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Ácido graxo aumenta a secreção de insulina e modula a expressão de genes envolvidos na biossíntese de insulina em ilhotas de ratos submetidos à desnutrição protéica

Tese apresentada ao Instituto de Biologia para obtenção do Título de Doutor em Biologia Funcional e Molecular, na área de Fisiologia

Orientador: Prof. Dr. Antonio Carlos Boschero

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LISTA DE ABREVIATURAS

SIGLAS

°C	degrees centigrades (graus centígrados)
ANOVA	analysis of variance (análise de variância)
C group	control group (grupo controle)
cDNA	complementary deoxyribonucleic acid (ácido deoxiribonucléico complementar)
dl	deciliter (decilitro)
DTT	dithiothreitol (ditiotreitol)
EDTA	ethylenediaminetetraacetic acid (ácido etilendiaminotetracético)
EGTA	ethylene glycol-bis (β-aminoethyl ether) (etileno-glicol-bis(β-aminoetil éter)
FFA	free fatty acids (ácidos graxos llivres)
GLUT-2	glucose transporter 2 (transportador de glicose facilitador isoforma 2)
IGF-II	insulin-like growth factor-i (fator de crescimento semelhante à insulina-i)
IR	insulin receptor (receptor de insulina)
IRS-1	insulin receptor substrate-1 (substrato 1 do receptor de insulina)
kDa	kilodaltons (quilodatons)
L ou l	liter (litro)
LP group	low protein group (grupo hipoprotéico)
μg	microgram (micrograma)

μm^2	micrometers (micrômetros quadrados)
mg	milligram (miligrama)
min	minute (minuto)
mL	milliliter (mililitro)
mmol	millimolar (milimolar)
MODY4	maturity onset diabetes of the young (diabetes tipo 1 com início na idade adulta)
mRNA	messenger ribonucleic acid (ácido ribonucléico)
p38/SAPK2	stress activated protein kinase 2 (proteína quinase ativada por stress)
PBS	phosphate buffered saline (tampão fosfato salina)
PCR	polymerize chain reaction (reação de cadeia polymerase)
PDX-1	pancreatic-duodenal homeobox (pancreato-duodenal homeobox 1)
PHAS-1	phosphorylated heat- and acid-stable protein regulated by insulin (proteína termo-ácido estável fosforilada regulada pela insulina)
PI3-kinase	phosphatidylinositol 3-kinase (fosfatidilinositol 3-quinase)
PMSF	Phenylmethylsulfonylfluoride (fenilmetilsulfonilfluoreto)
PPI	preproinsulin (pré-pró-insulina)
R group	recovered group (grupo recuperado)
RNA	ribonucleic acid (ácido ribonicléico)
RT	reverse transcription (transcrição reversa)
SDS	sodium dodecyl sulphate (sulfato dodecil de sódio)
SEM	standard error of the mean (erro padrão da media)
TBE	tris borate EDTA (tris borato EDTA)

TTBS	tris tween 20 buffered saline (tampão salina tris-tween 20)
v	volum (volume)

RESUMO

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Em animais, a desnutrição intra-uterina exerce efeitos marcantes sobre o desenvolvimento fetal e pós-natal. Sabe-se que animais desnutridos apresentam níveis elevados de ácidos graxos plasmáticos e esses, por sua vez, são responsáveis por alterar a secreção de insulina. Neste trabalho, verificamos a expressão do fator de transcrição PDX-1, da p38/SAPK2, o metabolismo da glicose e a secreção de insulina em ilhotas de ratos mantidos durante o período fetal e da lactação com uma dieta normoprotéica (17% de proteína) ou hipoprotéica (6% de proteína). Cultivamos as ilhotas por 48 horas em meio de cultura contendo 5.6 mM/L de glicose, na ausência ou presença de0.6 mM/L de ácido palmítico. A secreção de insulina em ilhotas isoladas em resposta 16,7 mmol/L de glicose foi reduzida em ratos desnutridos, no entanto, quando na presença de ácido graxo, observou-se um aumento. Em 2.8 mmol glicose/L,houve diminuição do metabolismo da glicose em ilhotas de desnutridos .Entretanto, quando estimuladas com 16.7 mmol/L de glicose, tanto as ilhotas de desnutridos como as do controle, apresentaram acentuada redução na oxidação da glicose, na presença de ácido graxo. Os níveis de mRNA do PDX-1 e da insulina aumentaram significativamente quando na presença de ácido graxo em ambos os grupos. O efeito do ácido palmítico sobre a expressão protéica de PDX-1 e da p38/SAPK2 apresentou-se

similar em ambos os grupos, mas o aumento foi muito mais evidente em ilhotas de desnutridos. Esses resultados demonstram a complexa relação entre nutrientes no controle da secreção de insulina e mostram queos ácidos graxos desempenham um papel importante na homeostasia da glicose, por afetar mecanismos moleculares e as vias de acoplament-secreção de insulina..

ABSTRACT

ABSTRACT

A severe reduction in insulin release in response to glucose is consistently noticed in protein-deprived rats and is attributed partly to the chronic exposure to elevated levels of free fatty acids. Since the pancreatic and duodenal transcription factor homeobox 1 (PDX-1) is important for the maintenance of B-cell physiology, and since PDX-1 expression is altered in the islets of rats fed a low protein diet, we assessed PDX-1 and insulin mRNA expression, as well as PDX-1 and p38/SAPK2 protein expression, in islets from young rats fed low (6%; LP) or normal (17%; C) protein diets and maintained for 48 h in culture medium containing 5.6 mmol glucose/L with or without 0.6 mmol palmitic acid/L. We also measured glucose-induced insulin secretion and glucose metabolism. Insulin secretion by isolated islets in response to 16.7 mmol glucose/L was reduced in LP compared to C rats. In the presence of free fatty acids, there was an increase in insulin secretion in both groups. At 2.8 mmol glucose/L, the metabolism of this sugar was reduced in LP islets, regardless of the presence of this fatty acid. However, when challenged with 16.7 mmol glucose/L, LP and C islets showed a severe reduction in glucose oxidation in the presence of free fatty acid. The PDX-1 and insulin mRNA were significantly

higher when free fatty acid was added to the culture medium in both groups of islets. The effect of palmitic acid on PDX-1 and p38/SAPK2 protein levels was similar in LP and C islets, but the increase was much more evident in LP islets. These results demonstrate the complex interrelationship between nutrients in the control of insulin release and support the view that fatty acids play an important role in glucose homeostasis by affecting molecular mechanisms and stimulus/secretion coupling pathways.

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INTRODUÇÃO

INTRODUÇÃO

É amplamente conhecido que influências dietéticas durante os estágios iniciais de desenvolvimento do organismo, são consideradas fatores de risco para o surgimento de doenças crônicas.

O diabetes mellitus do tipo 2 é considerado uma dessas doenças, desde que Hales & Barker em 1992 formularam a hipótese do "thrifty phenotype". De acordo com essa hipótese, enfermidades que se manifestam tardiamente como o diabetes mellitus, a hipertensão arterial e a doença isquêmica do coração são determinadas por fatores ambientais e possivelmente originadas *in utero* ou durante a infância. Acredita-se que a desnutrição intra-uterina provoca uma permanente reprogramação do metabolismo fetal.

Entretanto, é apropriado considerar também, uma hipótese mais antiga, que é conhecida como "thrifty genotype", introduzida por Neel em 1962. Nesta, o autor sugere que doenças como o diabetes mellitus e obesidade tem origem genética. De fato, as bases genéticas da relação entre desnutrição e desenvolvimento do diabetes na vida adulta têm sido estudadas e, recentemente, alguns resultados quanto à identificação de genes de susceptibilidade ao diabetes tipo 2 têm sido relatados (Elbein, 1997; Neel, 1999).

Como já descrito anteriormente, a hipótese do "thrifty phenotype" considera o ambiente intrauterino como fator preponderante no desenvolvimento de doenças (ex: diabetes) na vida adulta. No entanto, muitos geneticistas assumem que o diabetes tipo 2 é, em grande parte, determinado geneticamente. A formulação inicial da hipótese do "thrifty genotype" propõe que a explosão da prevalência de diabetes nas sociedades ocidentais deve-se não somente a fatores ambientais, como também à seleção de genes candidatos ao desenvolvimento do diabetes tipo 2 (Neel, 1962). Esses genes proporcionariam uma

vantagem seletiva em um ambiente de escassez de alimento, mas tornar-se-iam prejudiciais quando o suprimento alimentar passasse a ser adequado ou abundante. Nos tempos atuais, quando indivíduos geneticamente susceptíveis são expostos a fatores de risco como consumo elevado de alimentos calóricos e sedentarismo, desenvolvem obesidade, hiperinsulinemia, resistência à insulina com conseqüente descompensação das células beta pancreáticas e instalação do diabetes (Zimmet e cols, 1990). Os mesmos fatores genéticos que provocam redução na secreção e resistência aumentada à insulina podem alterar o crescimento intrauterino e a tolerância à glicose na vida adulta, possibilitando uma ligação entre eles. Terauchi e cols (2000) investigaram camundongos que apresentavam uma mutação no gene da glicoquinase e observaram que esses animais nasciam com baixo peso e susceptibilidade ao diabetes tipo 2. Corroborando com essas observações, Guillam e cols (1997) avaliaram camundongos com mutação no gene do GLUT-2 (transportador de glicose isoforma 2) e concluíram que esses animais perdiam a primeira fase de secreção de insulina.

As células beta do pâncreas respondem ao aumento das concentrações circulantes de glicose, secretando mais insulina e promovendo o controle glicêmico através do aumento da captação de glicose pelos tecidos periféricos (principalmente pelos tecidos muscular e adiposo). Entretanto, a insulina deve ser reposta por ressíntese. À curto prazo, isso é possível através da tradução de moléculas de mRNA da insulina pré-existentes, mas a longo prazo esse processo é possível somente através da estimulação da transcrição do gene da insulina (Macfarlane e cols, 1997).

O fator de transcrição PDX-1 é responsável pela diferenciação das células da ilhota pancreática e pela transcrição do gene da insulina. Sua expressão é limitada às células beta na idade adulta, e algumas células que produzem somatostatina nas ilhotas de Langherans.

Entretanto, existem períodos do desenvolvimento em que o PDX-1 é expresso nas células do ducto pancreático (McKinnon e cols, 2001). O PDX-1 também é conhecido como IPF-1, IDX-1, STF-1, IUF-1, GSF e se liga a 4 sítios (A1, A2, A3, A4) com a seqüência consenso C (C/T) TAATG no promotor da insulina humana (Clark e cols, 1993). Em pâncreas de camundongos adultos, o PDX-1 é seletivamente expresso na célula beta pancreática; liga-se e transativa o promotor da insulina, promovendo a transcrição desse gene (Ohlsson e cols, 1993). Jonsson e cols (1994), mostraram que camundongo homozigoto para uma mutação seletiva no gene PDX-1 apresenta agenesia pancreática. Esses neonatos mutantes sobrevivem ao desenvolvimento fetal, mas morrem poucos dias após o nascimento. Muitos estudos indicam que o PDX-1 regula genes envolvidos na manutenção da função e identidade da célula beta. Entre esses, a insulina, GLUT-2, glicoquinase e polipeptídeo amilóide da ilhota (Gremlich e cols, 1997). PDX-1 está envolvido no mecanismo pelo qual a glicose estimula a transcrição do gene da insulina. Incubação de ilhotas de ratos adultos em meio contendo elevadas concentrações de glicose, proporcionam a fosforilação do PDX-1 e subseqüente translocação para o núcleo, onde se liga ao promotor do gene da insulina e promove a transcrição do mesmo. Essa ativação, dependente de glicose, ocorre através de um mecanismo que envolve a PI3-Kinase (Fosfatidilinositol 3 kinase) e a p38/SAPK2 (Macfarlane e cols, 1999). Outros nutrientes metabolizáveis que induzem a secreção de insulina (incluindo frutose, piruvato e xilitol) e até mesmo a própria insulina ativam o PDX-1 (Wu e cols, 1999). Rafiq e cols (2000), demonstraram que as células beta de ilhotas pancreáticas expostas à glicose, responderam ao estímulo com a transcrição do gene da insulina, através da translocação do PDX-1 da periferia do núcleo para o nucleoplasma. Ainda, concluíram que a ativação da PI3-kinase é importante para a regulação do PDX-1, uma vez que, inibidores dessa via bloqueiam a

ativação do PDX-1. Marcfarlane e cols (1997) demonstraram que a via da p38/SAPK2 também é necessária para ativação do PDX-1,uma vez que, utilizando inibidores específicos para essa enzima, não há ativação do PDX-1.

A função do PDX-1 no diabetes mellitus tem sido documentado na última década. Mutação nesse gene provocou diabetes tipo 1, porém com surgimento na idade adulta, conhecido como MODY4. Resultados semelhantes foram encontrados em uma população da Inglaterra (Macfarlane e cols, 1999) e mesmo em camundongos knockout para o PDX-1 nas células beta (Ahlgren e cols, 1998).

Assim como o PDX-1 é ativado pela presença de glicose no meio, acredita-se que seja inibido na presença de ácidos graxos livres (AGL) (Gremlich e cols, 1997). Altos níveis de AGL induzem um estado de resistência à insulina em músculo cardíaco e esquelético, através de uma diminuição no metabolismo da glicose via ciclo inibitório glicose-ácidos graxos, proposto por Randle e cols (1988).

Animais submetidos à desnutrição protéica durante a fase intra-uterina apresentam alterações características dessa restrição, tais como baixo peso ao nascer, hipoinsulinemia, redução da secreção de insulina estimulada por glicose e elevados níveis de AGL plasmáticos. A cinética de secreção de insulina exibida por esses animais desnutridos mostra que a glicose induziu um pico de secreção de insulina imediato, porém menor, que se manteve, ou seja, houve uma drástica redução da primeira e segunda fases de secreção do hormônio. Curiosamente, essa cinética de secreção de insulina foi comparável àquela exibida por ilhotas de animais adultos submetidos ao jejum (Tamarit-Rodrigues et al. 1984), e por ilhotas de neonatos amamentados por ratas que receberam dieta rica em gordura (Bliss & Sharp, 1992). Nestes neonatos as alterações do

padrão de secreção de insulina foram atribuídas a um efeito inibitório do metabolismo da glicose em função da oxidação elevada de ácidos graxos.

Ácidos graxos de cadeia longa exercem efeitos agudo e crônico divergentes sobre as células beta pancreáticas. A administração aguda de ácidos graxos potencializa a secreção de insulina, induzida pela glicose, tanto *in vivo* quanto *in vitro*, enquanto que a exposição crônica causa aumento da secreção à baixa concentração de glicose (2-5 mM) e redução da secreção estimulada pela glicose (Assimacopoulos-Jeannet et al. 1997). Teoricamente, a alta concentração de ácidos graxos circulantes poderia influenciar a secreção de insulina se o ciclo ácido graxo-glicose estivesse operando nas células beta. De acordo com esse conceito, a elevação da oxidação de ácidos graxos inibe o metabolismo de glicose através de um mecanismo de competição por substrato (Randle 1998). Tendo em vista que a insensibilidade à glicose persiste *in vitro* na ausência de ácidos graxos, é provável que esses ácidos causem alterações a longo prazo na expressão de enzimas chaves envolvidas no metabolismo da glicose, oxidação de ácidos graxos e na formação de um suposto fator de associação metabólica do malonil-CoA (Brun et al. 1997). Estudos com linhagem de células de insulinoma (INS-1) mostraram redução da secreção de insulina estimulada pela glicose, inibição da expressão da enzima acetil-CoA carboxilase (ACC) e aumento da expressão da carnitina palmitoiltrnasferase-I (CPT-I), após exposição crônica dessas células aos ésteres acil-CoA graxos de cadeia longa. (Assimacopoulos-Jeannett et al. 1997, Brun et al. 1997). Gremlich e cols (1997), demonstraram que ilhotas pancreáticas de ratos, submetidos a altas concentrações de ácidos graxos, apresentaram redução de 70% no mRNA e na proteína PDX-1.

Em células beta pancreáticas, os AGL do citoplasma são convertidos à acil-CoA. A molécula de acil-CoA de cadeia longa (LC-CoA) é transportada para a mitocôndria via carnitina-palmitoil-transferase-1 (CPT-1), onde é beta oxidada, em condições basais. Na

presença de concentrações elevadas de glicose, este processo é inibido e ocorre aumento na concentração de LC-CoA no citoplasma (Prentik e cols, 1992; Unger, 1995). Esse efeito se deve ao aumento da concentração de malonil-CoA resultante do aumento do metabolismo da glicose. Malonil-CoA inibe a CPT-1, permitindo o referido acúmulo de LC-CoA no citoplasma (Chen e cols, 1994; De Fronzo, 1997). Esses ácidos graxos aumentam diretamente a exocitose de insulina por estimular o retículo endoplasmático a liberar cálcio, promovendo aumento do cálcio citoplasmático (Prentik e cols, 1992; Deeney e cols, 1992; Warnote e cols, 1994).

O aumento citoplasmático de LC-CoA, ácido fosfatídico e DAG, resultantes do estímulo com glicose, pode modular diretamente a atividade de enzimas como PKC (Nishizuka, 1992; Littman e cols, 2000) ou modificar o estado de acilação de proteínas-chaves que participam na regulação da atividade de canais iônicos e da exocitose (Rothman & Orci, 1992; Linder e cols, 1993). Os LC-CoA inibem a atividade da glicoquinase (Tippett & Neet, 1982; Powell e cols, 1985), da glicose-6-fosfatase (Fulceri, 1995) e a conversão do acetil-CoA em malonil-CoA pela acetil-CoA carboxilase (Powell e cols, 1985).

Acilação de proteínas parece ser essencial no processo de sinalização via GTP-proteínas carregadoras (proteínas G), possivelmente direcionando essas proteínas aos locais apropriados da membrana (Schmidt, 1989). As proteínas G são constituídas de 3 sub-unidades, α estimulatória (G_s) ou inibitória (G_i), β e γ, e regulam a ação do AMPc por sua associação ao complexo hormônio-receptor na membrana celular e subseqüente ativação de moléculas efetoras como adenilato ciclase e PLC. Todas as sub-unidades α são modificadas

por LC-CoA, como moléculas de palmitato ou miristato. Mutações nos locais de palmitoilação das sub-unidades α alteram sua função regulatória (Bouvier e cols, 1995; Linder e cols, 1993; Casey , 1995).

O mecanismo pelo qual os LC-CoA estimulam a liberação da insulina parece ocorrer também por efeito direto na exocitose, independente dos moduladores conhecidos desse processo. Os LC-CoA podem aumentar a fusão dos grânulos secretórios com a membrana da célula beta com subseqüente descarga da insulina, como demonstrado por mensuração da capacitância da membrana de células beta pancreáticas de camundongo. Este efeito é fisiologicamente relevante e não está limitado a células pancreáticas clonais. Assim, a fusão aumentada dos grânulos à membrana plasmática permitiria um maior número de sítios ancoradouros disponíveis para incorporação dos grânulos do *pool* de reserva, regulando a exocitose de insulina na célula beta (Deeney e cols, 2000).

Os estudos de modulação dos AGL nas células B apontam que as moléculas lipídicas desempenham papéis fisiológicos ou patofisiológicos. Num determinado momento, a secreção de insulina será governada não apenas pela concentração de glicose sérica, mas também pela concentração e natureza dos ácidos graxos circulantes (McGarry & Dobbins, 1999). O efeito dos ácidos graxos varia muito, elevando a secreção de insulina com o aumento do comprimento da cadeia e aumentam com o grau de insaturação (Stein ecols, 1997). Ácidos graxos de cadeia longa como palmitato, ácido linoléico e linolênico potencializam a secreção de insulina em resposta à concentração basal de glicose (3 mM). Dietas ricas em ácidos graxos saturados (banha de porco) reduzem a responsividade das

ilhotas à glicose, enquanto que dietas ricas em AG monoinsaturados (óleo de oliva) e poli-insaturados (óleo de soja) aumentam esta resposta (Picinato e cols, 1998).

Assim, podemos sugerir que os efeitos dos AGL sobre a secreção de insulina induzida pela glicose manifestam-se por alguns mecanismos diferentes, incluindo: a) dessensibilização dos transportadores de glicose, b) inibição da fosforilação da glicose pela hexoquinase, c) inibição da fosfofrutoquinase pelo acúmulo do citrato com conseqüente queda da glicólise e d) inibição da PDH reduzindo a oxidação de glicose.

As evidências encontradas na literatura mostram as influências do estado nutricional e dos ácidos graxos sobre os eventos que controlam a homeostase glicêmica e a expressão de genes. Neste trabalho, propusemos estudar, em ratos, os efeitos da restrição protéica durante as fases importantes de crescimento e desenvolvimento do animal associados à exposição ao palmitato sobre o metabolismo da glicose, secreção de insulina e expressão gênica e protéica do PDX-1, insulina e p38/SAPK2.

OBJETIVOS

OBJETIVOS

1-Objetivo geral

- Avaliar a expressão do PDX-1, insulina e p38/SAPK2 em ilhotas de ratos submetidos à restrição protéica e cultivadas em meio contendo palmitato por 48 horas.

2- Objetivos específicos

- Avaliar a secreção de insulina por ilhotas de ratos cultivadas em meio contendo 5.6 mmol/L de glicose, na ausência ou presença de 0.6 mmol/L de palmitato.
- Avaliar o metabolismo da glicose em ilhotas de ratos cultivadas em meio contendo 5.6 mmol/L de glicose, na ausência ou presença de 0.6 mmol/L de palmitato.
- Avaliar a expressão do mRNA do fator de transcrição PDX-1 e da insulina em ilhotas de ratos cultivadas em meio contendo 5.6 mmol/L de glicose, na ausência ou presença de 0.6 mmol/L de palmitato.
- Avaliar a expressão protéica do PDX-1 e da p38/SAPK2 em ilhotas de ratos cultivadas em meio contendo 5.6 mmol/L de glicose, na ausência ou presença de 0.6 mmol/L de palmitato.

ARTIGO 1

ARTIGO 1.

Palmitic acid increase levels of PDX-1 and p38/SAPK2 in islets from rats maintained in a low protein diet.

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Key Words: PDX-1, p38/SAPK2, low protein, islet

Running title: Effect of FFA on glucose metabolism and insulin secretion

Abbreviations used: C, control group; FFA, free fatty acids; GSIS, glucose-stimulated insulin secretion; LC-CoA, long-chain acyl-CoA; LCFA, long-chain fatty acid; LP, low protein group; NEFA, non-esterified fatty acids; PCR, polymerase chain reaction; RIA, radioimmunoassay; RT, reverse transcription; PDX-1, pancreatic duodenal homeobox –1; p38/SAPK2, stress-activated protein kinase.

ABSTRACT

A severe reduction in insulin release in response to glucose is consistently noticed in protein-deprived rats and is attributed partly to the chronic exposure to elevated levels of free fatty acids. Since the pancreatic and duodenal transcription factor homeobox 1 (PDX-1) is important for the maintenance of B-cell physiology, and since PDX-1 expression is altered in the islets of rats fed a low protein diet, we assessed PDX-1 and insulin mRNA expression, as well as PDX-1 and p38/SAPK2 protein expression, in islets from young rats fed low (6%; LP) or normal (17%; C) protein diets and maintained for 48 h in culture medium containing 5.6 mmol glucose/L with or without 0.6 mmol palmitic acid/L. We also measured glucose-induced insulin secretion and glucose metabolism. Insulin secretion by isolated islets in response to 16.7 mmol glucose/L was reduced in LP compared to C rats. In the presence of free fatty acids, there was an increase in insulin secretion in both groups. At 2.8 mmol glucose/L, the metabolism of this sugar was reduced in LP islets, regardless of the presence of this fatty acid. However, when challenged with 16.7 mmol glucose/L, LP and C islets showed a severe reduction in glucose oxidation in the presence of free fatty acid. The PDX-1 and insulin mRNA were significantly higher when free fatty acid was added to the culture medium in both groups of islets. The effect of palmitic acid on PDX-1 and p38/SAPK2 protein levels was similar in LP and C islets, but the increase was much more evident in LP islets. These results demonstrate the complex interrelationship between nutrients in the control of insulin release and support the view that fatty acids play an important role in glucose

homeostasis by affecting molecular mechanisms and stimulus/secretion coupling pathways.

INTRODUCTION

Alterations in the maternal metabolic milieu during pregnancy influence the development and functional maturation of B-cells (Metzger, 1991). Protein restriction during pregnancy and/or lactation in rats produces a variety of structural changes in the pancreas of offspring, including a reduction in islet size (Arantes *et al. 2002*) and in the vascular bed (Snoeck *et al. 1990*). In addition, there is a persistent alteration in B-cell mass (Swenne *et al.1987;* Snoeck *et al. 1990;* Metzger, 1991) and insulin secretion (Latorraca *et al. 1999*). Impaired insulin secretion in protein-restricted rats has also been related to alterations in different stages of the secretory mechanism itself (Rasschaert *et al. 1995;* Latorraca *et al. 1998;* Barbosa *et al. 2002*).

Rats fed a low protein diet have elevated serum FFA levels (Latorraca *et al. 1998*), and several studies have shown that long-term exposure to high FFA levels contributes to the inhibition of glucose-induced insulin secretion (Zhou *et al. 1994*). The detrimental effects of elevated FFA levels on B-cell function have been demonstrated in several animal models of diabetes, including FA/FA and DB/DB mice (Alstrup *et al. 2004*), cell lineages (Ritz-Laser et al. 1999), and in the fasting state (Zhou *et al. 1996*). The mechanisms involved include the stimulation of fatty acid oxidation with consequent inhibition of glucose metabolism (Randle *et al. 1988*), decreased PDX-1 mRNA and protein levels and reduced binding activity to the cognate cis-regulatory elements of the insulin gene promoter (Gremlich *et al. 1997*).

PDX-1 plays an important role in the development of the pancreas and in islet cell ontogeny (Jonsson *et al. 1994*; Guz *et al. 1995*), and is an essential component of the mechanisms by which glucose controls insulin promoter activity and endogenous insulin mRNA levels. The DNA-binding activity of PDX-1 is modulated by glucose via a pathway involving stress-activated protein kinase 2 (SAPK2) (Macfarlane *et al. 1997*). SAPK2, also known as RK/p38, is a member of an expanding family of kinases related to mitogen-activated protein kinase and is activated in response to stimuli such as heat, osmotic shock, UV light and DNA-damaging reagents, as well as by proinflammatory cytokines produced under stress (Cohen, 1997). The activation of SAPK2 leads to the phosphorylation of an inactive, 31 kDa, cytoplasmic form of PDX-1 that is tranformed into a 46 kDa active form and translocated to the nucleus (Macfarlane *et al. 1999*). Currently, it is unclear whether FFA alters the SAPK2 pathway.

The aim of this study was to investigate glucose-induced insulin-secretion and glucose metabolism in islets from rats fed a low protein diet, with particular emphasis on the effects of FFA on the expression of PDX-1, insulin and p38/SAPK2.

MATERIALS AND METHODS

Animals

The animal experiments were approved by the institutional Committee for Ethics in Animal Experimentation (CEEA/IB/UNICAMP). Virgin female Wistar rats (80-90 days old) were obtained from the breeding colony at UNICAMP. Mating was done by housing females with males overnight, and pregnancy was confirmed by examining vaginal smears for the presence of sperm. Pregnant females were separated at random and fed an isoenergetic diet containing 6% protein (low protein diet, LP) or 17% protein (control diet, C) from day 1 of pregnancy until the end of lactation. The protein in the LP diet was replaced by the same amount of carbohydrate as described elsewhere (Reis *et al. 1997)*; Table 1). Sixty-day-old rats were assigned to two groups: *1*) a control group (C) consisting of rats born to and suckled by dams fed a control diet during pregnancy, lactation and after weaning, and *2*) an LP group consisting of the offspring of dams fed an LP diet during pregnancy, lactation and after weaning. During the experimental period, rats consumed their respective diets ad libitum and had free access to water. The rats were housed on a 12 h light:dark cycle at 24°C. At the end of the experimental period, the rats were killed by decapitation.

Islet isolation, culture and insulin secretion

The pancreas was removed and digested with collagenase as described elsewhere (Boschero *et al. 1995*). Isolated islets were cultured for 48 h in RPMI 1640 medium containing glucose (5.6 mmol/L) and supplemented with 5% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 µg/mL) (Anderson, 1978), with the addition of palmitate (0.6 mmol/L) in an atmosphere of 5% CO_2 at 37°C. Albumin-bound palmitate was prepared by stirring the fatty acid Na+ salt at 45°C with defatted bovine serum albumin (BSA) (Sigma Fraction V). After adjustment to pH 7.4, the solution was filtered through a 0.22 µm filter, and the fatty acid concentration was measured using a NEFA C kit (Wako Chemicals GmbH). The medium was renewed every 24 h to maintain a constant concentration of fatty acid. After 48 h, groups of five islets each were incubated for 90 min at 37°C in Krebs-bicarbonate buffer containing glucose (2.8 mmol/L or 16.7 mmol/L) balanced 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 bovine serum albumin (3 g/L). The insulin released was measured by radioimmunoassay (Scott *et al. 1981*).

Glucose metabolism

Glucose oxidation was measured in isolated islets as previously described (Malaisse *et al. 1974*). Briefly, groups of 30 islets, cultured for 48 h with glucose (5.6 mmol/L) in the absence or presence of palmitate (0.6 mmol/L) were placed in wells containing Krebs-bicarbonate buffered medium (50 µL) supplemented with trace

amounts of D-[U-^{14}C] glucose (10 μCi/mL) plus non-radioactive glucose at a final concentration of 2.8 mmol/L or 16.7 mmol/L. The wells were suspended in 20 ml scintillation vials that were gassed with 5% O_2 and 95% CO_2 and capped airtight with rubber membranes. The vials were then shaken continuously for 2 h at 37°C in a water bath. After incubation, 0.1 ml of 0.2 N HCl and 0.2 ml of 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 4°C, 3 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.

Western blot

After islets were cultured for 48h with glucose (5.6 mmol/L) in the absence or presence of palmitate (0.6 mmol/L), a pool of at least 1000 clean islets from each experimental group was homogenized by sonication (15 s) in an anti-protease cocktail (10 mmol/L imidazole, pH 8.0, 4 mmol/L EDTA, 1 mmol/L EGTA, 0.5 mg/L pepstatin A, 2 mg/L aprotinin, 2.5 mg/L leupeptin, 30 mg/L trypsin inhibitor, 200 μmol/L DL-dithiothreitol and 200 μmol/L phenylmethylsulfonyl fluoride). After sonication, an amount of extract was collected and the total protein content was determined using a dye-binding protein assay kit (Bio-Rad Laboratories, Hercules, CA). Samples of crude membrane preparations (70 μg of protein) from each experimental group were incubated for 5 min at 80°C with 5X concentrated Laemmli sample buffer (1 mmol/L sodium phosphate, pH 7.8, 0.1% bromophenol blue, 50% glycerol, 10% SDS, 2% mercaptoethanol) (4:1, v/v) and then run on 10%

polyacrylamide gels. Electrotransfer of proteins to nitrocellulose membranes (Bio-Rad) was done for 1 h at 120 V (constant) in buffer containing methanol and SDS. After checking the efficiency of the transfer by Ponceau S staining, the membranes were blocked with dry skimmed milk (50 g/L) in Tris-Tween buffered saline (TTBS, 10 mmol/L Tris, 150 mmol/L NaCl, 0.5% Tween 20) overnight at 4°C. PDX-1 and p38/SAPK2 were detected in the membrane after a 2 h incubation at room temperature with a rabbit polyclonal antibody against PDX-1 and p38/SAPK2 (diluted 1:2500 in TTBS plus dry skimmed milk, 30 g/L). Detection was done using enhanced chemiluminescence (SuperSignal West Pico, Pierce) after incubation with a horseradish peroxidase-conjugated secondary antibody. Band intensities were quantified by optical densitometry (Scion Image, Frederick, MD) of the developed autoradiogram.

mRNA expression

Total RNA from 500 islets, isolated and separated as described for western blot analysis, was extracted using TriZol reagent (Life Technologies, Auckland, New Zealand). For polymerase chain reaction (PCR) analysis, RNA (1 μg) was reverse-transcribed using oligo (DT) primers. The resulting cDNA was amplified by PCR using oligonucleotides complementary to sequences in the PDX-1 gene (5'-CCGAATGGAACCGAGACTGG-3' and 5'-AGGTGGTGGCTTTGGCAATG-3'), insulin gene (5'-TTGCAGTAGTTCTCCAGTT-3' and 5'-ATTGTTCCAACATGGCCCTGT-3') and β-actin gene (5'-CTGCTGGCTGCTTTGCTCAC-3' and 5'-ACGGCATAGACAGGAAGTGGG-3'),

with the latter as the internal control. The PCR was done in a 25 μL reaction volume containing 1 μL of cDNA, 0.05 mmol/L of each cold dNTP (dATP, dCTP, dGTP, dTTP), 0.37 mmol/L $MgCl_2$, 0.25X PCR buffer, 0.1 μmol/L of appropriate oligonucleotides primers, and 1 U of *Taq* polymerase (Life Technologies). The PCR amplification conditions for PDX-1 were as follows: 3 min at 94°C followed by 29 cycles (30 s each) at 94^o, 59^o and 72°C, and for insulin gene 3 min at 94°C followed by 23 cycles (30 s each) at 94^o, 57^o and 72°C, and for β-actin gene (internal control), 2 min at 94°C followed by 25 cycles (30 s each) at 94^o, 58^o and 72°C. The PCR products were separated on a 1.5% agarose gel in Tris-borate-EDTA buffer 1X (TBE 1X) and stained with ethidium bromide. All assays included a negative control. The absence of contamination was confirmed by reverse transcription-negative RNA samples. The relative band intensities were determined by densitometry and the ratio of PDX-1 and insulin to β-actin gene expression was calculated for each sample.

Statistical analysis

The results were expressed as the means ± SEM. Student's two-tailed unpaired *t*-test was used to compare biochemical parameters and body weight in the C and LP groups. Levene's test for the homogeneity of variances was initially used to check the normality of the data before testing with a parametric two-way ANOVA (nutritional status and the presence of palmitate, or glucose concentration and the presence of palmitate). When necessary, these analyses were complemented by the LSD test to determine the significance of individual differences. A P-value < 0.05 indicated a

significant difference. All statistical analyses were done using a statistical software package (Statsoft, Tulsa, OK).

RESULTS

The main values for body weight, serum total protein, serum glucose, FFA and insulin concentrations are shown in Table 2. At the end of the experimental period, the body weight, serum total protein and insulin concentrations were significantly lower ($p < 0.05$) whereas the serum FFA level was significantly higher ($p<0.05$) in the LP group compared to the C group; there was no difference in the serum glucose levels of the two groups.

After 48 h in culture with 5.6 mmol/L glucose without palmitate, insulin secretion in the presence of 2.8 mmol/L glucose was similar in both groups (216 ± 26 pmol/islets.90 min and 275 ± 46 pmol/islet.90 min, respectively). The addition of palmitate did not change the insulin release by LP islets but raised the secretion 3.2-fold in C islets (203 ± 20 pmol/islets.90 min and 641±2 pmol/islet.90 min, respectively) (**Fig 1A**). At a higher glucose concentration (16.7 mmol/L), insulin secretion was 10-fold higher than with low glucose in both groups (**Fig 1B**); however, the insulin release in LP islets was 4.5-fold lower than in C islets cultured in medium containing only 5.6 mmol/L glucose (668 ± 57 pmol/islets.90 min and 2992 ± 19 pmol/islet.90 min, respectively). The exposure to palmitate increased the insulin secretion 3.6-fold in the LP group and 1.7-fold in the C group (2442 ± 393 pmol/islets.90 min and 5008 ± 209 pmol/islet.90 min, respectively) (**Fig 1B**).

Glucose metabolism in the presence of 2.8 mmol/L glucose, with or without palmitate, was influenced only by the nutritional status ($F_{1,17}$=14.8, p<0.01), i.e., islets from LP rats metabolized less glucose than islets from C rats (**Fig 2A**). Raising the glucose concentration of the medium from 2.8 to 16.7 mmol/L significantly increased the rate of glucose metabolism in both groups, in the absence or presence of palmitate. In 16.7 mmol/L glucose, two-way ANOVA revealed a significant effect of nutritional status ($F_{1,20}$ = 104.34; P = 0.00) and palmitate concentration ($F_{1,20}$ = 71.47; P = 0.00), as well as interaction between these factors ($F_{1,20}$= 9.0; P = 0.007) (**Fig 2B**). The glucose oxidation in islets incubated in the presence of 16.7 mmol/L glucose was 66.24 ± 6.98 pmol/islet.2 h and 33 ± 6.3 pmol/islet.2 h in C and LP islets, respectively (**Fig 2B**). The presence of palmitate in the culture medium reduced the $^{14}CO_2$ production in both groups of islets, but the magnitude of this effect was higher in LP than in C islets (11.0 ± 1.73 pmol/islet.2 h and 39.0 ± 2.8 pmol/islet.2 h, respectively) (**Fig 2A and B**). Hence, the effect of palmitate on glucose oxidation was also enhanced by protein restriction.

The PDX-1 and insulin mRNA levels were influenced only by palmitate ($F_{1,14}$=25.8, p=0.0002 and $F_{1,10}$= 7.64, p=0.02, respectively). Islets cultured in medium containing palmitate showed higher PDX-1 (**Fig 3A**) and insulin (**Fig 3B**) mRNA expression than islets cultured without palmitate, regardless of the nutritional status. p38/SAPK2 protein expression was modified by palmitate ($F_{1,8}$= 199.5, p=0.000) and by interaction between the nutritional status and palmitate ($F_{1,8}$=9.08, p=0.01). LP and C islets maintained in culture medium without palmitate had similar levels of p38/SAPK2 expression (3394 ± 374 and 5829 ± 750, respectively), and the

addition of palmitate increased the p38/SAPK2 expression by the same magnitude in both groups (17774 ± 1350 and 15151 ± 1313, respectively) (**Fig 4A**). PDX-1 expression was influenced by the nutritional status ($F_{1,8}$=49.2, p=0.0001) and by palmitate ($F_{1,8}$=68.2, p=0.000) such that PDX-1 expression in islets from LP rats was lower than in islets from C rats. In both groups, PDX-1 expression was higher in islets maintained with palmitate than in those cultured in medium containing only glucose (**Fig 4B**).

TABLE 1. Composition of the control and low protein diets[1]

Ingredient	Control protein (17% protein)	Low protein (6% protein)
	(g/kg)	
Casein (84% protein)	202.0	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 chlorohydrate	2.5	2.5

[1]See (Reeves *et al 1993*) for more details

TABLE 2. Body weight, serum total protein, glucose and insulin concentrations of young adult rats maintained on control (C) or low-protein (LP) diets during fetal life, lactation and after weaning.

	Groups	
Variable	C	LP
Body weight (g)	156±5 (17)	41±1* (20)
Total protein (g/l)	126±3 (17)	94±5* (17)
Glucose (mmol/l)	9.6±0.4 (17)	7.6±2.7 (19)
FFA (mmol/l)	0.3±0.02 (17)	0.6±0.04* (19)
Insulin (pmol/l)	1440±262 (16)	556±131* (17)

Values are the mean ± SEM of the number of rats shown in parentheses. *$p < 0.05$ compared to the C group (unpaired *t* test).

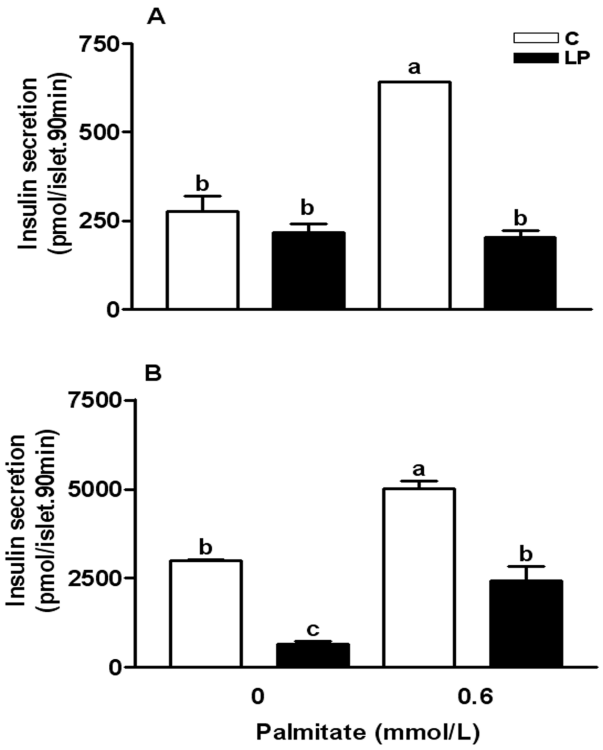

FIGURE 1. Effect of palmitate on insulin secretion by islets from control (C) and low protein (LP) groups in response to 2.8 mmol/L (A) or 16.7 mmol/L (B) glucose. Both groups of islets were cultured for 48 h with the indicated concentration of palmitate. The columns are the mean ± SEM for six batches of islets in each group. Means without a common letter differ significantly ($P < 0.05$).

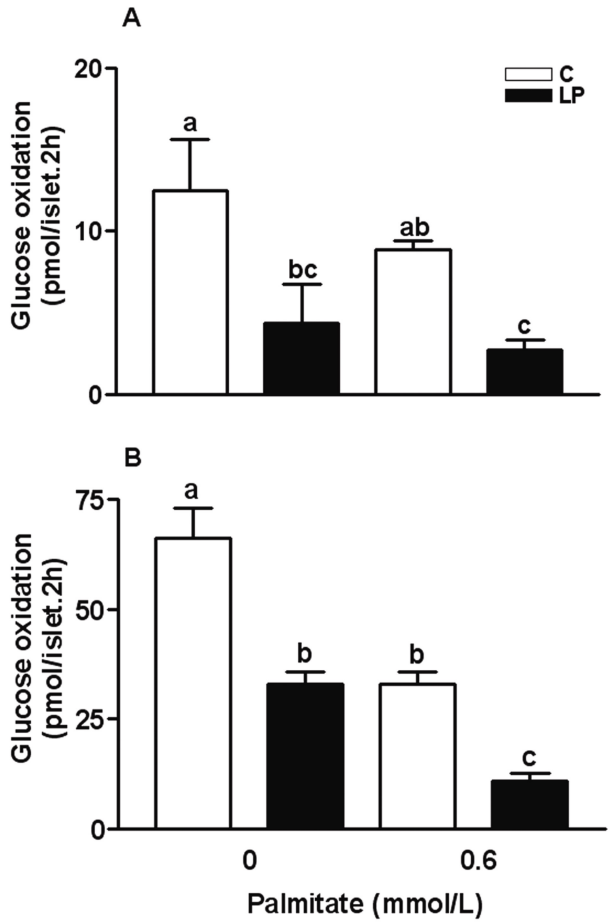

FIGURE 2. Effect of palmitate on glucose oxidation by islets from control (C) and low protein (LP) rats. Islets were cultured for 48 h in RPMI 1640 medium with the indicated concentration of palmitate. The rate of glucose oxidation was estimated by measuring the $^{14}CO_2$ production in groups of 30 islets for 2 h at 37°C in the presence of a trace amount of D-[U-^{14}] glucose. The final glucose concentration was 2.8 mmol/L (A) or 16.7 mmol/L (B). The columns are the mean ± SEM for six batches of islets in each group. Means without a common letter differ significantly ($P < 0.05$).

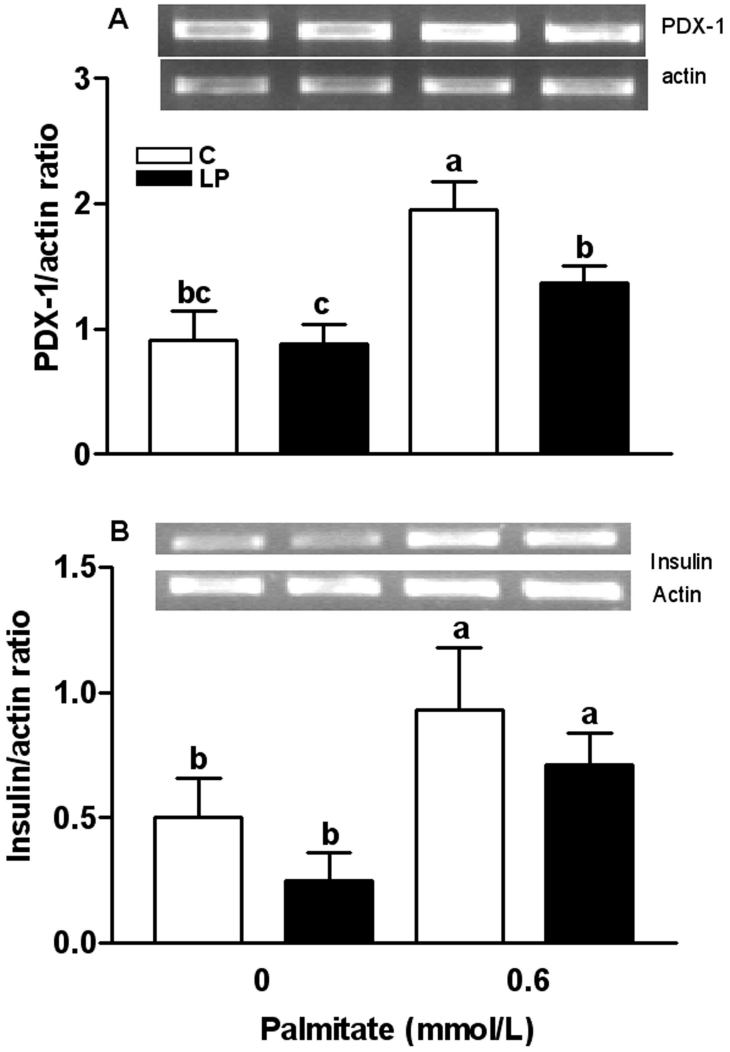

FIGURE 3. Pancreatic duodenal homeobox 1 (PDX-1) **(A)** and insulin **(B)** mRNA levels in pancreatic islets from control (C) and low protein (LP) rats. Total RNA was extracted from ~500 islets for each group and reverse-transcribed. Equal amounts of cDNA were subjected to PCR using primers complementary to the 5' and 3' ends of the PDX-1, insulin and actin genes. The mRNA concentrations of PDX-1 and insulin were expressed relative to actin mRNA. The columns are the means ± SEM of three independent experiments. Means without a common letter differ significantly ($P < 0.05$).

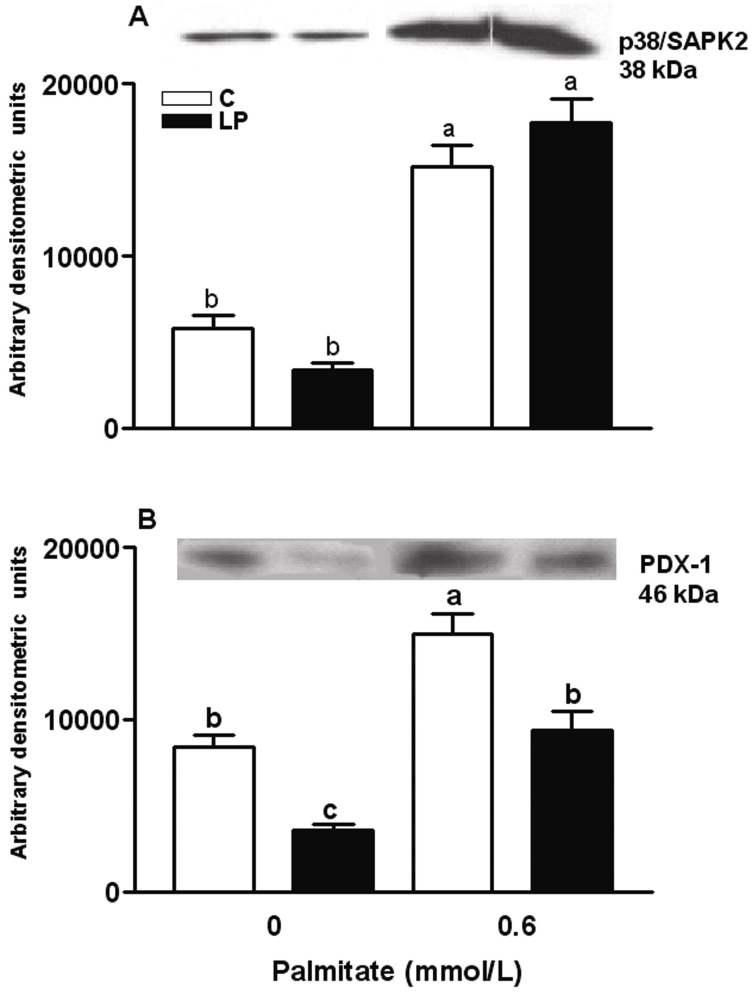

FIGURE 4. p38 **(A)** and Pancreatic duodenal homeobox 1 (PDX-1) **(B)** protein content in islets from control (C) and low protein (LP) rats. Each lane contained 50 μg of protein extracted from isolated islets cultured for 48 h in RPMI 1640 medium with the indicated concentration of palmitate. PDX-1 and p38 were detected by immunoblotting with polyclonal anti-PDX-1 and anti-p38 antibodies, respectively. The columns are the mean ± SEM of three independent experiments. Means without a common letter differ significantly ($P < 0.05$).

DISCUSSION

In this study, LP rats showed typical features of protein malnutrition, including a low body weight, low serum insulin and total protein concentrations, a high FFA level and normoglycemia, as previously reported for this same animal model (Latorraca *et al. 1998*).

We have shown elsewhere (Arantes *et al. 2002*) that a 6% protein diet during gestation and lactation reduced PDX-1 protein expression but did not modify the mRNA expression in pancreatic islets from weaned rats. Since high concentrations of FFA in the culture medium also reduced PDX-1 protein expression in control islets (Gremlich *et al. 1997*), we decided to evaluate the effect of palmitate on PDX-1 expression in islets derived from LP rats that had been continuously exposed to high concentrations of FFA *"in vivo"*.

In LP and C islets cultured in medium containing 5.6 mmol/L glucose for 48 h, the PDX-1 protein expression and insulin secretion in response to glucose were similar to those seen in fresh isolated islets (Arantes *et al. 2002*), indicating that culture "per se" did not alter the parameters analyzed here. In contrast, and somewhat unexpectedly, we verified that palmitate enhanced PDX-1 mRNA and protein expression in LP and C islets. Since the effect of palmitate on islet physiology is apparently glucose-dependent (Gremlich *et al. 1997*), an explanation for this seemingly contradictory result could be the different glucose concentrations used in the culture medium. Moreover, although Gremlich *et al*. (1997) have shown an inhibitory effect of palmitate on PDX-1 mRNA expression in islets cultured in the

presence of 5.6 mmol/L glucose, the inhibitory effect on protein expression was evaluated only in the presence of 30 mmol/L glucose.

p38/SAPK2 is a protein involved in the phosphorylation and activation of PDX-1 that in turn regulates the expression of various genes (Gremlich *et al. 1997*). Low protein diets have been reported not to modify p38/SAPK2 expression but reduced the PDX-1 levels (Martin *et al. 2004*). As shown here, protein restriction also did not alter p38/SAPK2 protein levels whereas the addition of FFA to the medium significantly increased the expression of this protein in both groups. The increase in p38/SAPK2 expression in the presence of FFA seen here provides important new information about the role of FFA in regulating P38/SAPK2 expression. Considering the lower PDX-1 protein expression in LP islets compared to C islets, and the increase in insulin mRNA in both groups in the presence of palmitate, we believe that the higher expression of p38/SAPK2 enhanced the activation of PDX-1 that consequently increased the insulin mRNA levels.

Although still incompletely understood, there is a relationship between the coupling factors that link glucose metabolism to insulin gene expression (Alarcon *et al. 2002*). However, as shown here, glucose metabolism in the absence or presence of elevated FFA levels was unrelated to the alterations seen in PDX-1 and insulin mRNA levels in both groups.

Glucose oxidation is influenced by the chronic exposure of B-cells to FFA. Almost all studies reported in the literature indicate that elevated FFA levels reduce glucose metabolism in islets (Sako & Grill, 1990; Zhou *et al. 1994*; Assimacopoulos-

Jeannete *et al. 1997*). Consequently, high concentrations of FFA may shift the metabolism of lipids and glucose into a relatively more pronounced FFA oxidation that could explain the impaired insulin secretion in response to glucose (Alstrup *et al. 2004*). In contrast, we observed an increase in glucose-induced insulin secretion, despite the lower glucose metabolism in the presence of elevated FFA in both groups. Based on this observation, we suggest that in LP islets and in the presence of high glucose, FFA may be preferentially used for insulin secretion. Considering that exogenous FFA can acutely potentiate glucose-stimulated insulin secretion, possibly by providing additional acyl groups for LC-CoA formation or complex lipid synthesis (Corkey *et al. 2000*), and since LP rats are chronically exposed to elevated serum FFA levels, it is possible that the intracellular level of acyl-CoA may be elevated in the islets of these rats and could activate enzymes, such as PKC (Alcazar *et al. 1997*) and SNAP-25 (Zraika *et al. 2004*), that are involved in the insulin secretory and exocytotic machinery.

In conclusion, our results indicate that the elevated FFA levels found in protein restriction models can account for the contribute to insulin secretion. The decreased glucose metabolism in the presence of palmitic acid indicates a shift from glucose to FFA as the oxidative fuel. Finally, the increase in insulin mRNA expression provoked by FFA may be linked to an increase in p38/SAPK2 and PDX-1 protein expression.

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ARTIGO 2

ARTIGO 2.

The increased lipid incorporation and the maintenance of lipid oxidation as main contributors to the glucose-stimulated insulin secretion in pancreatic islets from rats fed on a low protein diet

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Abbreviations used: C, control group; C and C + Pal, pancreatic islets from C group cultured in 5.6 mmol glucose/L in the absence or presence of 0.6 mmol palmitate/L; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; LP, low-protein group; LP and LP + Pal, pancreatic islets from LP groups cultured in 5.6 mmol glucose/L in the absence or presence of 0.6 mmol palmitate/L; TG, triacylglicerols

ABSTRACT

Reduced glucose-stimulated insulin secretion (GSIS) associated with intrauterine growth restriction is well established, however, the mechanistic basis of this secretory defect in pancreatic islets is poorly understood. Rodents fed on a low-protein diet during neonatal life show, in addition to impaired GSIS, chronically elevated levels of serum fatty acids. Since numerous studies attribute to high fatty acid levels a deleterious role on GSIS, we carried out a metabolic study of pancreatic islets of rats submitted to a low-protein (6% protein, LP group) or a control diet (17% protein, C group) during fetal life, suckling and after weaning. The islets from C and LP rats (40 d old) were cultured at 5.6 mmol glucose/L in the absence (C and LP) or presence of 0.6 mmol palmitate/L (C + Pal and LP + Pal). After 72 h, insulin secretion from C + Pal islets, at 2.8 mmol glucose/L, was higher than C islets but significantly lower than C islets at 16.7 mmol glucose/L. The insulin secretion from LP and LP + Pal islets was similar between each other but significantly lower than the secretion observed for C islets. Despite this, no significant difference was seen in the glucose oxidation at 16.7 mmol glucose/L between C and C + Pal, while LP and LP + Pal islets showed lower $^{14}CO_2$-glucose production. At 16.7 mmol glucose/L, LP islets showed higher palmitate oxidation compared with C islets, but no difference was observed among LP, LP + Pal and C + Pal islets. The incorporation of 1-^{14}C-palmitate in total lipids was higher in LP and LP + Pal compared with C islets. CPT-1 mRNA was increased in LP islets and no difference was observed in UCP2 mRNA expression. Those results suggest that protein undernutrition causes an early damage to the glucose metabolism, which can account for the reduced GSIS. The opposite, maintenance of the lipid oxidation as well as a

higher lipid incorporation, allowing the fatty acids to be available for exocytosis of insulin, indicate an important role of the free fatty acids in the maintenance of the GSIS in LP islets.

INTRODUCTION

Type 2 diabetes mellitus and coronary heart diseases in adult life in humans, as well as in rodents, can originate from intrauterine fetal growth restriction (1). Low-protein diet imposed to rats during early fetal life leads to a profound impairment in the structural development of the endocrine pancreas, such as reduced islet size, islet-cell proliferation, and islet vascularisation (2, 3). These structural alterations impair glucose-stimulated insulin secretion (GSIS) as detected in fetal (4-8), as well as in adult rats (9, 10). However, the exact mechanism underlining such secretory defect is still poorly understood. Proteome analysis of pancreatic fetal islets of dams fed on a low-protein diet showed altered expression of proteins involved in the secretion mechanism (4). In addition, a reduced expression of protein kinase (PK)Cα, phospholipase C (PLC) and PKAα were described in islets from rats fed on a low protein diet after weaning (11, 12).

Alterations in the metabolism of proteins, carbohydrate and lipids observed in different tissues contribute to the development of features typical of protein malnutrition, such as, hypoalbuminemia, normoglycemia, high serum free fatty acid (FFA) levels, and a greater liver concentration of glycogen (10). Numerous studies attributed to chronically elevated FFA levels an important role in the reduced GSIS observed in type 2 diabetes (13). FFA exerts its deleterious effect on glucose metabolism, on the expression of genes involved in such a metabolism and in lipid partitioning.

Thus, to better understand the relationship between the mechanism of the reduced GSIS and the chronic action of fatty acids on it, we carried out a metabolic study of pancreatic islets of control rats as well as of rats submitted to intra-uterine protein

undernutrition. We studied pancreatic islets after 72 h exposure to the fatty acid (0.6 mmol/FFA/L due to serum FFA concentration observed in LP rats, and 72 h is a long enough time to establish the secretory defect described previously), and 5.6 mmol/glucose/L, due to normoglicemic feature of LP rats. Then, we ascertained whether changes in lipid partitioning, alterations in glucose oxidation, malonyl-CoA and acetyl-CoA concentrations, or modulation of carnitine palmitoyltransferase-1 (CPT-1) and UCP2 expression in islets were involved.

MATERIALS AND METHODS

Animals and diet

All of the animal experiments were approved by the institutional Committee for Ethics in Animal Experimentation (Instituto de Biologia, Universidade Estadual de Campinas). Virgin female Wistar rats (85-90 d old) were obtained from the University´s own breeding colony. Mating was performed by housing males with females overnight and pregnancy was confirmed by the examination of vaginal smears for the presence of sperm. Pregnant females were separated at random and maintained from the first day of pregnancy until the end of lactation on isocaloric diets containing 17% protein (control diet) or 6% protein (low-protein diet) as described previously (14). Two groups of young male rats (40 d old) were used in this study: 1) a control group (C) consisting of rats born to and suckled by mothers fed on a control diet, and subsequently fed on a control diet after weaning, 2) a low-protein group (LP) consisting of the offspring of mothers fed on a low-protein diet during both pregnancy and lactation and subsequently fed on the same diet after weaning. The entire offspring were weaned at the fourth week after birth. Throughout the

experimental period, rats were given free access to food and water. They were kept under standard lighting conditions (12-h light:dark cycle), at a temperature of 22°C. At the end of the experimental period, one group of rats was killed by decapitation. Blood samples were collected, allowed to clot, and the sera stored at –20°C for the subsequent measurement of insulin by radioimmunoassay (RIA). The following determinations were performed immediately after decapitation: serum glucose (15), serum free fatty acids (Wako NEFA C) and albumin (16). Body weight was evaluated at the end of experimental period.

Islet isolation, culture and insulin secretion

The pancreata were removed aseptically from 40 d old C and LP rats and the islets were isolated by collagenase digestion. Briefly, the pancreas was inflated with Hanks solution containing 0.7 to 0.9 g collagenase/L, excised and then maintained at 37°C for 20 min. The digested tissue was harvested and the islets were collected by handpicking and distributed onto Petri dishes containing RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 5% heat-inactivated fetal bovine serum, antibiotics (penicillin 0.2 X 104 U/L, streptomycin 0.2 g/L) and 5.6 mmol glucose/L in the absence or presence of 0.6 mmol/palmitate/L for 72 h. The batches of islets were designated: C and LP to pancreatic islets from Control and Low-protein groups, respectively, and C + Pal and LP + Pal to pancreatic islets from control and low-protein groups, respectively, cultured in the presence of palmitate/albumin. Albumin-bound palmitate was prepared by stirring the fatty acid Na+ salt at 45°C with defatted bovine serum albumin (BSA) (Sigma Fraction V). After adjustment to pH 7.4, the solution was filtered through a 0.22 μm filter, and the fatty acid concentration was measured using a NEFA C kit (Wako Chemicals GmbH). The medium

was changed daily to maintain a constant concentration of fatty acid.

Following islet culture for 72 h, groups of five islets were first incubated for 45 min at 37°C in 0.5 mL of Krebs-bicarbonate buffer of the following composition (in mmol/L): NaCl, 115; KCl, 5; $CaCl_2$, 2.56; $MgCl_2$, 1; $NaHCO_3$, 24; and glucose, 2.8; the buffer was supplemented with 3 g of bovine serum albumin/L and equilibrated with a mixture of 95%O_2: 5%CO_2, pH 7.4. This medium was then replaced with fresh buffer and the islets incubated for 90 min in the presence of 2.8 or 16.7 mmol glucose/L. The insulin content of the medium was measured by RIA at the end of the incubation period.

Glucose and palmitate metabolism

Glucose oxidation was measured in cultured islets as previously described (17). Briefly, groups of 15 islets, were placed in wells containing Krebs-bicarbonate buffered media (100 µL) supplemented with trace amounts of D-[U-^{14}C] glucose (10µCi/mL) plus non-radioactive glucose to a final concentration of 2.8 or 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 ^{o}C in a water bath. After incubation, 0.1 mL HCl (0.2 mol/L) 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, 2 mL of scintillation fluid was added to the hyamine and the radioactivity was counted. The rate of glucose oxidation was expressed as pmol/islet.h.

The procedure used to measure the generation of $^{14}CO_2$ from [1-^{14}C]palmitate (0,5 μCi/[1-^{14}C]-palmitate/mL) was similar to that described previously (18), except for the number of islets (batches of 100 islets/condition), and 12 h incubation for trapping $^{14}CO_2$.

Malonyl and acetyl-CoA measurements

The total content of acetyl-CoA and malonyl CoA was determined by the HPLC method (19). Briefly, cultured islets were incubated with 1 mL of Krebs bicarbonate buffer containing 16.7 mmol glucose/L for 30 min at 37°C. Followed by the addition of 1 mL of trichloroacetic acid (10%), the precipitated proteins were then removed by centrifugation. The resulting supernatant was neutralized by extracting the acid five times with ether. The aqueous samples were dried and resuspended in water.

Measurement of palmitate incorporation into total lipids and triacylglycerol content

Groups of 100 islets were placed in wells containing Krebs-bicarbonate buffered media (200 μL) supplemented with trace amounts of [1-^{14}C] palmitate (0,5μCi/mL), carnitine, BSA, plus non-radioactive 0.125 mmol palmitate/L and glucose to a final concentration of 16.7 mmol/L. After 2 h incubation, media were discarded and the islets were washed twice in methanol: PBS (2:3). Then the islets were centrifuged at 700x *g*, and washed once with PBS. Two hundred μL of 0.2 mol NaCl/L were added to the islets pellet and the mixture was immediately frozen in liquid N_2. The lipid and aqueous soluble products were separated by the following procedure. To the thawed islets suspension, 750 μL of chloroform: methanol (2:1) and 50 μL of 0.1 mol KOH/L was added and, after

vigorous vortexing, the phases were separated by centrifugation at 2000 x *g* for 20 min. The top aqueous layer was removed and the bottom lipid-soluble layer was washed with 200 µL of methanol: water: chloroform (48:47:3). Two hundred µL of lipid soluble phase were added to a scintillation mixture and incorporation of radiolabel into total lipids was measured (20). The cellular triacylglycerols content was measure after lipid extraction as described previously (18), followed by reconstitution of dried-samples in isopropanol and determination by Triglycerides GPO-PAP kit (Roche Diagnostics).

Measurement of gene expression

Expression of the genes of CPT-1 and UCP2 in cultured pancreatic islets was determined using semi quantitative reverse transcription-polymerase chain reaction (RT-PCR). Total RNA from 500 islets was extracted using TRIzol reagent (Life Technologies, Auckland, New Zealand). For polymerase chain reaction (PCR) analysis, RNA (1 µg) was reverse-transcribed using Oligo (DT) Primers. The resulting cDNA was amplified by PCR using oligonucleotides complementary to gene sequences and the β-actin gene used as internal control (CPT-1 gene 5'-TATGTGAGGATGCTGCTTCC-3' and 5'-CTCGGAGAGCTAAGCTTGTC-3', UCP2 gene 5'-CCTTGCCACTTCACTTCTGCC-3' and 5'-ATCCCAAGCGGAAGGAAG-3', β-actin gene 5'-CTGCTGGCTGCTTTGCTCAC-3' and 5'-ACGGCATAGACAGGAAGTGGG-3'). The PCR was done in a 12.5 µL reaction volume containing 0.5 µL cDNA, 0.05 mmol/L each cold dNTP (dATP, dCTP, dGTP, dTTP), 0.37 mmol $MgCl_2$/L, 0.25 x PCR buffer, 0.1 µmol/L of appropriate oligonucleotides primers, and 1 unit of Taq polymerase (Life

Technologies). The PCR amplification conditions were: 2 min at 94°C, 30s 94°C followed by 30s each cycle at 55 or 58°C, 45s at 72°C and 7 min at 72°C. The PCR products were separated on a 1.5% agarose gel in Tris borate EDTA buffer 1x (TBE 1x) and stained with ethidium bromide. All PCR included a negative control. The absence of genome contamination in the RNA samples was confirmed by the RT-negative RNA samples. The relative band intensities were determined by densitometry and the ratio of each gene to β-actin was calculated for each sample.

Statistical analysis

Results were presented as the means ± SEM for the number of rats (n) indicates. When comparing C and LP groups, Student's non-paired *t* test was used. Otherwise data were analyzed by two-way ANOVA including the Levene test for the homogeneity of variances, followed by the LSD test for individual differences between groups and conditions. When necessary, data were log-transformed to correct for variance heterogeneity or non-normality (21). The level of significance was set at $P < 0.05$. Data were analyzed using the Statistica software package (Statsoft).

RESULTS

Characteristics of the animals

LP rats showed features typical of protein malnutrition including hypoalbuminemia (22 ± 0.4 g/L, n = 15 for LP rats and 27 ± 0.6 g/L, n = 13 for C rats, $P < 0.05$), high serum free fatty acids (0.60 ± 0.06 mmol/L, n = 15 and 0.25 ± 0.02 mmol/L, n = 13, for LP and C rats, respectively, $P < 0.05$), and low body weight (LP, 47 ± 4 g, n = 13 and C, 120 ± 12 g, n = 10). Serum glucose and insulin concentrations were lower in LP group (glucose, 6.7 ± 0.21 mmol/L, n = 8 for LP, and 8.6 ± 0.3 mmol/L, n = 7 for C rats, $P < 0.05$, and insulin, 0.15 ± 0.02 nmol/L, n = 15 for LP and 0.32 ± 0.03 nmol/L, n = 13 for C rats, $P < 0.05$).

Insulin secretion in cultured islets

To study if the chronic and elevated FFA level observed in rats fed on a low protein diet plays a role in the reduced glucose-induced insulin secretion observed in those animals, C and LP islets were cultured in 5.6 mmol glucose/L in the presence or absence 0.6 mmol palmitate/L for 72 h. **Fig. 1A** shows that basal insulin secretion (2.8 mmol glucose/L) was significantly lower in LP compared with C islets ($P < 0.05$). Addition of palmitate in the culture medium significantly stimulated insulin secretion in C but not in LP islets. Insulin secretion stimulated by 16.7 mmol glucose/L was also significantly higher in C than in LP islets. However, the presence of palmitate in the culture medium significantly reduced the insulin secretion in C islets ($P < 0.05$). At 16.7 mmol glucose/L, palmitate did not alter insulin secretion in LP islets. Interestingly, the amount of insulin secreted by C islets, cultured in the presence of palmitate, was similar at 2.8 **(Fig. 1A)** and 16.7 mmol glucose/L

(Fig. 1B), whereas the ratio of insulin secretion was 6.9 fold higher at 16.7 mmol glucose/L compared with 2.8 mmol glucose/L in LP islets ($P < 0.05$). The result obtained with C islets was comparable to those observed by others (22 – 28) and suggests that the presence of palmitate in the culture medium affects insulin secretion in a higher magnitude in C than in LP islets.

Glucose oxidation

Since glucose oxidation is crucial for insulin secretion, we next evaluated the glucose oxidation in islets cultured in the absence or presence of palmitate. As shown in **Fig. 2A**, addition of palmitate to the culture medium did not alter the glucose oxidation in C + Pal islets under basal or stimulatory concentration of glucose. This is consistent with the lack of action of fatty acids on the glucose oxidation described in pancreatic islets (29, 30) and β cell lines (24, 31, 32). In agreement with previous data (Latorraca et al. unpublished data), we observed a reduced rate of glucose oxidation at 16.7 mmol glucose/L in LP islets treated or not with palmitate, compared with C islets.

Palmitate oxidation

In keeping with earlier reports (22, 27, 33) the oxidation of the labeled fatty acid in the presence of basal concentration of glucose in C islets, cultured in the absence or presence of palmitate was similar **(Fig. 2B).** Raising the glucose concentration to 16.7 mmol/L produced a marked inhibition of palmitate oxidation in C islets cultured in the absence of palmitate and, in a lesser extent, in palmitate-treated C islets. In LP islets cultured or not in the presence of palmitate, the oxidation of palmitate was also

significantly higher in 2.8 mmol compared with 16.7 mmol glucose/L. However, the oxidation of palmitate was higher in LP than in C islets cultured in the absence of palmitate at 16.7 mmol glucose/L.

Malonyl and Acetyl-CoA islet contents

Fig. 2C shows that the concentrations of malonyl-CoA in LP islets were lower than in C islets ($P < 0.05$), consistent with the reduced glucose oxidation in LP islets. Intracellular acetyl-CoA concentrations were similar between C and LP islets (0.515 ± 0.022 nmol/mg and 0.572 ± 0.022 nmol/mg, respectively) and was not affected by the presence of palmitate in the culture medium (C + Pal = 0.512 ± 0.036 and LP + Pal = 0.495 250 ± 0.015).

Incorporation of palmitate in total lipid and triacylglicerol content

Fig. 3 shows that the suppression of palmitate oxidation caused by 16.7 mmol glucose/L was accompanied by an increased incorporation of fatty acid into total lipids in LP related with C islets.

Intracellular TG content was similar between C and LP islets and was not affected by the presence of palmitate in the culture medium (C, 170 ± 30 ng/islet; 168 ± 51 ng/islet for C + Pal; LP, 165 ± 35 ng/islet and for LP + Pal, 171 ± 40 ng/islet). Our results did not agree with those previously described (22, 25). However, it is conceivable that this difference may be due to the higher concentration of FFA used by those authors (2 mmol/L) compared to the concentration used in this work.

The regulation of gene expression by palmitate

As expected, the presence 0.6 mmol palmitate/L for 72 h in the medium induced an increase of the CPT-1 gene expression in C + Pal islets, since LC-CoA is considered to be an endogenous stimulator of the enzyme **(Fig. 4A)** (34). Although the CPT-1 mRNA tended to be higher in LP than in the C islets, a further and significant increase was seen only when LP islets were cultured in the presence of palmitate. Since reduced glucose oxidation suggests a low ATP production, we investigated the expression of UCP2, the uncoupling protein present in pancreatic islets (26). However, neither LP nor C islets showed any significant change in UCP2 mRNA expression **(Fig. 4B).**

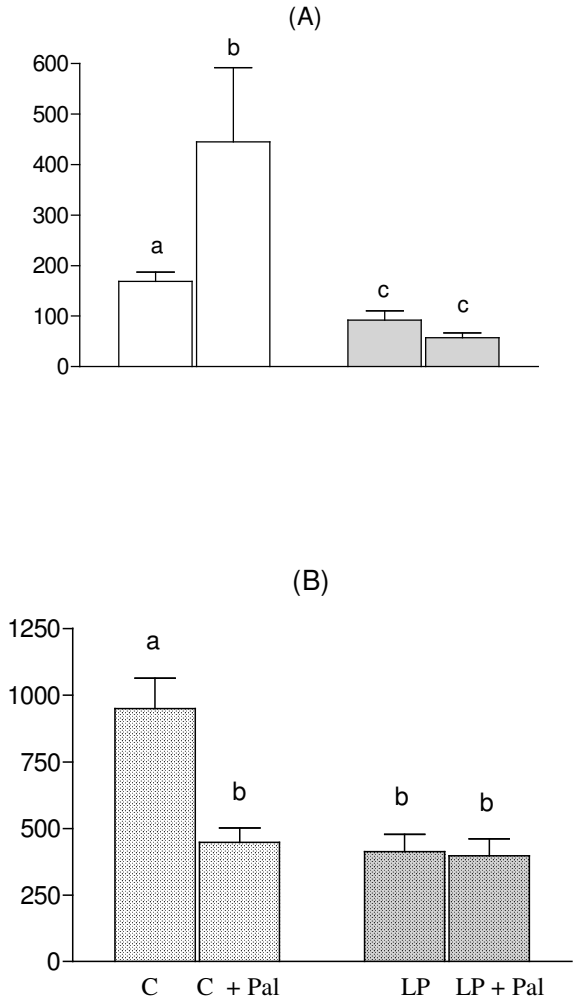

FIGURE 1

FIGURE 1. Effect of chronic palmitate exposure on GSIS. Pancreatic islets from C and LP rats were cultured for 72 h at 5.6 mmol glucose/L in the absence (C and LP) or presence of 0.6 mmol palmitate/L (C + Pal and LP + Pal). Following culture, islets were first incubated for 45 min at 37°C in 0.5 mL of Krebs-bicarbonate buffer, 2.8 mmol glucose/L and BSA. This medium was then replaced with fresh buffer and the islets incubated for 90 min in the presence of 2.8 and 16.7 mmol glucose/L, (A and B, respectively). Results are mean ± SEM of 3 experiments. Different letters indicate significant difference between groups and conditions (ANOVA, $P < 0.05$).

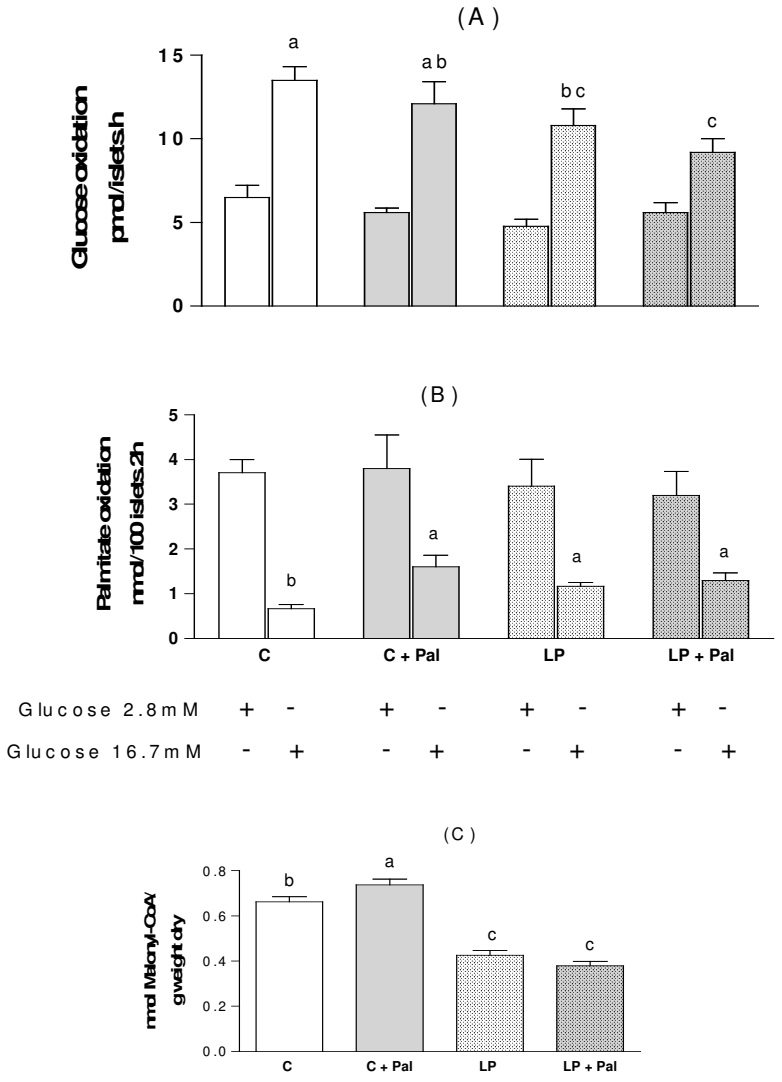

FIGURE 2

FIGURE 2. Effect of chronic palmitate exposure on glucose oxidation (A), palmitate oxidation (B) malonyl-CoA concentration (C). Pancreatic islets from C and LP rats were cultured for 72 h at 5.6 mmol glucose/L in the absence (C and LP) or presence of 0.6 mmol palmitate/L (C + Pal and LP + Pal). Following culture, islets were incubated for 2 h (glucose and palmitate oxidation) at 2.8 or 16.7 mmol glucose/L or incubated with 1 mL of Krebs bicarbonate buffer containing 16.7 mmol glucose/L for 30 min at 37 °C (malonyl-CoA concentration). Results are mean ± SEM of 5 experiments. Different letters indicate significant difference between groups and conditions (ANOVA, $P < 0.05$).

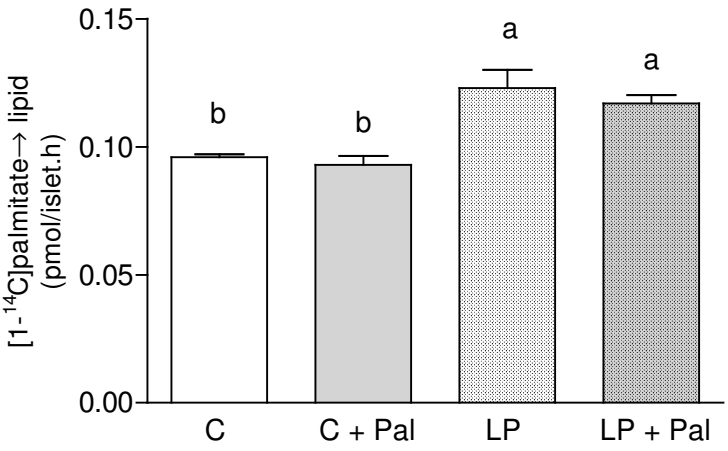

FIGURE 3

FIGURE 3. Effect of chronic palmitate exposure on [1-^{14}C] palmitate incorporation into total lipids. Pancreatic islets from C and LP rats were cultured for 72 h at 5.6 mmol glucose/L in the absence (C and LP) or presence of 0.6 mmol palmitate/L (C + Pal and LP + Pal). Following culture, islets were incubated for 2 h in the presence of 16.7 mmol glucose/L and [1-^{14}C] palmitate. Results are mean ± SEM of 3 experiments. Different letters indicate significant difference between groups and conditions (ANOVA, $P < 0.05$).

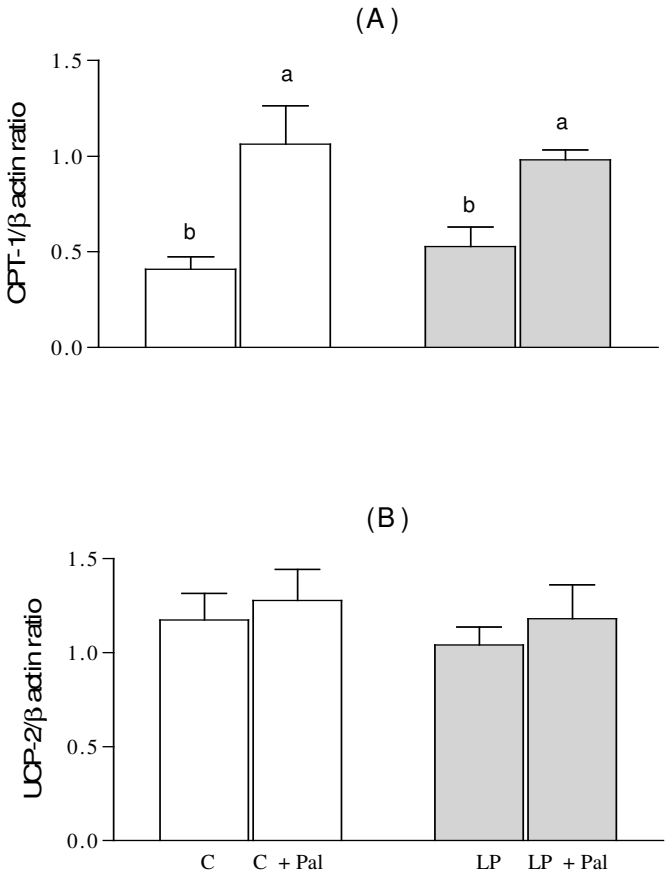

FIGURE 4

FIGURE 4. Effect of chronic palmitate exposure on CPT-1 (A) and UCP-2 (B) mRNA expression. Pancreatic islets from C and LP rats were cultured for 72 h at 5.6 mmol glucose/L in the absence (C and LP) or presence of 0.6 mmol palmitate/L (C + Pal and LP + Pal). Results are mean ± SEM of 3 experiments. Different letters indicate significant difference between groups and conditions (ANOVA, $P < 0.05$).

DISCUSSION

To study if the chronically elevated FFA serum concentration, observed in rats submitted to a low-protein diet from early life to 40 d old, plays a role in the reduced GSIS observed in those animals, C and LP islets were cultured at 5.6 mmol glucose/L in the presence or absence of 0.6 mmol palmitate/L for 72 h. Moreover, some other metabolic consequences of the long-term exposure of the pancreatic islets to FFA were evaluated. By culturing C islets at a physiological glucose concentration and 0.6 mmol palmitate/L, we were able to reproduce the deleterious effect of FFA on GSIS, which allowed us to compare them to the alterations observed in LP islets. The results obtained in this study on GSIS in C + Pal islets are in accordance with previous studies (25, 27) and argue against the hypothesis that increased glucose is a prerequisite for the deleterious effect of FFA on β-cell function.

Reduction of glucose metabolism induced by FFA is probably one of the mechanisms involved in the reduction of GSIS. However, the reciprocal relationship between glucose and FFA oxidation (Randle cycle) was refuted (27, 35) as it was shown that fat oxidation rate is lower than glucose oxidation, and so, the former would not inhibit the later. Moreover, the reduced pyruvate dehydrogenase (PDH) activity, attributed to exposition of β-cells to high FFA levels, was shown to be overcome by elevated production of pyruvate, due to enhanced trafficking through the malate-pyruvate shuttle (36). The similar rate of glucose oxidation observed between C and C + Pal corroborates that hypothesis. Concerning the LP islets, the reduced glucose oxidation in LP and LP + Pal islets could not be related to elevated FFA levels or high palmitate oxidation. Instead, a

reduced pyruvate descarboxylation observed in islets of undernourished rats (Latorraca et al. unpublished results) could account for the low rate of glucose oxidation.

The markedly reduced malonyl-CoA level observed in LP and LP + Pal islets is consistent with the diminished glucose oxidation. Malonyl-CoA is thought to participate in the signal transduction for insulin secretion as a regulator (37, 38). The physiological role of malonyl-CoA in the endocrine pancreas, unlike other tissues, is not a *de novo* synthesis of fatty acids but rather the regulation of CPT-1 activity (38). Recently, results have provided direct support for the hypothesis that the malonyl-CoA/CPT-1 interaction is a component of a metabolic signaling network that controls insulin secretion (37).

A specific effect of CPT-1 on insulin secretion has arisen through an interesting work with adenovirus-mediated overexpression of CPT-1 in INS1E cells (18). Through this maneuver the authors showed a specific effect on the reduced insulin secretion and no effect on glucose oxidation. In the presence of Etomoxir, an irreversible inhibitor of CPT-1 activity, fatty acid oxidation and insulin secretion were restored (18).

The increased palmitate oxidation and CPT-1 mRNA expression in association to a reduced insulin secretion were observed in LP, LP + Pal and C + Pal islets. LP + Pal islets showed to be capable of increasing the mRNA CPT-1 expression, despite no significant increase in palmitate oxidation. Such results could indicate some damage in the CPT-1 translation, in the enzymatic activity or a protective mechanism against a higher fat oxidation.

As previously described, a high palmitate oxidation rate could be related to the depletion of a critical lipid content (FFA, long-chain fatty acyl-CoA, phospholipids and/or DAG), which could act as a signal molecule (18, 33, 38). Fatty acyl-CoAs may act as coupling factors in insulin secretion by stimulating several isoforms of protein kinase C

(39), by stimulating the ATP-sensitive K^+ channel (40), and by acetylating proteins to target them to appropriated membrane sites (41). This could be applied to C + Pal islets, which showed increased oxidation, reduced lipid incorporation and GSIS. On the contrary, higher lipid incorporation in LP could sustain the GSIS, despite it being low when compared with C islets. An important increase in the insulin secretion ratio (16.7/2.8 mmol glucose/L) observed in LP + Pal comparing to C + Pal (6.9 vs. 1 fold, respectively) may be a indicative of a major importance of lipid molecules to insulin secretion in islets from malnourished rats.

The correlation between TG content and loss of insulin secretion has been cited as a contributing factor to β-cell failure (42), we therefore decided to evaluate this parameter in our study. Since LP rats are chronically exposed to high FFA plasma levels, it would be expected that both LP and LP + Pal show higher TG content than C islets (at least). However, previous work has suggested that lipid-induced impairment of β cell function and accumulation of TG requires co-exposure to elevated glucose concentrations (42). Experiments recently reported (18, 42) show no actual correlation between TG content and loss of insulin secretion. The adenovirus-mediated overexpression of a cytosolically localized variant of malonyl CoA decarboxylase (MCD) resulted in a dramatic decrease of cellular TG stores, but no improvement in GSIS (38), while the adenovirus-mediated overexpression of liver CPT-1 in INS1E cells drastically reduced the GSIS with no significant alteration in TG content (18).

Finally, our results indicate that increased oxidation and reduced lipid incorporation account for the inhibitory action of FFA on insulin secretion in C + Pal islets. The opposite, that is, LP islets exposed to palmitate showed an improvement in insulin secretion, without

significant change in glucose and palmitate oxidation related to C + Pal islets. These results suggest that the protein undernutrition in utero causes an early damage in glucose metabolism in pancreatic islets, which account for reduced GSIS observed herein in LP islets. However, adaptive mechanisms that maintain the rate of lipid oxidation and, at the same time, increase lipid incorporation favoring a certain level of lipid molecules to be available for insulin exocytosis, could be an attractive hypothesis to afford the role of high FFA levels on GSIS in LP islets.

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CONCLUSÃO

CONCLUSÕES

ARTIGO 1

A restrição protéica durante a gravidez e lactação comprometeu o crescimento da prole e produziu alterações típicas da desnutrição, como baixo peso corpóreo e aumento da concentração de ácidos graxos livres.

Ratos desnutridos apresentaram glicemia normal, apesar das baixas concentrações de insulina sérica.

Ilhotas isoladas de ratos desnutridos, quando cultivadas com 5.6 mmol/L de glicose, na presença ou ausência de 0.6 mmol/L de palmitato por um período de 48 h, apresentaram aumento da secreção de insulina, provavelmete por conta da presença de LC-CoA.

Em presença de concentrações estimulatórias de glicose (16.7 mmol/L) o metabolismo da glicose foi alterado tanto nas ilhotas de animais controles quanto nas de desnutridos. O dano foi mais exacerbado em ilhotas de animais desnutridos, levando-nos a acreditar na hipótese de que essas ilhotas estavam metabolizando preferencialmente os ácidos graxos em detrimento da glicose.

Os níveis de mRNA da insulina, do PDX-1 e a expressão protéica da p38/SAPK2 apresentaram-se semelhantes em ilhotas de ratos desnutridos e controles, no entanto, quando cultivadas com palmitato por 48 h, observamos um aumento da expressão

desses genes, possivelmente por conta do aumento da expressão protéica da p38/SAPK2.

Nossos resultados sugerem que as ilhotas de animais desnutridos possivelmente conseguem manter a secreção de insulina por conta da presença de LC-CoA, ativando vias alternativas tais como: a via da PKC e extrusão dos grânulos de insulina, e a via que envolve a p38/SAPK2, responsável por estimular o PDX-1 e transcrever o gene da insulina.

ARTIGO 2

Neste trabalho, avaliamos o efeito crônico do palmitato (72 horas de exposição) sobre: a secreção de insulina e a oxidação da glicose e do palmitato, a concentração de malonil-CoA e, a expressão de mRNA da CPT-1 e UCP-2 em ilhotas de ratos submetidos à restrição potéica durante a vida intra-uterina até os 40 dias de vida.

Observamos que o palmito reduziu a secreção de insulina por ilhotas de animais controle em concentraçãoes estimulatórias de glicose.

O metabolismo da glicose em ilhotas de animais desnutridos, expostas ao plamitato, não foi diferente quando comparado com ilhotas cultivadas apenas com glicose, o que nos leva a crer que a presença do ácido graxo não altera a oxidação da glicose. Provavelmente, este efeito, seja devido à redução na descarboxilação do piruvato.

Encontramos níveis reduzidos de malonil-coA em ilhotas de animais desnutridos, independente da presença de palmitato no meio, o que é consistente com menor metabolismo da glicose observado nessas ilhotas, em comparação a ilhotas controle.

Na presença de palmitato, ilhotas de animais desnutridos apresentaram maior expressão de mRNA da CPT-1, embora nenhum aumento no metabolismo do palmito tenha sido observado. Provavelmente esse resultado seja em decorrência de alterações no processo de tradução dessa enzima, ou mesmo na atividade enzimática

O conteúdo de TG de ilhotas de animais desnutridos não diferiu do encontrado em ilhotas controle.

Nossos resultados indicam que a aumentado oxidação de ácidos graxos e reduzida incorporação de lipídeos provocaram menor secreção de insulina em ilhotas de animais controle na presença de palmitato.

Efeitos opostos aos mencionados acima foram encontrados em ilhotas de animais desnutridos, sugerindo que mecanismos adaptativos responsáveis por manter a taxa de oxidação de ácidos graxos e a incorporação de lipídeos favorecem, por disponibilizar moléculas lipídicas, a exocitose da insulina.

Esta pode ser uma hipótese atrativa para explicar a função dos ácidos graxos na secreção de insulina induzida por glicose em animais desnutridos.

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