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Low protein diet confers resistance to the inhibitory effects of interleukin 1β on insulin secretion in pancreatic islets☆

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Abstract

High protein content in the diet during childhood and adolescence has been associated to the onset insulin-dependent diabetes mellitus. We investigated the effect of interleukin-1β (IL-1β) on insulin secretion, glucose metabolism, and nitrite formation by islets isolated from rats fed with normal protein (NP, 17%) or low protein (LP, 6%) after weaning. Pretreatment of islets with IL-1β for 1 h or 24 h inhibited the insulin secretion induced by glucose in both groups, but it was less marked in LP than in NP group. Islets from LP rats exhibited a decreased IL-1β-induced nitric oxide (NO) production, lower inhibition of D-[U^{14}C]-glucose oxidation to $^{14}CO_2$ and less pronounced effect of IL-1β on α-ketoisocaproic acid-induced insulin secretion than NP islets. However, when the islets were stimulated by high concentrations of K^+ the inhibitory effect of IL-1β on insulin secretion was not different between groups. In conclusion, protein restriction protects β-cells of the deleterious effect of IL-1β, apparently, by decreasing NO production. The lower NO generation in islets from protein deprived rats may be due to increased free fatty acids oxidation and consequent alteration in Ca^{2+} homeostasis.

Keywords: Protein malnutrition; Pancreatic islets; Insulin secretion; Interleukin 1β; β cell; Cytokines

1. Introduction

Cytokines have been implicated as immunological effector molecules that induce dysfunction and destruction of the pancreatic β-cell [1]. β-cell dysfunction and damage may result from direct contact with islet-infiltrating macrophages and T-cells and/or by exposure to soluble products of these cells, such as cytokines and free radicals [2,3]. IL-1β is one of the main cytokines released by macrophages [4]. The cellular mechanism by which IL-1β mediates its inhibitory effect on islet function and induce islet destruction involves the expression of nitric oxide synthase (NOS) and the subsequent increased production of nitric oxide (NO) from arginine [5,6]. In rodent islets, NO generation seems to be the main mechanism behind cytokine-induced β-cell dysfunction. Two of the most important effects of NO in these islets are the inhibition of the mitochondrial enzyme aconitase, leading to decreased oxidative metabolism and adenosine triphosphate (ATP) production, and the induction of nuclear DNA damage [6–9]. Based on these data, NO has been suggested to be an important mediator of β-cell destruction in type 1 diabetes.

Of particular interest is the fact that children with severe protein malnutrition show a significant suppression of cellular immunity with low IL-1β activity [10]. Epidemiological studies have showed reduced incidence of type 1 diabetes during famine period [11]. Large differences in incidence rates between two genetically similar populations suggested a relationship between higher socioeconomic status and type 1 diabetes [12]. Moreover, the increase in type

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1 diabetes among migrant population in Polynesia and Micronesia suggests a relationship to nutritional status [13]. Finally, there is an association between high linear growth rates among children and increased risk of type 1 diabetes [14].

Thus, the aim of this study was to evaluate whether protein deprivation protects the pancreatic islets from the inhibitory effect of IL-1β on insulin secretion. It was also investigated whether NO generation induced by IL-1β is affected by protein restriction and if the protective effect against IL-1β is associated with glucose metabolism in islet cells.

2. Materials and methods

2.1. Materials

P Collagenase was from Boehringer Mannheim (Indianapolis, IN). Antiserum against insulin was kindly provided by Dr. Leclercq-Meyer (Faculty of Medicine, Brussels Free University, Brussels Belgium). Standard rat insulin was from Novo-Nordisk (Copenhagen, Denmark). Bovine serum albumin (fraction V), sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride (Griess reagent) and other chemicals were from Sigma Chemical Co. (St. Louis, MO). Human recombinant interleukin 1β (specific activity 2×108 μ/mg) from Genzyme Diagnostics (Cambridge, MA, USA) was kindly provided by Dr. D.L. Eisirik, Diabetes Research Center, Vrije Universiteit Brussels, Belgium. D-[U-^{14}C]-Glucose (306 mCi/mmol) was obtained from Amersham International (Amersham, United Kingdom).

2.2. Animals

All of the animal experiments were approved by the State University of Campinas Ethics Committee. Wistar rats were obtained from the University's breeding colony. Offspring (28 days old) born to and suckled by dams fed a control diet (17% protein), were separated at random and maintained for eight weeks on isocaloric diets containing 6% protein [low protein (LP) group] or 17% protein [normal protein (NP) group] as described in Table 1 [15]. Throughout the experimental period, the rats were allowed free access to food and water and were housed a 12 h light/dark cycle at 24°C. At the end of the experimental period, the rats were weighed and killed by decapitation and blood samples were collected. The adequacy of the animal model used in this work was evaluated by the following determinations, performed immediately after decapitation: serum glucose [16] serum albumin [17] serum protein [18]. Sera samples were stored at −20°C for the subsequent measurement of insulin by radioimmunoassay [19].

Table 1
Composition of the normal and low protein diets (g/Kg)

Ingredient	Normal protein (17% protein)	Low protein (6% protein)
Casein (84% protein)	202.2	71.5
Cornstarch	397.0	480.0
Dextrinized cornstarch	130.5	159.0
Sucrose	100.0	121.0
Soybean oil	70.0	70.0
Fiber	50.0	50.0
Mineral mix (AIN-93G)*	35.0	35.0
Vitamin mix (AIN-93G)*	10.0	10.0
L-Cystine	3.0	1.0
Choline Choridrate	2.5	2.5

* Detailed composition given by Reeves et al.

2.3. Islet isolation, culture and insulin secretion

Pancreas was removed and digested with collagenase as described elsewhere [20]. In the first series of experiments, isolated islets were cultured for 1 or 24 h in complete culture media RPMI 1640 supplemented with 10% fetal-bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin, containing 10 mmol/L glucose [21] with addition of 0, 3, 10 or 30 U/ml IL-1β under an atmosphere of 5% CO_2 at 37°C. Following the culture period groups of five islets each were preincubated for 45 min at 37°C in Krebs-bicarbonate buffer containing glucose 5.6 mmol/L equilibrated with a mixture of 95% O_2-5% CO_2, pH 7.4. The incubation medium contained 115 mmol/L NaCl, 5 mmol/L KCl, 24 mmol/L $NaHCO_3$, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, and albumin (3 g/L; bovine serum albumin). The solution was then replaced with fresh buffer containing glucose 2.8 mmol/L (basal concentration) or 16.7 mmol/L, and the islets were incubated for 1 h. In the second series of experiments insulin secretion was measured from islets cultured by 24 h with 0 or 30 U/ml IL-1β. After preincubation as described above, the buffer was changed to a medium containing glucose 2.8 mmol/L supplemented with the following test agents: 25 mmol/L KIC (α-ketoisocaproic acid) or 40 mmol/L K^+. The insulin released was measured by radioimmunoassay [19].

2.4. Nitrite formation

Groups of 100 islets each were cultured at 37°C for 24 h in 0.3 ml of RPMI-1640 medium supplemented with 10 mmol/L glucose, 10% fetal calf serum, 2 mmol/L glutamine, 100 IU penicillin/ml, 100 μg streptomycin/ml, without or with 30 U/ml IL-1β. After the end of this period, 100 μl of the medium were mixed with 100 μl of Griess reagent (1% w/v sulfanilamide in 0.1M HCl and 0.1% w/v N-(1-naphthyl) ethylenediamine dihydrochloride). Nitrite formation was determined by measuring the absorbance at 540 nm using a Multiskan bichromatic reader (version 1.06

Table 2
Final weight body and serum total protein, albumin, glucose and insulin concentration of normal (NP) and low protein (LP) diet fed rats for eight weeks[1,2]

Group	Body weight g	Total protein[1] g/L	Albumin[1] g/L	Glucose[1] mmol/L	Insulin[1] nmol/L
NP	315 ± 12	88 ± 1.4	45 ± 1.7	7.0 ± 0.4	0.11 ± 0.01
	(16)	(12)	(12)	(11)	(6)
LP	163 ± 12*	77 ± 2.9*	40 ± 1.2*	6.0 ± 0.7	0.09 ± 0.01
	(16)	(11)	(11)	(10)	(7)

[1] Values for 12 h food restricted rats.
[2] All results are the mean ± SEM of number of rats shown in parentheses.
* $P < 0.05$ compared to NP group.

P). The rate of nitrite formation was expressed as pmol/islet.24 h [22].

2.5. Glucose metabolism

Glucose oxidation was measured in isolated islets as previously described [23]. Briefly, groups of 15 islets, cultured by 24 h with 0 or 30 U/ml IL-1β, were placed in wells containing Krebs-bicarbonate buffered media (50 μl) supplemented with trace amounts of D-[U-^{14}C] glucose (10 μCi/ml) plus non-radioactive glucose to a final concentration of 16.7 mmol/L. The wells were suspended in 20 ml scintillation vials, which were gassed with 5% O_2 and 95% CO_2 and capped airtight with rubber membranes. The vials were shaken continuously for 2 h at 37°C in a water bath. After incubation, 0.1 ml HCl (0.2 N) and 0.2 ml hyamine hydroxide were injected through the rubber cap into the glass cup containing the incubation medium and into the counting vial, respectively. After 1 h at room temperature, 6 ml of scintillation fluid was added to the hyamine and the radioactivity was counted. The rate of glucose oxidation was expressed as pmol/islet.2 h.

2.6. Statistical analysis

The results are presented as the mean ± SEM. Body weight, and serum total protein, albumin, glucose and insulin levels were compared by Student's t-test for unpaired data. Linear regressions were done (using individual data) to verify the acute and chronic effects of IL-1β on insulin secretion in LP and NP groups. It was tested whether there was a difference in the slopes of the regressions of IL-1β concentrations on insulin secretion between the two groups, using two separated linear regression models. Both variable were log-transformed (Log_{10} variable + 1) to make the relationships linear. To verify if the magnitude of inhibition of insulin secretion (in response to glucose, KIC and K^+), glucose oxidation, and the increase in nitrite production in presence of IL-1β were different between groups, the data were analyzed by ANOVA using contrast of means of specific interest. Bartlett's test for the homogeneity of variances was initially used to check the fit of the data to the assumptions for parametric analysis of variance. To correct for variance heterogeneity or non-normality data were log-transformed [24]. P values <0.05 were considered to indicate a significant difference.

3. Results

Rats from LP group showed body weight, total serum protein and serum albumin significantly lower than those from NP group ($P < 0.001$). These features are commonly found in protein deprivation. No difference was noted in serum glucose levels between LP and NP groups (Table 2).

After 1 or 24 h culture, LP islets, exposed to 2.8 mmol/L glucose, exhibited a 2.5-fold lower insulin secretion than NP islets. At higher glucose concentration (16.7 mmol/L), insulin secretion was 2- and 3-fold lower in LP than in NP islets, after 1 and 24 h of culture, respectively. The acute exposure (1h) to IL-1β suppressed insulin secretion in the NP group by 67%, independently of dose. In the LP islets the degree of inhibition was less pronounced (43% in 3U IL-1β and 53% in 30 U IL-1β). The presence of IL-1β (3U) for 24 h suppressed insulin secretion by 46% and 42% in the NP and LP islets, respectively. At higher concentration of IL-1β (30U) the suppressive effect reached 69% in the NP islets and 57% in the LP islets (Table 3). Finally, the slopes of the relationship between the log-insulin secretion in response to IL-1β concentration were significantly lower in the LP than in the NP group, either in acute (LP group: $r = -0.769$, $F_{(1,22)} = 31.82$, $p = 0.000011$; NP group: $r = -0.816$, $F_{(1,22)} = 43.85$, $p = 0.000001$; $b_{NP\ group}$: -0.250, $b_{LP\ group}$: -0.146, $T = -2.26$, $0.010 < p < 0.025$) or chronic treatment (LP group: $r = -0.478$, $F_{(1,60)} = 18.94$, $p = 0.000050$; NP group: $r = -0.645$, $F_{(1,60)} = 42.77$, $p = 0.000000$; $b_{NP\ group}$: -0.140, $b_{LP\ group}$: -0.076, $T = -2.28$, $0.010 < p < 0.025$).

Since the deleterious effect of IL-1β on pancreatic islets is mediated by nitric oxide production [25] we measured the nitrite accumulation in the incubation medium in the presence and absence of the cytokine. In the absence of IL-1β, LP and NP islets exhibited a comparable nitrite production. Addition of IL-1β increased nitrite production in both

Table 3
Effect of acute (1 h) or chronic (24 h) exposure to IL-1β on glucose-induced insulin secretion in islets from normal (NP) and low protein (LP) groups

Time of culture (h)	Incubation conditions		Insulin secretion (ng/islet.1 h)	
	IL-1β (U/mL)	Glucose (mmol/L)	NP	LP
1	0	2.8	0.5 ± 0.03 (6)	0.2 ± 0.04 (6)
	0	16.7	8.1 ± 0.31 (6)	3.0 ± 0.27 (6)
	3	16.7	2.8 ± 0.19 (6)	1.7 ± 0.18 (6)
	10	16.7	2.9 ± 0.19 (6)	1.6 ± 0.18 (6)
	30	16.7	2.7 ± 0.17 (6)	1.4 ± 0.12 (6)
24	0	2.8	0.5 ± 0.07 (19)	0.2 ± 0.03 (23)
	0	16.7	1.3 ± 0.14 (18)	0.7 ± 0.19 (15)
	3	16.7	0.7 ± 0.12 (15)	0.4 ± 0.05 (23)
	10	16.7	0.5 ± 0.06 (17)	0.3 ± 0.04 (13)
	30	16.7	0.4 ± 0.06 (12)	0.3 ± 0.08 (11)

Values are mean + SEM of the number of the experiments in parentheses.

The data were analyzed by linear regression (except 2.8 mmol/L), followed by test to verify difference in the slopes of the regression IL-1β concentration on insulin secretion between two groups, using two separated linear regression models.

groups of islet; however, the rise in nitrite accumulation in LP group was significantly lower than that found in islets from NP group ($F_{(1, 22)} = 10.26225$, $P = 0.0040$) (Fig. 1).

The glucose oxidation in islets incubated in the presence of 16.7 mmol/L glucose were 113.9 ± 6.3 and 47.5 ± 1.7 pmol/islet.2 h in NP and LP islets, respectively. The presence of IL-1β during culture induced a 2.4- and 1.8-fold reduction in the $^{14}CO_2$ production (69.6 ± 4.5 pmol/islet.2 h for NP and 38.5 ± 2.5 pmol/islet.2 h for LP islets), respectively ($F_{(1, 58)} = 7.751505$, p = 0.0072) (Fig. 2). Hence, the effect of the cytokine on glucose oxidation was also reduced by protein restriction.

At basal concentrations of glucose (2.8 mmol/L) IL-l β had no effect on insulin secretion, whereas 25 mmol/L KIC stimulated insulin release in both groups although to a lesser extent in LP islets (0.3 ± 0.04 ng/islet.h, n = 12, 1.6 ± 0.39 ng/islet.h, n = 11 in LP group and 0.57 ± 0.09 ng/islet.h, n = 9, 4.35 ± 0.48 ng/islet.h, n = 13 in NP group; $F_{(1,41)} = 10.73021$, p = 0.0021). When KIC was combined with IL-1β the insulin response was also reduced to a lesser extent in LP than in NP group (0.91 ± 0.20 ng/islet.h, n = 9 and 1.63 ± 0.31 ng/islet.h, n = 12, respectively; $F_{(1,41)} = 4.6561$, p = 0.03685) (Fig. 3). These findings suggest that protein restriction attenuate the specific inhibitory effect of the cytokine on the Krebs' cycle.

Fig. 4 illustrates the effect of high concentrations of K^+ on insulin secretion. As already shown, insulin secretion in

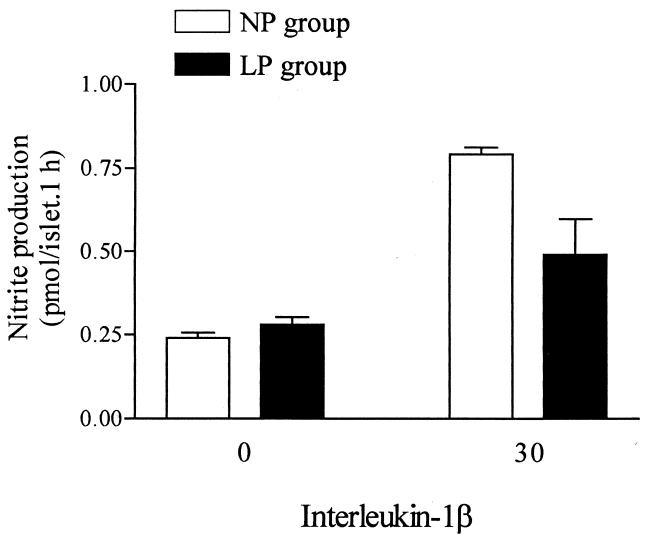

Fig. 1. Effect of IL-1β on nitrite production by islets from normal (NP) and low protein (LP) islets. Both groups of islets were cultured for 24 h with the indicated concentration of IL-1β. The medium was removed, and its nitrite content was determined. Values are ±SEM for 5–10 batches of islets in each group.

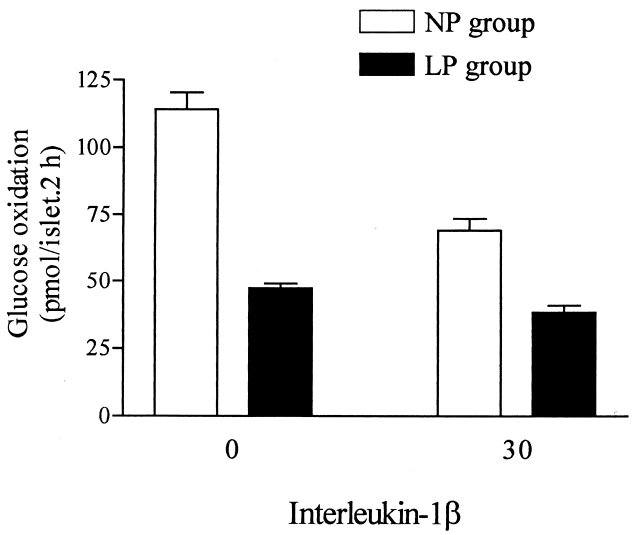

Fig. 2. Effect of IL-1β on glucose oxidation by islets from NP (normal) and LP (low protein) groups. Islets were cultured for 24h in RPMI 1640 with the indicated concentration of IL-1β. The rate of glucose oxidation was measured in groups of 15 islets for 2 h at 37°C with addition of D-[U-^{14}] glucose. The final glucose concentration was 16.7 mmol/L. Production of $^{14}CO_2$ was then measured and used to calculate the rate of glucose oxidation. Values are mean ± SEM for 14–17 batches of islets in each group.

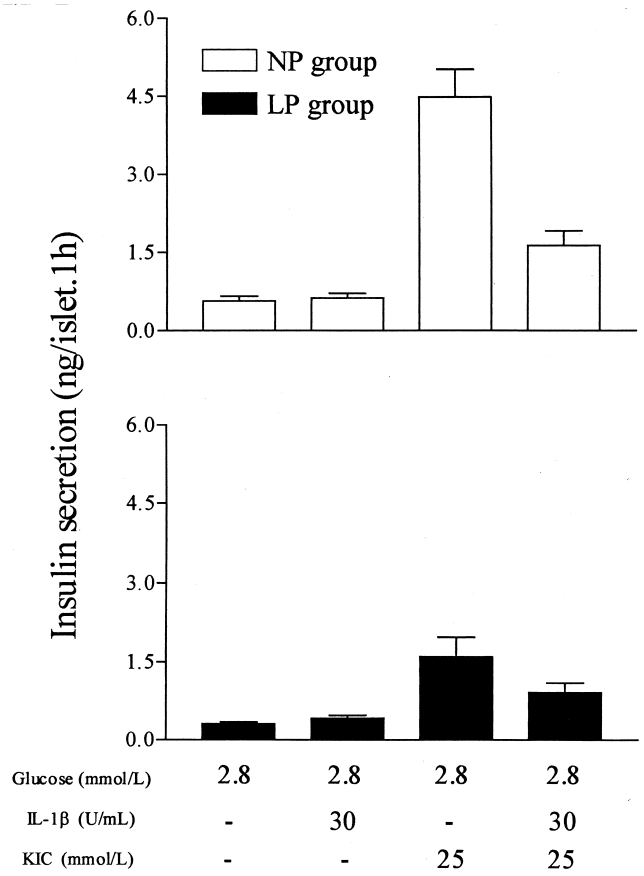

Fig. 3. Effect of IL-1β on KIC-induced insulin secretion by islets from NP (normal) and LP (low protein) groups. Groups of 100 islets were cultured for 24 h in RPMI 1640 with the indicated concentration of IL-1β. Islets insulin-secretory responsiveness was determined after incubation in medium containing 2.8 mmol/L glucose in the absence or presence of 25 mmol/L KIC. Values are mean ± SEM for 9–12 batches of islets in each group.

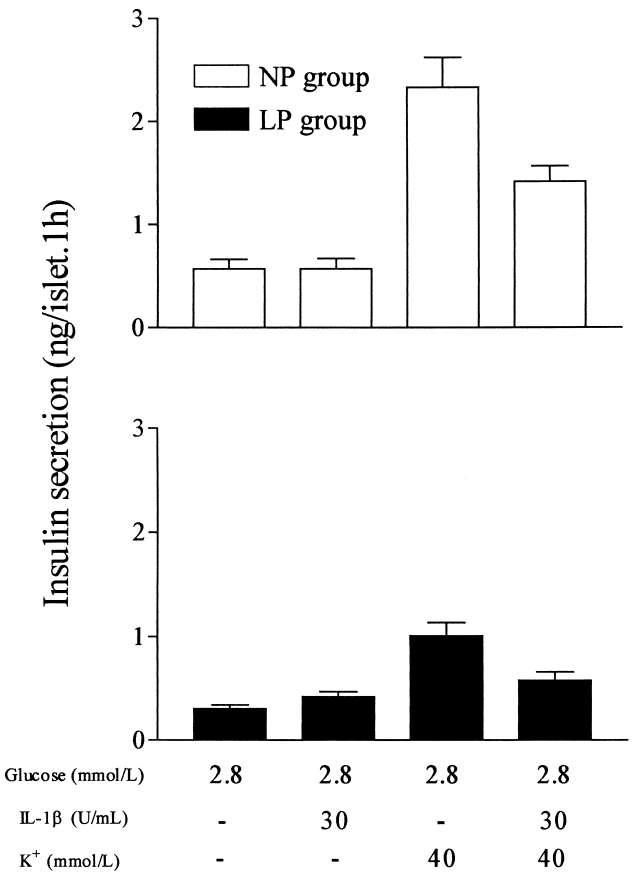

Fig. 4. Effect of IL-1β on K^+-induced insulin secretion by islets from NP (normal) and LP (low protein) groups. Groups of 100 islets were cultured for 24 h in RPMI 1640 with the indicated concentration of IL-1β. Islets insulin-secretory responsiveness was determined after incubation in medium containing 2.8 mmol/L glucose in the absence or presence of 40 mmol/L K^+. Values are mean ± SEM for 8–10 batches of islets in each group.

the presence of 5.0 mmol/L K^+ was not affected by IL-1β (0.30 ± 0.04 ng/islet.h, n = 12 and 0.42 ± 0.05 ng/islet.h, n = 11 in LP group and 0.57 ± 0.09 ng/islet.h, n = 9 and 0.57 ± 0.10 ng/islet.h in NP group; $F_{(1,67)} = 0.6103$, p = 0.4374). High concentrations of K^+ increased insulin secretion in both groups, but the magnitude of this effect was higher in LP than in the NP islets ($F_{(1,67)} = 9.343781$, p = 0.003212). IL-1β exerted comparable suppressive effects on K^+-induced insulin secretion in the two groups ($F_{(1,67)} = 0.5323$, p = 0.4682). The latter observation reinforce the involvement of metabolic mechanisms in the resistance of protein deprived islets to the inhibitory effect of IL-1β.

4. Discussion

Epidemiological observations suggest that exposure to a high food intake during childhood and adolescence could favor the development of type 1 diabetes [11–13]. The present data show that low protein content in the diet partially protected rat pancreatic islets from the deleterious effects of IL-1β. This conclusion is based on the observations that acute (1 h) or chronic (24 h) treatment with IL-1β resulted in a lower inhibition of glucose-stimulated insulin secretion in islets from LP than NP rats. The protection against IL-1β conferred by protein deprivation seems to be mediated by lower NO generation in LP islets. These data agree with the observation that in protein malnourished rats alloxan (a generator of oxygen free radicals) fails to induce diabetes [26].

The reduced production of NO by IL-1β treated LP islets correlated with a lower inhibition of glucose oxidation to CO_2, and was also accompanied by a less pronounced effect of KIC-induced insulin release. It is noteworthy that when both type of islets were challenged with high concentrations of K^+, the reduction of insulin secretion was similar between groups. Thus, the benefits brought about by protein deprivation to functional activity of islets exposed to IL-1β appear to be related to a diminished in NOS activity, reduction of NO production and its consequent action on glucose metabolism. Since KIC is metabolized directly in the mito-

chondria [27], the lower inhibition of KIC-induced insulin release in islets from LP rats indicate that protein deprivation apparently turns the β-cells less sensitive to the deleterious effects of IL-1β on Krebs' cycle.

As mentioned before, NO is the product of the oxidation of arginine to citrulline, a reaction catalyzed by NOS [28]. Islets of Langerhans are equipped with NOS in two distinct forms: a constitutive form (cNOS) Ca^{2+}-dependent and a Ca^{2+}-independent cytokine inducible form (iNOS) [29,30]. Since islets derived from malnourished rats show altered Ca^{2+} handling [31] it is conceivable that the low amount of NO produced by islets from malnourished rats may be, at least in part, a consequence of decreased activation of cNOS.

It is important to note that protein deprived rats exhibit normoglycemia and high serum levels of free fatty acids (FFAs) [31]. We have suggested that in islets from malnourished rats chronic exposure to elevated levels of FFAs associated to euglycemia contribute to the enhanced FFA oxidation and a decrease of their esterification rate [32]. In addition, it has been shown that procedures that deplete islets fat, such as hyperleptinemia [33,34]; the use of troglitazone, an enhancer of FFA oxidation; or tricsin C, an inhibitor of fatty acyl-CoA synthetase [35] provide striking protection against IL-1β cytotoxicity. All together, this protection appears to be the result of reduced expression of iNOS mRNA and consequent inhibition of NO production [35].

In conclusion, our results indicate that low protein diet protects pancreatic islets against the inhibitory effects of IL-1β on glucose-stimulated insulin secretion. This protection appears to be related to a decrease production of NO that ultimately affects mitochondrial metabolism. Although further work is need, we would like to suggest that metabolic abnormalities, i.e. the activation of the glucose-fatty acid cycle and consequent alteration Ca^{2+} homeostasis involved in poor secretory response in LP islets, could afford for the observed protection against IL-1β.

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