, particularly, in non-β-cells.

Glucose-induced Ca^{2+} signals are altered in the β-cells of LP mice

The stimulus-secretion coupling in pancreatic β-cells is well established. Glucose-induced depolarization leads to Ca^{2+} influx through voltage-dependent channels, rise of intracellular Ca^{2+}, and exocytosis of insulin granules (28). To check Ca^{2+} handling in islets from LP mice, we next measured intracellular Ca^{2+} signals by fluorescence microscopy. Islets exposed to 11 mM glucose produced a rapid transient rise in Ca^{2+} followed by oscillations (Fig. 4A). The analysis of the AUC, which gives an estimation of the global Ca^{2+} entry (23), indicated that intracellular Ca^{2+} in the presence of 11 mM glucose was decreased in islets from LP mice compared with islets from NP mice ($P < 0.05$) (Fig. 4A). Because glucose-induced Ca^{2+} signals depend on electrical activity, these findings are in part consistent with the effects on membrane potential (Fig. 2). However, we do not discard other factors such as protein

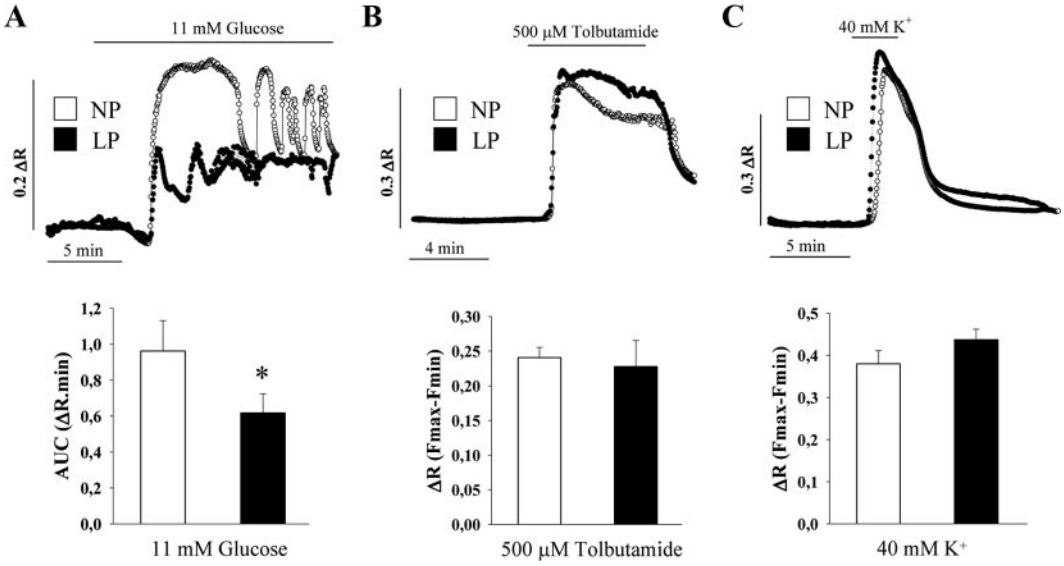


FIG. 4. Glucose-induced Ca^{2+} signaling is diminished in islets from LP mice. Intracellular Ca^{2+} signals were measured in intact islets by conventional fluorescence microscopy and Fura-2. Each figure shows a representative trace of the Ca^{2+} signal in both groups, the NP and LP mice. Either the AUC or the increase of fluorescence (ΔF) indicate the magnitude of the Ca^{2+} signal during the stimulus (23, 24). A, Exposure of islets to 11 mM glucose produced the characteristic transient rise in Ca^{2+} followed by oscillations. The analysis of the Ca^{2+} signals indicated a lower response in LP (n = 7) compared with NP mice (n = 9). B, Tolbutamide induced similar responses in islets from NP and LP mice (n = 6, n = 10, respectively). C, Similar levels of Ca^{2+} were also obtained with 40 mM K^+ (n = 9 in NP; n = 6 in LP). Statistical significance *vs.* control (NP): *, $P < 0.05$.

kinases that can also modulate glucose-induced Ca^{2+} signaling. In this regard, it has been reported that protein kinase A levels are reduced in the islets of protein-malnourished rats (12). There were no differences in the Ca^{2+} signals of both groups when islets were exposed to nonmetabolic stimuli such as tolbutamide or 40 mM of K^+ (Fig. 4, B and C). In agreement with these latter experiments, tolbutamide did not produce different profiles of secretion between both groups (Supplemental Fig. 2), in contrast with the effect of glucose (Fig. 1).

Cell-to-cell synchrony among β-cells is reduced in the islets of LP mice

It has been shown that Ca^{2+} oscillations induced by glucose are synchronous in all β-cells within the mouse islet and this coordination is essential for pulsatile insulin release (31–33). The cell-to-cell communication and the degree of synchrony among mouse β-cells depends on the gap-junctions made of connexin 36 (34). Therefore, we next analyze the level of synchrony among Ca^{2+} signals in β-cells within intact islets as we have previously performed (22, 25), as well as the mRNA expression of connexin 36 in islets from NP and LP mice. Figure 5A shows individual Ca^{2+} signals in β-cells from NP islets. Increasing glucose from low (0.5 mM) to stimulatory concentrations (11 mM) produced synchronous Ca^{2+} oscillations in the totality of β-cells of all the islets analyzed in NP mice (Fig. 5D). Figure 5B shows that β-cells from LP islets also synchronized individual Ca^{2+} signals in the presence of high glucose in some islets, but we also found several islets with low level of coupling (Fig. 5C). The analysis of the percentage of synchronized cells clearly shows that β-cells from LP islets exhibit a reduction of approximately 30% in the number of coupled cells. Additionally, the relative mRNA levels of connexin 36 were significantly decreased by approximately 35% in LP islets compared with controls (Fig. 5A), suggesting that the lower level of coupling levels of the Ca^{2+} oscillations in islets from LP mice might be explained by decreased levels of connexin 36.

Glut-2 and glucokinase expression is reduced in islets from malnourished mice

Previous studies in malnourished rodents have shown several alterations related to glucose metabolism including glucose oxidation and utilization as well as changes in the expression of mitochondrial genes (35–39). Consistent

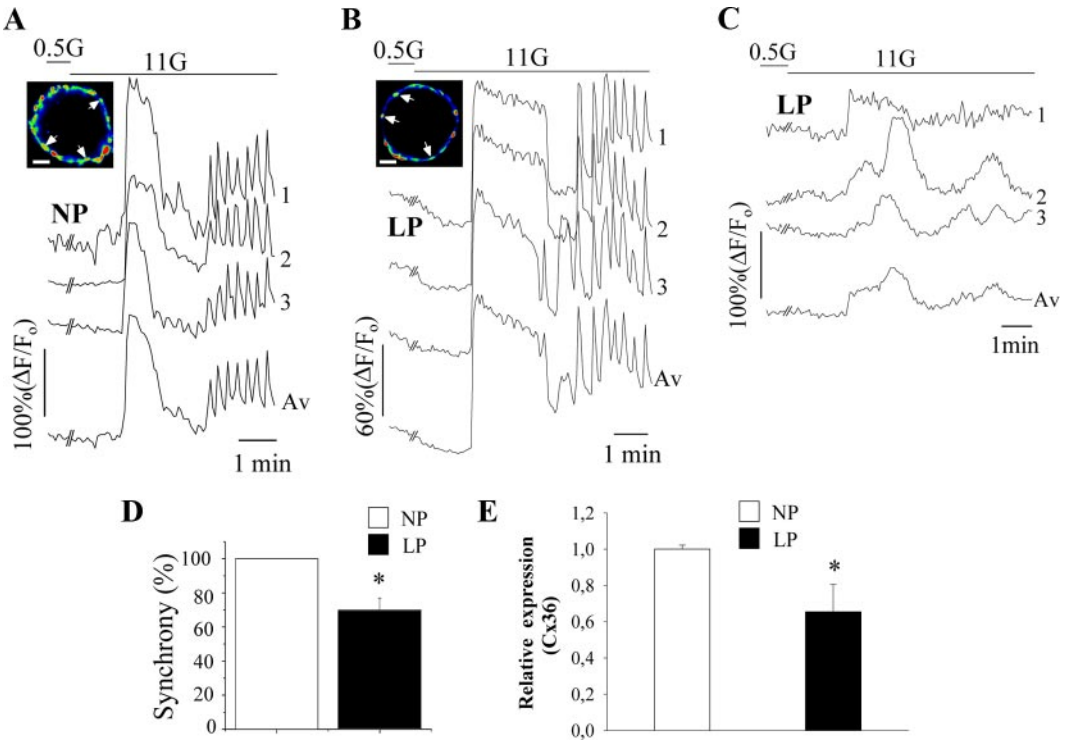


FIG. 5. Individual Ca^{2+} signals exhibit lower cell-to-cell synchrony in islets from LP mice. Individual Ca^{2+} signals were measured in thin optical sections (8 μm) of intact islets by confocal microscopy and Fluo-4. A–C, Each figure shows the individual Ca^{2+} signal of three different β-cells within the same islet in NP (A) and LP mice (B and C). The change from low (0.5 mM) to stimulatory glucose concentrations (11 mM) induced the typical Ca^{2+} oscillations. Note that Ca^{2+} oscillations exhibit a high level of coupling in A and B but not in C. The average (Av) of the three records is also shown, further indicating that signals are synchronized in A and B. The images illustrate the islet optical sections where Ca^{2+} signals were recorded in A and B. Analyzed cells are indicated by arrows. *Scale bar*, 30 μm. D, Analysis of cell-to-cell synchrony was performed in both groups. The analysis shows the percentage of synchronized cells, which was obtained from 47 cells of 7 NP islets and 69 cells of 15 LP islets. E, Real-time PCR analysis of connexin 36. The relative quantification indicates a decrease of approximately 35% in connexin 36 mRNA levels in the islets of LP mice (n = 3). G, glucose. Statistical significance *vs.* control (NP): *, $P < 0.05$.

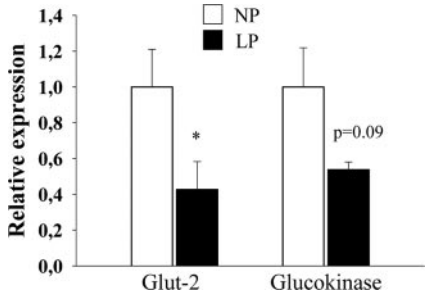


FIG. 6. Glut-2 and glucokinase expression in islets from LP mice. Real-time PCR analysis of Glut-2 and glucokinase in the islets of LP mice and controls (n = 8 for Glut-2 in each group; n = 6 for glucokinase in each group). Statistical significance *vs.* control (NP): *, $P < 0.05$.

with these previous findings, we observed an approximate 57% decrease in GLUT-2 expression in LP islets compared with controls ($P < 0.05$; Fig. 6). Additionally, we found a trend to decrease in the expression of glucokinase ($\downarrow$ 47%; $P = 0.09$). In agreement with previous studies of several groups, including ours (36, 38, 39), we also observed that glucose oxidation was reduced (Supplemental Fig. 3). Overall, these findings indicate that glucose metabolism is altered in malnourished animals.

Discussion

Protein deficiency has been considered one of the pathogenic factors involved in malnutrition-related diabetes that may occur in developing countries (2). Malnutrition during intrauterine period or during early postnatal life can predispose to development of T2DM and obesity during adulthood (40, 41). We have previously demonstrated that rats subjected to LP diet have several alterations in their metabolic parameters as well as glucose homeostasis (6, 15). In the present work, we show that mice fed with LP diet have similar metabolic abnormalities as those found in malnourished rats including lower body weight, lower total plasma protein, hypoalbumineamia, and lower plasma insulin levels compared with mice fed with NP diet (Table 1; Fig. 1). Because plasma glucagon levels were not altered, it seems that functional changes in LP islets probably occur only in β-cells and in insulin release. The lower plasma insulin levels observed in LP mice were consistent with the reduced GSIS found in isolated islets from these animals. A reduced insulin secretion (6, 7, 39) together with diminished number of β-cells per islet and decreased insulin amount per β-cell (8, 42, 43) have been demonstrated in several models of malnutrition. Despite low plasma insulin levels and a remarkable reduction in GSIS, LP mice had increased glucose tolerance and higher glucose disappearance rate than NP mice as judged by the insulin and glucose tolerance tests. This seems to be a compensatory mechanism in the peripheral tissues to maintain normoglycemia that is also found in malnourished rats (9). We have previously proposed that the increased insulin sensitivity in malnourished rats is due to an increase in insulin-induced tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate-1 in the skeletal muscle (9). However, further studies should be performed to determine what is taking place first, the alteration in the islet secretory response or in the peripheral insulin sensitivity. A time-course study on this subject could be helpful to analyze the temporal and causal relationship between both processes. In any case, we show that LP mice exhibit a coordinated cross talk between islet function and peripheral insulin sensitivity, which allows for the coupling of the insulin demand with the amount of insulin secreted to maintain normal glucose levels. Previous studies supported this idea (9). In this line, we can also suggest that islets from LP mice may have adapted to their smaller body weight, and thus the reduced insulin secretion could be sufficient to maintain glucose homeostasis in response to stimuli. Therefore, all these results indicate that protein deficiency during early age in mice alters glucose homeostasis, diminishing GSIS and increasing peripheral insulin sensitivity. Because malnourished mice have all the metabolic features of most animal models of protein-deficient nutritional status, we used this model to further study the alterations in the stimulus-secretion coupling of pancreatic β-cells.

Several theories have emerged to explain the reduced insulin secretion in response to glucose in animal models of protein-deficient diets. Decreased insulin release has been related to changes in glucose metabolism (35–39, 44), defects in the ability of glucose to increase Ca^{2+} uptake in β-cells (6), and alterations in K_{ATP} channel pathways (45). However, no study on the electrophysiological properties has been performed in pancreatic β-cells from malnourished animal models. Using the patch-clamp technique, we found that β-cells from LP mice had a more hyperpolarized resting membrane potential than controls in the absence of glucose. This hyperpolarization may be explained by the higher basal K_{ATP} channel activity found in LP mice. In addition, LP β-cells exhibited less efficiency to depolarize and generate action potentials as well as a higher time delay for the initiation of depolarization in response to stimulatory glucose levels (Fig. 2; Table 2). The alterations in the generation of action potentials in LP β-cells were consistent with the inability of the sugar to decrease the K_{ATP} channel activity to the same extent as that found in NP β-cells (Fig. 3). Glucose effects on membrane potential and electrical activity through the K_{ATP} channel occur within a narrow window of channel activity (*i.e.* 8 mM glucose produces almost 90% inhibition of K_{ATP} channels) (29, 30). Thus, the higher (2-fold) K_{ATP} channel

activity with glucose in β-cells from LP mice (Fig. 3) may explain the lower glucose sensitivity of the membrane potential in these cells (Fig. 2). The diminished capacity of glucose to affect the stimulus-secretion coupling in LP β-cells might be related to alterations in glucose metabolism. Actually, it has been shown that oxidative metabolism in β-cells from malnourished rats is impaired (36, 38, 39) and might be explained by altered activity of m-GDH and L-leucine transamination to 2-ketoisocaproate (37). Our results shown in Fig. 6 further indicate that glucose metabolism is altered in malnourished animals. On the other hand, it has been proposed that the defects in insulin secretion in malnourished rats are located in both K_{ATP} channel dependent and independent pathways (45). Here, we found that the effect of tolbutamide to induce depolarization, action potentials, and K_{ATP} channel inhibition in β-cells (Figs. 2 and 3) as well as insulin secretion in intact islets (Supplemental Fig. 2) was similar in LP and NP mice. Thus, our findings indicate that the alterations in the islets of LP animals might be more related to alterations in glucose metabolism rather than nonfunctional K_{ATP} channels.

The K_{ATP} channel is essential for proper GSIS by coupling glucose metabolism to electrical activity (28). The channel is composed of the subunit Kir6.2, responsible for ion selectivity, and SUR1, which controls most of the channel sensitivity to nucleotides (46, 47). Interestingly, we found that islets from LP mice exhibit 2.77-fold more SUR1 protein content than NP mice (Fig. 3). Immunocytochemical experiments revealed that, although a trend to increase was also observed in LP β-cells, non-β-cells were mainly contributing to the enhanced SUR1 levels in LP islets. The physiological importance of this difference in islet cells is not known, but it has been suggested in neurons that SUR1 overexpression may enhance coupling with endogenous Kir6.X subunits, which would elevate the density of K_{ATP} channels, leading to hyperpolarization in response to metabolic stress (48). Whether a similar process would occur in islet cells, it would be possible that SUR1 overexpression may contribute to changes in the electrical response as those observed here, although the altered glucose metabolism may have a key role (35, 44).

The alterations in the stimulus-secretion coupling in LP β-cells was also observed in other signaling events involved in GSIS. Actually, β-cells of malnourished mice exhibited lower ability to increase intracellular Ca^{2+} in response to glucose (Fig. 4). However, no differences were observed between both groups in the presence of nonmetabolic stimuli such as tolbutamide and high K^+ concentrations. These results further point to an abnormal glucose metabolism in LP β-cells (35–39) and are in agreement with our previous work in islets from malnourished rats, which exhibited poor insulin secretory response to nutrients due to altered ^{45}Ca uptake (6).

Proper insulin secretion requires a fine coordination among β-cells within pancreatic islets. One of the most important proteins involved in this coordination is connexin 36, which allows for the formation of gap-junctions that connect adjacent β-cells in the mouse islet (33). This cell-to-cell communication ensures an electrical and chemical coupling, favoring the synchrony of electrical and Ca^{2+} signals between neighboring β-cells (34, 49). In the present work, we found lack of synchrony in 30% of β-cells of LP mice, which might be due the approximate 35% reduction in the relative mRNA levels of connexin 36 (Fig. 5). These results resemble the data published with $Cx36^{-/-}$ mice. Pancreatic islets from these animals displayed irregular Ca^{2+} oscillations and lack of pulsatile insulin release during glucose stimulation (34). Thus, the present work shows for the first time that LP diet leads to alterations in important genes involved in the pancreatic β-cell function as well as changes in several signaling steps involved in GSIS such as electrical activity, Ca^{2+} signals, and islet synchrony. All these alterations in the stimulus-secretion coupling of β-cells from malnourished mice may explain the endocrine pancreas dysfunction and development of diabetes in malnutrition state.

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References

1. **Venables MC, Jeukendrup AE** 2009 Physical inactivity and obesity: links with insulin resistance and type 2 diabetes mellitus. Diabetes Metab Res Rev 25 (Suppl 1):S18–S23
2. **Rao RH** 1988 Diabetes in the undernourished: coincidence or consequence? Endocr Rev 9:67–87

3. **López-Jaramillo P** 2009 Enfermedades cardiometabólicas en Iberoamérica: papel de la programación fetal en respuesta a la desnutrición materna. Rev Esp Cardiol 62:670–676
4. **Desai M, Babu J, Ross MG** 2007 Programmed metabolic syndrome: prenatal undernutrition and postweaning overnutrition. Am J Physiol Regul Integr Comp Physiol 293:R2306–R2314
5. **Hales CN, Barker DJ** 1992 Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35:595–601
6. **Carneiro EM, Mello MAR, Gobatto CA, Bochero AC** 1995 Low protein diet impairs glucose-induced insulin secretion from and 45 Ca uptake by pancreatic rat islets. J Nutr Biochem 6:314–318
7. **Okitolonda W, Brichard SM, Henquin JC** 1987 Repercussions of chronic protein-calorie malnutrition on glucose homeostasis in the rat. Diabetologia 30:946–951
8. **Rao RH** 1990 Chronic undernutrition may accentuate the β cell dysfunction of type 2 diabetes. Diabetes Res Clin Pract 8:125–130
9. **Reis MA, Carneiro EM, Mello MA, Boschero AC, Saad MJ, Velloso LA** 1997 Glucose-induced insulin secretion is impaired and insulin-induced phosphorylation of the insulin receptor and insulin receptor substrate-1 are increased in protein-deficient rats. J Nutr 127:403–410
10. **Heywood WE, Mian N, Milla PJ, Lindley KJ** 2004 Programming of defective rat pancreatic β-cell function in offspring from mothers fed a low-protein diet during gestation and the suckling periods. Clin Sci (Lond) 107:37–45
11. **Hugh-Jones P** 1955 Diabetes in Jamaica. Lancet 269:891–897
12. **Ferreira F, Barbosa HC, Stoppiglia LF, Delghingaro-Augusto V, Pereira EA, Boschero AC, Carneiro EM** 2004 Decreased insulin secretion in islets from rats fed a low protein diet is associated with a reduced PKAα expression. J Nutr 134:63–67
13. **Giozzet VA, Rafacho A, Boschero AC, Carneiro EM, Bosqueiro JR** 2008 Dexamethasone treatment in vivo counteracts the functional pancreatic islet alterations caused by malnourishment in rats. Metabolism 57:617–624
14. **Filiputti E, Ferreira F, Souza KL, Stoppiglia LF, Arantes VC, Boschero AC, Carneiro EM** 2008 Impaired insulin secretion and decreased expression of the nutritionally responsive ribosomal kinase protein S6K-1 in pancreatic islets from malnourished rats. Life Sci 82:542–548
15. **Latorraca MQ, Reis MA, Carneiro EM, Mello MA, Velloso LA, Saad MJ, Boschero AC** 1998 Protein deficiency and nutritional recovery modulate insulin secretion and the early steps of insulin action in rats. J Nutr 128:1643–1649
16. **Arantes VC, Teixeira VP, Reis MA, Latorraca MQ, Leite AR, Carneiro EM, Yamada AT, Boschero AC** 2002 Expression of PDX-1 is reduced in pancreatic islets from pups of rat dams fed a low protein diet during gestation and lactation. J Nutr 132:3030–3035
17. **Delghingaro-Augusto V, Ferreira F, Bordin S, do Amaral ME, Toyama MH, Boschero AC, Carneiro EM** 2004 A low protein diet alters gene expression in rat pancreatic islets. J Nutr 134:321–327
18. **Wolfson WQ, Cohn C** 1948 Studies in serum proteins; clinical studies employing rapid chemical fractionation procedures, with particular reference to the frequency and significance of hypoalbuminemia. J Lab Clin Med 33:1276–1288
19. **Doumas BT, Watson WA, Biggs HG** 1971 Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31:87–96
20. **Eizirik DL, Sandler S, Welsh N, Cetkovic-Cvrlje M, Nieman A, Geller DA, Pipeleers DG, Bendtzen K, Hellerström C** 1994 Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. J Clin Invest 93:1968–1974
21. **Bonora E, Manicardi V, Zavaroni I, Coscelli C, Butturini U** 1987 Relationships between insulin secretion, insulin metabolism and insulin resistance in mild glucose intolerance. Diabetes Metab 13:116–121
22. **Quesada I, Todorova MG, Alonso-Magdalena P, Beltrá M, Carneiro EM, Martin F, Nadal A, Soria B** 2006 Glucose induces opposite intracellular Ca2+ concentration oscillatory patterns in identified α- and β-cells within intact human islets of Langerhans. Diabetes 55:2463–2469
23. **Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquié M, Gauthier BR, Nef S, Stefani E, Nadal A** 2008 Pancreatic insulin content regulation by the estrogen receptor ER α. PLoS One 3:e2069
24. **Rafacho A, Marroquí L, Taboga SR, Abrantes JL, Silveira LR, Boschero AC, Carneiro EM, Bosqueiro JR, Nadal A, Quesada I** 2010 Glucocorticoids *in vivo* induce both insulin hypersecretion and enhanced glucose sensitivity of stimulus-secretion coupling in isolated rat islets. Endocrinology 151:85–95
25. **Nadal A, Quesada I, Soria B** 1999 Homologous and heterologous asynchronicity between identified α-, β- and δ-cells within intact islets of Langerhans in the mouse. J Physiol 517:85–93
26. **Tudurí E, Marroquí L, Soriano S, Ropero AB, Batista TM, Piquer S, López-Boado MA, Carneiro EM, Gomis R, Nadal A, Quesada I** 2009 Inhibitory effects of leptin on pancreatic α-cell function. Diabetes 58:1616–1624
27. **Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonák J, Lind K, Sindelka R, Sjöback R, Sjögreen B, Strömbom L, Ståhlberg A, Zoric N** 2006 The real-time polymerase chain reaction. Mol Aspects Med 27:95–125
28. **Ashcroft FM, Rorsman P** 1990 ATP-sensitive K+ channels: a link between B-cell metabolism and insulin secretion. Biochem Soc Trans 18:109–111
29. **Soriano S, Ropero AB, Alonso-Magdalena P, Ripoll C, Quesada I, Gassner B, Kuhn M, Gustafsson JA, Nadal A** 2009 Rapid regulation of K(ATP) channel activity by 17β-estradiol in pancreatic β-cells involves the estrogen receptor β and the atrial natriuretic peptide receptor. Mol Endocrinol 23:1973–1982
30. **Göpel S, Kanno T, Barg S, Galvanovskis J, Rorsman P** 1999 Voltage-gated and resting membrane currents recorded from B-cells in intact mouse pancreatic islets. J Physiol 521:717–728
31. **Gylfe E, Ahmed M, Bergsten P, Dansk H, Dyachok O, Eberhardson M, Grapengiesser E, Hellman B, Lin JM, Sundsten T, Tengholm A, Vieira E, Westerlund J** 2000 Signaling underlying pulsatile insulin secretion. Ups J Med Sci 105:35–51
32. **Gilon P, Henquin JC** 1992 Influence of membrane potential changes on cytoplasmic Ca2+ concentration in an electrically excitable cell, the insulin-secreting pancreatic B-cell. J Biol Chem 267:20713–20720
33. **Charollais A, Gjinovci A, Huarte J, Bauquis J, Nadal A, Martín F, Andreu E, Sánchez-Andrés JV, Calabrese A, Bosco D, Soria B, Wollheim CB, Herrera PL, Meda P** 2000 Junctional communication of pancreatic β cells contributes to the control of insulin secretion and glucose tolerance. J Clin Invest 106:235–243
34. **Ravier MA, Güldenagel M, Charollais A, Gjinovci A, Caille D, Söhl G, Wollheim CB, Willecke K, Henquin JC, Meda P** 2005 Loss of connexin36 channels alters β-cell coupling, islet synchronization of glucose-induced Ca2+ and insulin oscillations, and basal insulin release. Diabetes 54:1798–1807
35. **Sener A, Reusens B, Remacle C, Hoet JJ, Malaisse WJ** 1996 Nutrient metabolism in pancreatic islets from protein malnourished rats. Biochem Mol Med 59:62–67
36. **Martín MA, Fernández E, Pascual-Leone AM, Escrivá F, Alvarez C** 2004 Protein calorie restriction has opposite effects on glucose metabolism and insulin gene expression in fetal and adult rat endocrine pancreas. Am J Physiol Endocrinol Metab 286:E542–E550
37. **Rasschaert J, Reusens B, Dahri S, Sener A, Remacle C, Hoet JJ, Malaisse WJ** 1995 Impaired activity of rat pancreatic islet mitochondrial glycerophosphate dehydrogenase in protein malnutrition. Endocrinology 136:2631–2634
38. **Filiputti E, Rafacho A, Araújo EP, Silveira LR, Trevisan A, Batista TM, Curi R, Velloso LA, Quesada I, Boschero AC, Carneiro EM** 2010 Augmentation of insulin secretion by leucine supplementation in malnourished rats: possible involvement of the phosphatidylino-

sitol 3-phosphate kinase/mammalian target protein of rapamycin pathway. Metabolism 59:635–644
39. **Vieira EC, Carneiro EM, Latorraca MQ, Delguingaro-Augusto V, Amaral ME, Bosqueiro JR, Boschero AC** 2001 Low protein diet confers resistance to the inhibitory effects of interleukin 1β on insulin secretion in pancreatic islets*. J Nutr Biochem 12:285–291
40. **Hales CN** 1997 Non-insulin-dependent diabetes mellitus. Br Med Bull 53:109–122
41. **Hales CN, Barker DJ** 2001 The thrifty phenotype hypothesis. Br Med Bull 60:5–20
42. **Okitolonda W, Brichard SM, Pottier AM, Henquin JC** 1988 Influence of low- and high-protein diets on glucose homeostasis in the rat. Br J Nutr 60:509–516
43. **Rafacho A, Giozzet VA, Boschero AC, Abrantes JL, Cestari TM, Carneiro EM, Bosqueiro JR** 2009 Reduced pancreatic β-cell mass is associated with decreased FoxO1 and Erk1/2 protein phosphorylation in low-protein malnourished rats. Braz J Med Biol Res 42:935–941
44. **Martín MA, Alvarez C, Goya L, Portha B, Pascual-Leone AM** 1997 Insulin secretion in adult rats that had experienced different underfeeding patterns during their development. Am J Physiol 272:E634–E640
45. **Wilson MR, Hughes SJ** 1998 Impaired glucose-stimulated insulin release in islets from adult rats malnourished during foetal-neonatal life. Mol Cell Endocrinol 142:41–48
46. **Seino S, Miki T** 2003 Physiological and pathophysiological roles of ATP-sensitive K+ channels. Prog Biophys Mol Biol 81:133–176
47. **Yan FF, Casey J, Shyng SL** 2006 Sulfonylureas correct trafficking defects of disease-causing ATP-sensitive potassium channels by binding to the channel complex. J Biol Chem 281:33403–33413
48. **Hernández-Sánchez C, Basile AS, Fedorova I, Arima H, Stannard B, Fernandez AM, Ito Y, LeRoith D** 2001 Mice transgenically overexpressing sulfonylurea receptor 1 in forebrain resist seizure induction and excitotoxic neuron death. Proc Natl Acad Sci USA 98:3549–3554
49. **Calabrese A, Zhang M, Serre-Beinier V, Caton D, Mas C, Satin LS, Meda P** 2003 Connexin 36 controls synchronization of Ca2+ oscillations and insulin secretion in MIN6 cells. Diabetes 52:417–424

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