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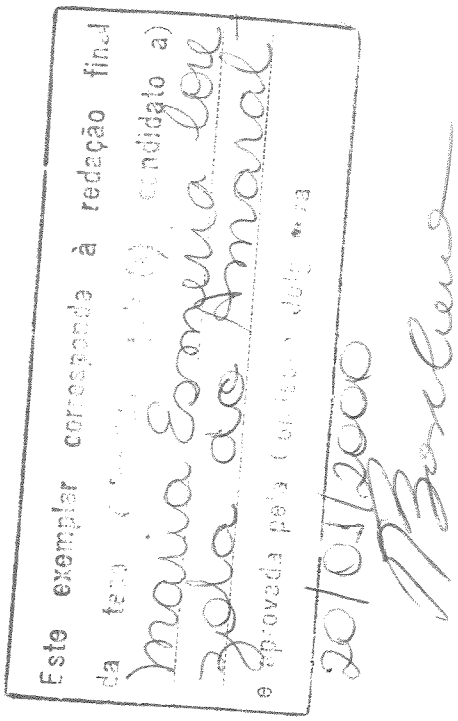
REGULAÇÃO DA GLICEMIA E SECREÇÃO DE INSULINA EM CAMUNDONGOS TRANSGÊNICOS HIPERTRIGLICERIDÊMICOS



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SUMÁRIO

Páginas

Resumo

Neste trabalho, estudamos a homeostasia da glicose e a secreção de insulina em camundongos transgênicos que superexpressam a apolipoproteína CIII humana (apo CIII tg). Esses animais possuem altos níveis de ácidos graxos livres (AGL), de colesterol e de triglicérides plasmáticos em relação aos controles. Peso corporal, concentração de glicose e insulina plasmáticas, taxa de desaparecimento da glicose, após sobrecarga de insulina (Kitt), e área sob a curva glicêmica, após o Teste de Tolerância à Glicose (GTT), não diferiram entre os grupos transgênicos e controles. Contudo, animais transgênicos, que tiveram aumento adicional de AGL plasmático induzido pelo tratamento com heparina, apresentaram menor Kitt durante o Teste de Tolerância à Insulina (ITT) e maior área sob a curva glicêmica durante o GTT, quando comparados aos controles. A secreção de insulina por ilhotas isoladas de animais transgênicos e controles, expostas a concentrações basais ou estimulatórias de glicose, foi semelhante. Contudo, a secreção de insulina por ilhotas isoladas de animais transgênicos tratados com heparina, incubadas durante 15min em presença de baixas ou altas concentrações de glicose, foi menor que em ilhotas de controles heparinizados. Após 60min de incubação, a redução da secreção da insulina das ilhotas dos transgênicos heparinizados só foi observada em presença de altas concentrações de glicose. Concluindo, a hipertrigliceridemia, *per se*, mesmo com moderado aumento da concentração de AGL plasmático, não modifica a homeostasia da glicose nos camundongos transgênicos para apolipoproteína CIII humana. No entanto, incremento adicional da concentração de AGL plasmático por administração de heparina, que, sabidamente, aumenta a atividade da lipoproteína lipase, alterou a homeostasia da glicose nos transgênicos. Esse efeito parece

ser decorrente de uma redução na secreção de insulina, associada a um aumento de resistência periférica ao hormônio.

Abstract

Glucose homeostasis and insulin secretion in transgenic mice overexpressing the human apolipoprotein CIII gene (apo CIII tg) were studied. These mice have elevated plasma levels of triglycerides, free fatty acids (FFA) and cholesterol compared with control mice. The body weights, plasma glucose, and insulin levels, glucose disappearance rates and areas under the intraperitoneal Glucose Tolerance Test (*ip*GTT) curve for adult (4-8 month old) and aged (20-24 month old) apo CIII tg mice were not different from those of control animals. However, an additional elevation of plasma FFA by treatment with heparin for 2-4 h impaired the *ip*GTT responses in transgenic mice compared to saline-treated mice (areas under the *ip*GTT curves; 18.7 ± 1.0 mmol/L.120 min and 13.6 ± 1.2 mmol/L.120 min for heparin- and saline-treated transgenic mice, respectively; n = 10 each; $p < 0.01$). The glucose disappearance rate in heparin-treated transgenic mice (2.88 ± 0.49 %/min; n=11) was slightly lower than in heparin-treated controls (4.12 ± 0.46 %/min; n =10 ; $p < 0.05$). Glucose (22.2 mmol/L) stimulated insulin secretion in isolated islets to the same extent in saline-treated control and apo CIII tg mice. In islets from heparin-treated apo CIII tg mice, the insulin secretion at 2.8 and 22.2 mmol glucose/L was lower than in heparin-treated control mice. In conclusion, hypertriglyceridemia *per se* or a mild elevation in FFA did not affect insulin secretion or insulin resistance in adult or aged apo CIII tg mice. Nonetheless, an additional elevation of FFA induced by heparin in hypertriglyceridemic mice impaired the *ip*GTT by reducing insulin secretion, and was probably associated with a marginal increase in insulin resistance.

I. INTRODUÇÃO

Resistência à insulina tem sido apontada como componente fundamental na patogênese do Diabetes Mellitus Tipo 2. Essa anomalia é freqüentemente associada a alterações tais como: obesidade, hipertrigliceridemia, baixas concentrações de HDL (lipoproteína de alta densidade) e hipertensão. Fatores genéticos bem como adquiridos têm sido utilizados para explicar o Diabetes Mellitus Tipo 2. A provável contribuição de alterações genéticas na gênese dessa patologia direcionou os estudos sobre possíveis alterações nos genes do receptor de insulina, e de seus substratos e na atividade tirosina-quinase dos mesmos. Quanto aos fatores adquiridos, os mais citados são: obesidade, glicotoxicidade e lipotoxicidade. Merece destaque o conceito de lipotoxicidade que tem sido abordado para explicar o declínio progressivo na função da célula Beta. (DeFronzo, 1997).

SECREÇÃO DE INSULINA

A secreção de insulina é estimulada apenas por substratos energéticos metabolizáveis pela própria célula Beta. Sem dúvida, o agente estimulador mais importante é a glicose. O estímulo da síntese e secreção de insulina requer não somente a entrada de glicose na célula, mas seu metabolismo subseqüente. A glicose é transportada para o interior da célula Beta por uma proteína integral de membrana, GLUT 2, que possui um alto K_m (baixa afinidade para a glicose) e um V_{max} muito elevado. Isso permite que o transportador de glicose seja altamente eficiente, respondendo rapidamente aos aumentos das concentrações externas e internas de glicose. Após entrar na célula beta, a glicose é fosforilada em glicose-6-fosfato (G-6-P) por duas enzimas: uma glicoquinase (hexoquinase IV) de baixa afinidade (K_m entre 6 a 11mmol/L) e uma hexoquinase de alta afinidade (k_m

baixo). Entretanto, a enzima de alta afinidade é fortemente inibida por glicose-6-fosfato e, em menor grau, por glicose-1-6-difosfato, o que transfere para a glicoquinase o papel preponderante na fosforilação da glicose nas células Beta. Esse mecanismo funciona como "válvula de segurança", permitindo formação de glicose-6-fosfato apenas em concentrações fisiológicas e suprafisiológicas de glicose no sangue (Matschinsky, 1996; Boschero, 1996). A glicoquinase tem ainda papel fundamental na regulação do fluxo glicolítico e, portanto, na secreção de insulina, o que caracteriza essa enzima, em suma, como o sensor de glicose nas células secretoras de insulina. O destino preferencial de G-6-P dentro da célula Beta é a glicólise (Boschero, 1996). Menos que 10% de G-6-P vai para a via da pentose fosfato. Além disso, as enzimas para a síntese de glicogênio na célula Beta são limitadas (Perales *et al*, 1991). O piruvato formado no citoplasma é transportado à mitocôndria, onde é convertido em acetil Co-A pela piruvato desidrogenase (PDH). Subseqüentemente, o acetil Co-A entra no ciclo de Krebs levando a um aumento de NADH (nicotinamida adenina dinucleotídeo) e $FADH_2$ (flavina adenina dinucleotídeo). O metabolismo da glicose gera ATP (adenosina trifosfato) e a relação ATP/ADP (adenosina trifosfato/adenosina difosfato) aumenta no citoplasma (Matschinsky, 1996). Essa relação ATP/ADP aumentada provoca o fechamento dos canais de potássio e a conseqüente despolarização da membrana celular que abre canais de cálcio sensíveis à voltagem. O aumento do influxo de cálcio na célula Beta resulta em despolarização suplementar da membrana plasmática e estimulação da secreção de insulina (Prentki *et al*, 1996).

Ácidos graxos livres são transportados para a célula Beta e, no citoplasma, são convertidos em acil Co-A. Em condições basais, a molécula de acil Co-A, transportada para a mitocôndria via carnitina palmitoil transferase-1 (CPT-1), sofre aí a beta oxidação. Na

presença de elevadas concentrações de glicose, esse processo é inibido e ocorre aumento na concentração de acil Co-A de cadeias longas no citoplasma, o que estimula a secreção de insulina (Unger,1995; Prentki *et al*,1992). Esse processo se deve ao aumento dos níveis de malonil Co-A formado como resultado do aumento do metabolismo da glicose. Malonil Co-A inibe a CPT-1, permitindo o referido acúmulo de acil Co-A de cadeias longas no citoplasma (Chen *et al*,1994; DeFronzo,1997). Esses ácidos graxos aumentam diretamente a exocitose de insulina por estimular o retículo endoplasmático a liberar cálcio, promovendo aumento de cálcio citoplasmático (Prentki *et al*,1992 & Warnotte *et al*, 1994). Evidências indicam ainda que essas formas de ácidos graxos promovem o fechamento dos canais de potássio, potencializando a secreção de insulina (Prentki *et al*,1992). Assim, a exposição aguda das células Beta tanto à glicose quanto ao AGL aumenta a secreção de insulina. No entanto, exposição crônica a altas concentrações de AGL ou de glicose podem levar à inibição da secreção de insulina, via operação do ciclo de Randle (Randle, 1994). A noção de que altos níveis de AGL circulantes podem produzir resistência à insulina (Boden, 1997) foi proposta por Randle, há mais de 35 anos (Randle, 1963 & Randle, 1998).

CICLO DE RANDLE: Randle propôs a existência de um ciclo glicose-AGL baseado em estudos com coração e diafragma de rato isolados. Em princípio, observou-se que o aumento de AGL diminuía a oxidação de carboidratos e a captação da glicose por esses tecidos. Os pontos chaves para suporte desta hipótese podem ser encadeados da seguinte maneira: o aumento da concentração plasmática de AGL induz o processo de beta oxidação com aumento da produção de acetil Co-A, levando à inibição da piruvato desidrogenase e oxidação do piruvato. Ao mesmo tempo, o aumento do citrato inibe a

fosfofrutoquinase e a glicólise, resultando em acúmulo da G-6-P. Essa, por sua vez, leva à inibição da atividade da hexoquinase, com redução da captação e fosforilação da glicose.

Em seres humanos, o aumento da concentração de AGL plasmático foi induzido pela administração intravenosa concomitante de lipídeos e de um agente estimulador da lipoproteína lipase, a heparina. Geralmente, a infusão de lipídeos diminui a captação, oxidação, e estoques de glicose em músculos (Randle, 1998). Esse efeito inibitório da concentração de AGL plasmático aumentado sobre a oxidação de glicose foi significativo 2-4h após a infusão, sendo a queda nos estoques de glicose associada à redução da atividade da glicogênio sintase (Boden *et al* ,1991). Recentemente, e ao contrário do proposto por Randle, mostrou-se que, em seres humanos, o aumento do nível de AGL plasmático provoca resistência à insulina por inibição do transporte ou fosforilação da glicose e, conseqüente, redução da sua oxidação, diminuição da formação de G-6-P e da síntese de glicogênio no músculo (Roden *et al*, 1996; Roden *et al*, 1999).

Por outro lado, em ilhotas isoladas de ratos, submetidos à dieta rica em lipídeos, a oxidação da glicose e a secreção de insulina, frente a altas concentrações do açúcar e de lipídeos, foram significativamente menores quando comparadas as ilhotas controles (Takahashi *et al*, 1991). Observou-se ainda que a capacidade secretória de ilhotas isoladas de camundongos submetidos à dieta rica em gordura foi similar ao padrão de resposta da secreção de insulina de indivíduos com Diabetes Mellitus Tipo 2 (Capito *et al*,1992). O efeito de altas concentrações de AGL sobre a secreção de insulina é tempo-dependente. Observou-se aumento e diminuição da secreção quando as ilhotas foram expostas a altas concentrações de AGL por curto (3-4h) ou longo (24-48h) período de tempo, respectivamente (Zhou *et al*, 1994; Sako & Grill,1990). Ilhotas de ratos e de seres humanos

expostas aos ácidos graxos por 48h aumentaram a secreção de insulina frente a 3mM (concentração basal) de glicose e diminuíram a liberação de insulina quando a concentração de glicose foi elevada para 27mM (Zhou *et al*, 1995). Ácidos graxos de cadeia longa como palmitato, ácido linoléico e linolênico potencializam a secreção de insulina em resposta à concentração basal de glicose (5,6 mM). Contudo, após esse efeito potencializador, estes ácidos graxos promovem a dessensibilização da célula Beta a altas concentrações de glicose (Opara *et al*, 1993).

O AGL pode também interferir na produção de glicose hepática. Há evidências de que, *in vitro,* o AGL estimula a gliconeogênese. O mecanismo proposto para isso inclui aumento da produção de ATP e NADH e ativação da piruvato carboxilase pelo acetil Co-A gerado via oxidação do AGL (Morand *et al*, 1993). Assim, podemos concluir que os efeitos do AGL sobre a utilização de glicose se manifestam de quatro maneiras diferentes: a) a dessensibilização dos transportadores de glicose; b) a inibição da fosforilação da glicose pela hexoquinase; c) a inibição da fosfofrutoquinase pelo acúmulo do citrato com conseqüente queda da glicólise; d) a inibição da PDH reduzindo a oxidação de glicose. Essas descobertas têm importância clínica relevante, uma vez que o aumento de AGL poderia induzir em indivíduos com Diabetes Mellitus Tipo 2 a resistência periférica à insulina e queda da secreção de insulina (Boden & Gaissmaier, 1997).

I.1 MODELO ANIMAL

Um modelo de hipertrigliceridemia primária foi desenvolvido através da criação de camundongos transgênicos que superexpressam a apolipoproteína CIII humana (apo CIII tg) (Ito *et al*, 1990). Essa apo CIII é produzida em pequenas quantidades pelo fígado e intestino e secretada como constituinte das lipoproteínas ricas em triglicérides (VLDL – lipoproteína de muito baixa densidade e quilomícrons) (Garcia e Oliveira, 1992). A expressão do gene da apo CIII é normalmente reprimida pela insulina (Li *et al*, 1995). Os animais apo CIII tg exibem produção hepática elevada de triglicérides (VLDL) e concentrações plasmáticas de AGL aumentadas (Aalto-Setala *et al*, 1996). A presença de maior quantidade de apo CIII nas VLDL reduz a velocidade da sua remoção do compartimento plasmático, presumivelmente por dificultar sua interação com receptores específicos (Aalto-Setala *et al*, 1992). A insulina ativa a lipoproteína lipase, enzima ancorada no endotélio capilar dos tecidos periféricos, que hidrolisa os triglicerídeos presentes nas VLDL e quilomícrons, com conseqüente liberação de AGL e geração de IDL (lipoproteína de densidade intermediária). Por sua vez, a IDL é convertida em LDL (lipoproteína de densidade baixa) mais rica em colesterol. Desordens no metabolismo dessas lipoproteínas levam ao aumento das frações lipoproteicas ricas em triglicérides, VLDL e IDL (Steiner, 1994), à maior quantidade de LDL (Reaven *et al*, 1993) e diminuição do colesterol das HDL (Dunn, 1992). Situação como esta ocorre freqüentemente em indivíduos diabéticos do tipo 2 com dislipidemia severa. Segundo Piatti *et al*, 1995, níveis elevados apenas de triglicérides também podem diminuir a captação e oxidação da glicose bem como a produção hepática de glicose. Tanto a redução do catabolismo vascular da VLDL (Knudsen *et al*, 1995) quanto o aumento de sua secreção

hepática (Laws, 1996) contribuem para a hipertrigliceridemia do diabético. Na deficiência de insulina ocorre diminuição da lipoproteína lipase dos leitos capilares, o que aumenta a meia-vida da VLDL na circulação sangüínea (Knudsen *et al*, 1995). Além disso, há um aumento da lipase hormônio sensível do tecido adiposo gerando maior aporte de ácidos graxos livres para o fígado, os quais são substratos para a síntese e secreção de VLDL (Laws, 1996). Demonstrou-se também que a síntese *de novo* de ácidos graxos em hepatócitos pode estar aumentada na resistência à insulina ou à hiperinsulinemia (ou em ambos) em conseqüência de um efeito estimulatório da insulina sobre a ácido-graxo-sintase (Prip-Buus C *et al*, 1995). **Portanto, este modelo experimental transgênico para apoliproteína CIII humana permitirá testar a hipótese de que anomalias do metabolismo de triglicérides e ácidos graxos livres podem alterar a homeostasia da glicose. Uma eventual utilização preferencial dos ácidos graxos pelos tecidos periféricos em detrimento da glicose, representaria uma sobrecarga para o pâncreas, que, com o tempo, levaria o animal a um estado de resistência à insulina.**

II. CAPÍTULOS

II.1. Trabalho submetido à publicação

Os dados obtidos durante o desenvolvimento desta tese foram organizados no trabalho submetido à publicação em Janeiro/2000, para a revista: Journal of Lipid Research, apresentado a seguir:

PLASMA GLUCOSE REGULATION AND INSULIN SECRETION IN HYPERTRIGLYCERIDEMIC TRANSGENIC MICE

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Running title: Hypertriglyceridemia and glucose homeostasis

Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; ITT, insulin tolerance test; GTT, glucose tolerance test; FFA, free fatty acid; tg, transgenic; apo CIII, apolipoprotein CIII; VLDL, very low density lipoprotein.

Abstract

Glucose homeostasis and insulin secretion in transgenic mice overexpressing the human apolipoprotein CIII gene (apo CIII tg) were studied. These mice have elevated plasma levels of triglycerides, FFA and cholesterol compared with control mice. The body weights, plasma glucose, and insulin levels, glucose disappearance rates and areas under the *ip*GTT curve for adult (4-8 mo. old) and aged (20-24 mo. old) apo CIII tg mice were not different from those of control animals. However, an additional elevation of plasma FFA by treatment with heparin for 2-4 h impaired the *ip*GTT responses in transgenic mice compared to saline-treated mice (areas under the *ip*GTT curves; 18.7 ± 1.0 mmol/L.120 min and 13.6 ± 1.2 mmol/L.120 min for heparin- and saline-treated transgenic mice, respectively; n = 10 each; $p < 0.01$). The glucose disappearance rate in heparin-treated transgenic mice (2.88 ± 0.49 %/min; n=11) was slightly lower than in heparin-treated controls (4.12 ± 0.46 %/min; n =10 ; $p < 0.05$). Glucose (22.2 mmol/L) stimulated insulin secretion in isolated islets to the same extent in saline-treated control and apo CIII tg mice. In islets from heparin-treated apo CIII tg mice, the insulin secretion at 2.8 and 22.2 mmol/L glucose was lower than in heparin-treated control mice. In conclusion, hypertriglyceridemia *per se* or a mild elevation in FFA did not affect insulin secretion or insulin resistance in adult or aged apo CIII tg mice. Nonetheless, an additional elevation of FFA induced by heparin in hypertriglyceridemic mice impaired the *ip*GTT by reducing insulin secretion, and was probably associated with a marginal increase in insulin resistance.

Supplementary Key words: hypertriglyceridemic mouse, glucose homeostasis, insulin secretion, insulin resistance.

Introduction

Insulin resistance in type 2 diabetic subjects can be provoked by acquired and/or genetic factors. One of the most important of these factors is hyperlipidemia with elevated plasma FFA levels (1). Elevation of FFA oxidation leads to a reduction in glucose uptake, oxidation and storage, and an increase in glucose production (2-4). However, the precise mechanism by which elevation of FFA impairs glucose use is still controversial. According to the glucose-fatty acid cycle proposed by Handle 35 years ago, a rise in FFA oxidation increases intramitochondrial acetyl-CoA/CoA and $NADH/NAD^+$ ratios with a consequent inactivation of pyruvate dehydrogenase. These alterations raise the citrate concentration, thereby abolishing phosphofructokinase activity with accumulation of glucose-6-phosphate, which ultimately inhibits hexokinase activity and leads to a decrease in glucose uptake (5-6). Alternatively, the increased insulin resistance provoked by the elevation of FFA has been ascribed to the inhibition of glucose transporters and/or glucose phosphorylation with a subsequent reduction in the rate of glucose oxidation and muscle glycogen synthesis (7,8). Normal glucose homeostasis is dependent on the interaction between tissues sensitivity to insulin and insulin secretion. Thus, an increase in the insulin resistance requires progressive increases in the insulin secretion rate. If the compensatory hyperinsulinemia is not sufficient to maintain euglycemia, hyperglycemia can occur (1). On the other hand, an increase in FFA oxidation by pancreatic β-cells stimulates insulin secretion by raising the $[Ca^{2+}]_i$ associated with activation of PKC (9). A rise in FFA oxidation in β-cells also impairs glucose-induced insulin secretion (10-13).

Transgenic mice expressing the human apolipoprotein CIII (apo CIII tg), exhibit hypertriglyceridemia and elevated plasma cholesterol and FFA levels (14-16), and are a

suitable model for studying the association between hypertriglyceridemia and insulin resistance and secretion (17,18) . However, hypertriglyceridemic transgenic mice are neither insulin resistant nor hyperinsulinemic (19).

In the present work, we have confirmed and extended the observation that hypertriglyceridemia *per se* does not affect insulin resistance in adult (4-8 mo) or aged (20-24 mo) apo CIII tg mice. However, additional elevation of FFA, induced by heparin, impaired the *ip*GTT in transgenic but not in control mice. This effect is the result mainly of a reduction in insulin secretion.

METHODS

Materials

Collagenase was purchased from Boehringer. Antiserum against insulin was kindly provided by Dr Leclercq-Meyer (Free University of Brussels, Brussels, Belgium). Rat standard insulin was from Novo-Nordisk (Copenhagen) and human recombinant insulin (Humulin R) was from Eli Lilly Co. (Indianapolis, IN). Bovine serum albumin (fraction V) and chemicals were from Sigma Chemical Co. (St. Louis, MO). The anesthetics rompun and vetanarcol were from Bayer S.A. (Germany) and König (Brazil), respectively. Liquemine was from Roche (Brazil).

Animals

Human apo CIII transgenic mice (line 3707) kindly donated by Dr. Alan R. Tall (Columbia University, NY) were bred in the animal facilities of the Department of Physiology and Biophysics, UNICAMP. The experiments involving animals were approved by the university's Ethics Committee and were in accordance with the *Guidelines on the*

Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare (1992). The mice had access to standard laboratory chow and water *ad libitum* and were housed at 22 ± 2 ^{O}C on a 12 h light dark cycle. Male and female hemizygous apo CIII transgenic and non-transgenic littermates aged 4-24 months were used in this study. Blood for plasma glucose (20), triglyceride (21), cholesterol (22) and insulin (23) measurements was taken either from the retro-orbital plexus or the tail of anesthetized mice. FFA measurements were done using an enzymatic colorimetric method according to the manufacturer's instructions (Wako Chem. USA, Inc., VA).

Glucose-tolerance test.

Intraperitoneal glucose tolerance tests (*ip*GTT) were performed at 4-8 months or 20-24 months of age. After 12-15 h of fasting, the mice were injected with glucose (2 g kg^{-1}) of glucose intraperitoneally (24). Blood samples for glucose determinations were obtained from the cut tip of the tail at 0, 15, 30, 60 and 120 min. The glucose response during the glucose tolerance test were evaluated by estimating the total area under the glycemia *vs* time curves using the trapezoidal method (25).

Insulin-tolerance test

Intraperitoneal (*ip*) or intravenous (*iv*) insulin-tolerance tests (ITT) were performed in fed mice, 4-8 months old. The *ip*ITT consisted of a bolus intraperitoneal injection of regular insulin (0.75 U kg^{-1}). Blood samples were obtained from the cut tip of the tail at 0, 15, 30 and 60 min for glucose determinations (24). The *iv*ITT consisted of a bolus injection of regular insulin (0.1 U kg^{-1}) into the inferior cava vein of anesthetized mice previously laparotomized. Blood samples were obtained from the cut tip of the tail at 0, 4, 8, 12 and 16

min. The rate constant for serum glucose disappearance (Kitt) was calculated from the formula $0.693/t_{1/2}$. The serum glucose $t_{1/2}$ was calculated from the slope of the least square analysis of the serum glucose concentrations from 0-16 min after intravenous insulin injection [26].

Insulin secretion from isolated pancreatic islets

Fasted mice were killed by decapitation and the pancreas was removed. Islets were isolated by handpicking after collagenase digestion of the pancreas. Groups of five islets were first incubated for 30 min at 37 °C in Krebs-bicarbonate buffer containing 2.8 mmol glucose/L and equilibrated with 95% O_2 / 5% CO_2, pH 7.4. The solution was then replaced with fresh Krebs-bicarbonate buffer and the islets were further incubated for 1 h with medium containing 2.8 or 22.2 mmol glucose/L. The incubation medium contained (in mmol/L): NaCl 115, KCl 5, $NaHCO_3$ 24, $CaCl_2$ 2.56, $MgCl_2$ 1, and BSA 0.3% (w/v) [27]. The insulin released after 15 min and 1 h was quantified as previously described [23] using rat insulin as the standard.

Statistics

The results are presented as the mean ± SEM for the number of mice (*n*) indicated. When working with islets, *n* refers to the number of experiments performed (120 islets per group per experimental condition, distributed in groups of five islets each). The data were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test for individual differences between groups. Non-paired *t*-tests were employed whenever appropriate. p values <0.05 indicated a significant difference.

Results

The body weight, plasma glucose and insulin levels (Table 1), glucose disappearance rate (Fig.1A) and area under the *ip*GTT curve (Fig. 1B) of adult apo CIII tg mice (4-8 mo. old) were not different from those of control mice. However, the plasma cholesterol, triglyceride and FFA levels of apo CIII tg were higher than in control mice (Table 1). The differences in triglyceride, FFA and cholesterol levels between apo CIII tg and control mice persisted in aged animals (20-24 mo. old) whereas the plasma glucose levels as well as *ip*GTT values were not different between the two groups of aged mice.

Heparin significantly increased the plasma FFA levels of control and apo CIII tg mice (Fig. 2). The mean plasma FFA levels 90 to 240 min after heparin administration were 4.3 ± 0.2 and 2.8 ± 0.14 mmol/L for apo CIII tg and control mice, respectively (n=10 for both groups; $p < 0.01$). These values were significantly higher than those obtained prior to heparin administration. The administration of saline did not interfere with the plasma FFA levels in apo CIII tg mice but significantly reduced the levels in control mice at the end of the period of infusion (130-240 min, not shown). The glucose disappearance rates were 3.51 ± 0.34 %/min (n = 9) and 3.47 ± 0.27 %/min (n = 8) for saline-treated apo CIII tg and control mice, respectively. The corresponding rates for heparin-treated apo CIII tg and heparin-treated control mice were 2.88 ± 0.36 %/min (n = 11) and 4.12 ± 0.45 %/min (n =10), respectively. Although these values were significantly different ($P < 0.05$), they were not different from control or apo CIII tg mice treated with saline. In addition, the *ip*GTT showed an increased area under the glycemic curve when heparin-treated apo CIII tg mice were compared to saline-treated apo CIII tg mice (18.7 ± 1.0 mmol/L.120 min *vs* 13.6 ± 1.2 mmol/L.120 min; n = 10 for both groups; $p < 0.01$; Fig. 3).

In another series of experiments, the insulin secretion from isolated islets derived from both control and transgenic mice, previously treated with saline or heparin was examined. Basal insulin secretion in the presence of 2.8 mmol glucose/L after a 15 min or 1 h incubation was similar in islets from control and transgenic saline-treated mice (Fig. 4 A,B). After a 15 min incubation in the presence of 22.2 mmol glucose/L, the insulin secretion increased 1.6 ± 0.5 - and 2.4 ± 0.3 - fold in the control and transgenic saline-treated mice, respectively (NS). After a 1 h incubation at high concentrations of glucose, the increase in insulin secretion increased 3.0 ± 0.4 - and 3.8 ± 0.5 - fold in the saline-treated control and apo CIII tg islets, respectively (NS). However, basal (2.8 mmol glucose/L; Fig. 5A) and glucose (22.2 mmol/L)-stimulated (Fig 5 A,B) insulin secretion were lower in islets from heparin-treated apo CIII tg islets compared to heparin-treated controls ($p < 0.05$).

Discussion

Type 2 diabetes mellitus is frequently associated with obesity and hyperlipidemia (1). Together, these diseases are part of a pathophysiological state known as the plurimetabolic syndrome, but the cause-effect relationship between them is unclear. Two main aspects of this syndrome have been extensively explored during the past 15 years, namely, the insulin resistance provoked by increased FFA oxidation (2-4) and, more recently, β-cell lipotoxicity (10,12). In the present work, we studied the glucose homeostasis and insulin secretion in apo CIII tg mice, a model in which the primary defect is hypertriglyceridemia. Streptozotocin-induced diabetic mice show increased expression of apo CIII mRNA. This upregulation of apo CIII is abolished by insulin administration (28). These findings suggest that overexpression of the apo CIII gene could be responsible for

the hypertriglyceridemia observed in IDDM. Similarly, apo CIII tg mice have significantly higher plasma triglycerides, cholesterol and FFA levels than non-transgenic littermate control mice (14-16).

In agreement with previous results (19) the glycemia and insulinemia of adult (4-8 mo. old) apo CIII tg mice were similar to those of the controls. In aged apo CIII tg mice, the glycemic control may be hampered, although the data for 20-24 months-old transgenic mice were not significantly different from aged non-transgenic mice. Thus, hypertriglyceridemia with increased plasma FFA, at least to the levels observed in these apo CIII tg mice, did not affect the glucose homeostasis of adult and aged mice. Insulin resistance has been reported in humans with increased plasma FFA concentrations produced by the concomitant administration of triglycerides and heparin (7,29). The plasma FFA levels in these subjects were significantly lower than those found in apo CIII tg mice. However, the increase observed after treatment with heparin was much greater in humans, *i.e.*, four-fold *vs* less than two-fold in transgenic mice. We have no explanation for this difference between human and hypertryglyceridemic transgenic mice. Except that the effect of increased plasma FFA concentrations on insulin resistance my be species-specific (19). A further possibility is that elevated levels of plasma triglycerides, and in specially FFA, are necessary but not sufficient to provoke alterations in insulin action in target tissues. We addressed the latter possibility by provoking an additional increase in plasma FFA levels through the administration of heparin to control and transgenic mice. Both groups showed significant increases in their plasma FFA levels compared to the levels before heparin administration (Fig. 2). A deterioration of glucose homeostasis in transgenic heparin-treated mice was observed based on the increased area under the *ip*GTT curve and the lower Kitt values in these mice compared to the corresponding controls.

Parallel experiments showed that the glucose-induced insulin secretion by isolated islets incubated for 15min or 60 min was similar in transgenic- and saline- treated control mice. However, in heparin-treated mice, the insulin secretion at low (after a 15 min incubation) or high (after a 15 min and 1 h) concentrations of glucose was slightly, but significantly, reduced in transgenic mice when compared to control mice ($p < 0.05$). Thus, it seems that a reduction in insulin secretion associated with a diminished sensitivity to insulin could explain the impaired GTT in heparin-treated transgenic mice.

The precise mechanism underlying the reduction in insulin sensitivity produced by increased plasma FFA concentrations is not yet clear. According to the glucose-fatty acid cycle hypothesis, an increase in FFA oxidation is necessary to inhibit phosphofructokinase activity and cause the accumulation of glucose-6-phosphate, which in turn inhibits hexokinase activity and eventually reduces glucose uptake (2-4). An alternative explanation envisages increased FFA concentrations leading to the inhibition of glucose transport and/or glucose phosphorylation, followed by a reduction in the rate of glucose oxidation and muscle glycogen synthesis, and finally, a reduction in glucose-6-phosphate accumulation (7,8). Regardless of the precise mechanism, it is conceivable that an increase in FFA oxidation is always necessary to provoke insulin resistance. Increased levels of apo CIII protein in the plasma of transgenic mice decrease the fractional catabolic rate of VLDL-triglycerides and the uptake of particles by tissues (19). However, the increased levels of triglycerides and FFA in the plasma of transgenic mice may not necessarily indicate an increase in FFA oxidation. Furthermore, if FFA oxidation is indeed increased, it is apparently not sufficient to alter cellular metabolism and provoke insulin resistance in apo CIII transgenic mice.

Recently, lipoprotein lipase deficient mice were reported to have an increased insulin secretion and lower plasma glucose levels compared to wild type mice. In addition, insulin secretion in INS-1 cells (a rat insulinoma cell line) was inversely proportional to the level of lipoprotein lipase expression (30).

In conclusion, it is conceivable that the additional increase in plasma FFA levels as a consequence of enhanced lipoprotein lipase activity produced by heparin in apo CIII tg mice could compensate for the reduced insulin secretion by islets and the consequently impaired response to the *ip*GTT in these transgenic mice.

Acknowledgements

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Figure Legends

Figure 1

Insulin and glucose tolerance tests in apo CIII tg and control mice. In **A**, eight fed adult mice received a bolus intraperitoneal injection of regular insulin (0.75 U kg^{-1}). Blood samples were obtained from the cut tip of the tail at 0, 15, 30 and 60 min for glucose measurements. In **B**, twelve fasted adult mice received glucose (2 g kg^{-1}) intraperitoneally. Blood samples were obtained from the cut tip of the tail at 0, 15, 30, 60 and 120 min for glucose measurements. The points are the mean ± SE of 8-12 mice.

Figure 2

Plasma FFA levels in apo CIII tg and control mice. The mice received three bolus injections of heparin (100U kg^{-1}) at 0, 90 and 180 min. Blood samples were obtained from the cut tip of the tail at 0, 90, 120, 180 and 240 min. The points are the mean ± SE of 8-10 mice. *$P < 0.05$ and **$P < 0.01$ compared to the control at the corresponding time.

Figure 3

Glucose tolerance tests in saline- and heparin-treated apo CIII tg mice. The mice were treated with three subcutaneous injection of saline or heparin at 0, 90 and 180 min and received glucose (2 g kg^{-1}) intraperitoneally at 120 min. Blood samples were obtained from the cut tip of the tail at 120, 135, 150, 180 and 240 min for glucose measurements. The points are the mean ± SE of 10-11 mice per groups.

Figure 4

Glucose stimulation of insulin secretion in islets from saline-treated apo CIII tg and control mice. Groups of five islets were preincubated for 30 min in Krebs-bicarbonate medium containing 2.8 mmol glucose/L, after which the medium was replaced with Krebs solution containing 2.8 or 22.2 mmol glucose/L. The columns represent the cumulative 15 min (**A**) and 1 h (**B**) insulin secretion and are the mean ± SE of 20-35 groups of islets.

Figure 5

Glucose stimulation of insulin secretion in islets from heparin-treated apo CIII tg and control mice. Groups of five islets were preincubated for 30 min in Krebs-bicarbonate medium containing 2.8 mmol glucose/L, after which the medium was replaced with Krebs solution containing 2.8 or 22.2 mmol glucose/L. The columns represent the cumulative 15 min (A) and 1 h (B) insulin secretion and are the mean ± SE of 20-35 groups of islets. *$p < 0.05$ compared to the respective control.

Table I

Body weight and plasma glucose, insulin, cholesterol, triglyceride and FFA levels in fasted control and apo CIII tg mice.

	Control		Apo CIII tg	
Body weight (g) - M	29.4 ± 0.5	(15)	30.4 ± 1.0	(15)
F	23.8 ± 1.0	(15)	24.5 ± 1.0	(15)
Glucose (mg/dl) - fasted	89 ± 4.6	(15)	81 ± 2.8	(15)
fed	135 ± 4.4	(8)	142 ± 2.4	(8)
Insulin (ng/ml) - fasted	0.57 ± 0.1	(16)	0.6 ± 0.3	(16)
fed	0.96 ± 0.3	(16)	0.98 ± 0.2	(13)
Cholesterol (mg/dl)	97 ± 4.2	(10)	117 ± 5.4*	(10)
Triglycerides (mg/dl)	86 ± 5.5	(10)	573 ± 61*	(10)
FFA (mmol/L)	1.3 ± 0.17	(10)	3.0 ± 0.14*	(10)

Means ± SEM of *n* (in parenthesis)

*$p < 0.01$ *vs* control

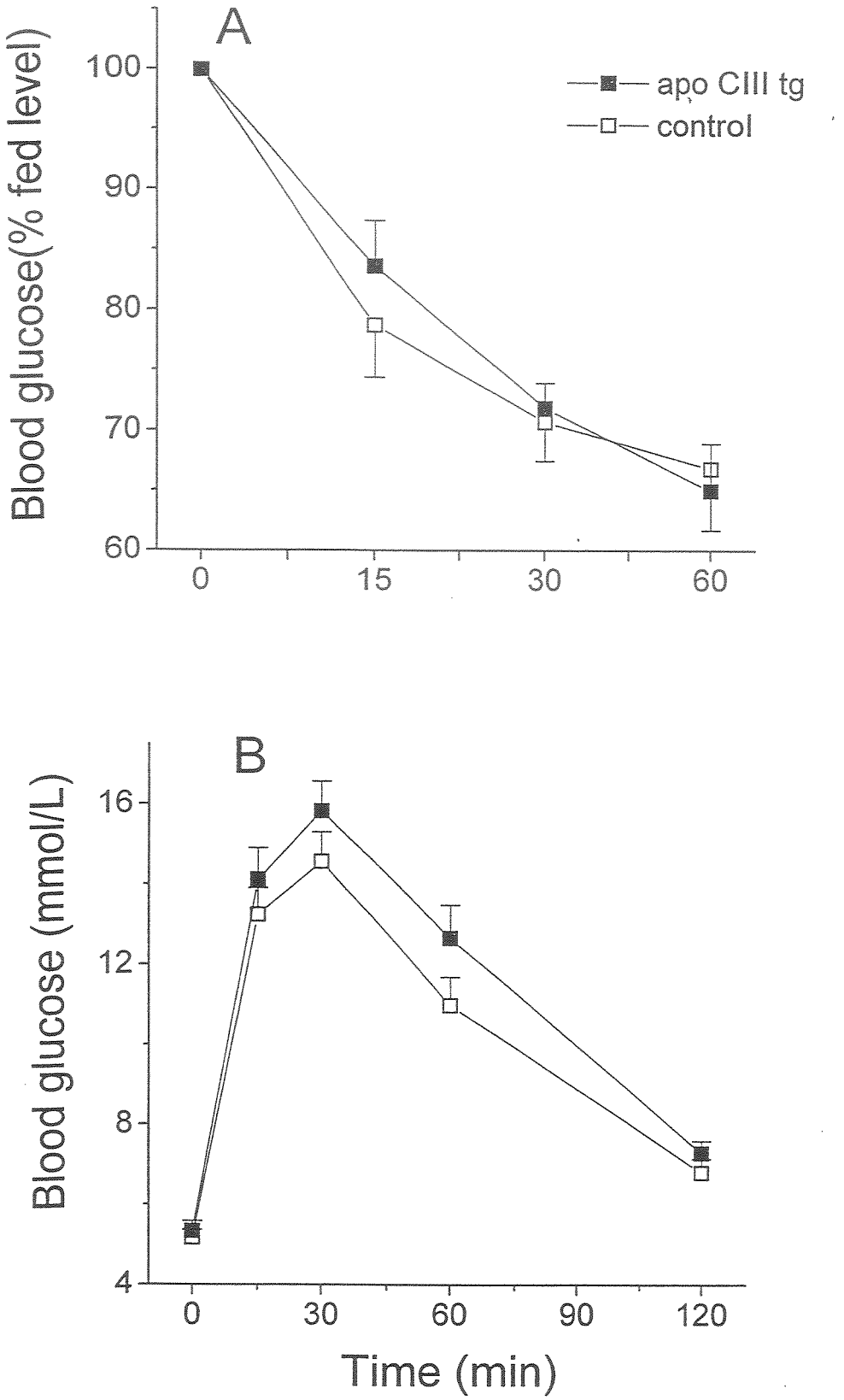

A
Blood glucose(% fed level)
100
90
80
70
60
0
15
30
60
apo CIII tg
control
B
Blood glucose (mmol/L)
16
12
8
4
0
30
60
90
120
Time (min)

Figure 1

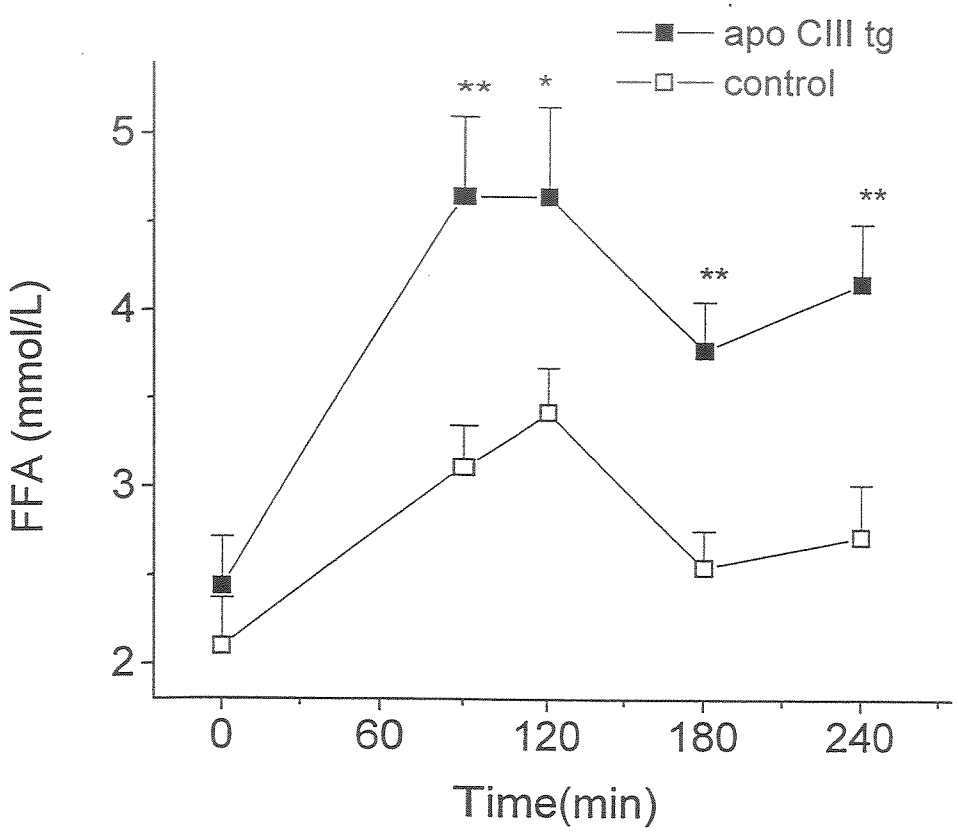


Figure 2

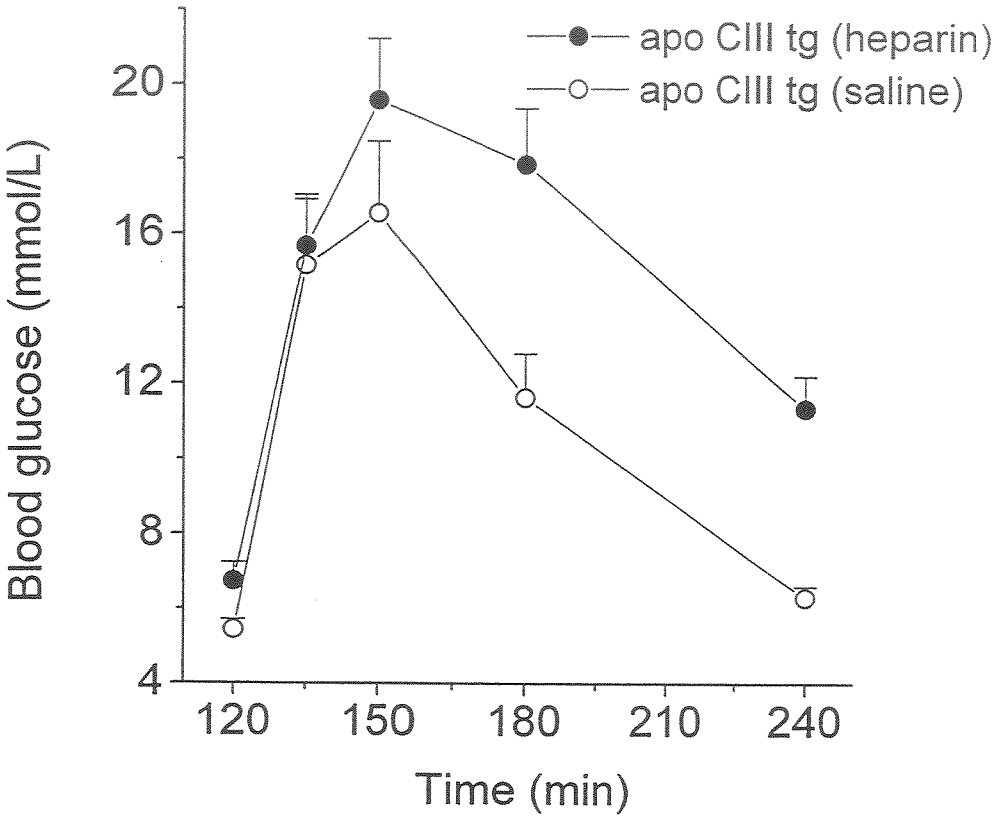

apo CIII tg (heparin)
apo CIII tg (saline)
Blood glucose (mmol/L)
20
16
12
8
4
120
150
180
210
240
Time (min)

Figure 3

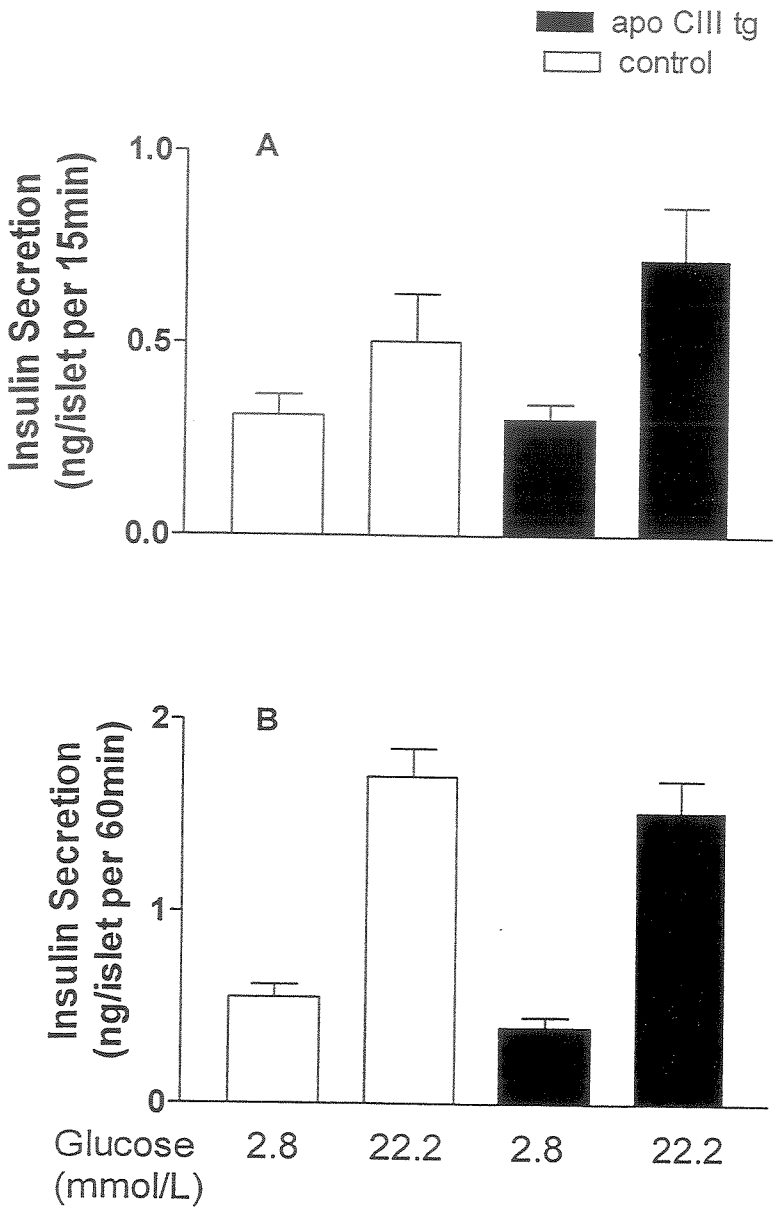

apo CIII tg
control
A
Insulin Secretion (ng/islet per 15min)
1.0
0.5
0.0
B
Insulin Secretion (ng/islet per 60min)
2
1
0
Glucose (mmol/L)
2.8
22.2
2.8
22.2

Figure 4

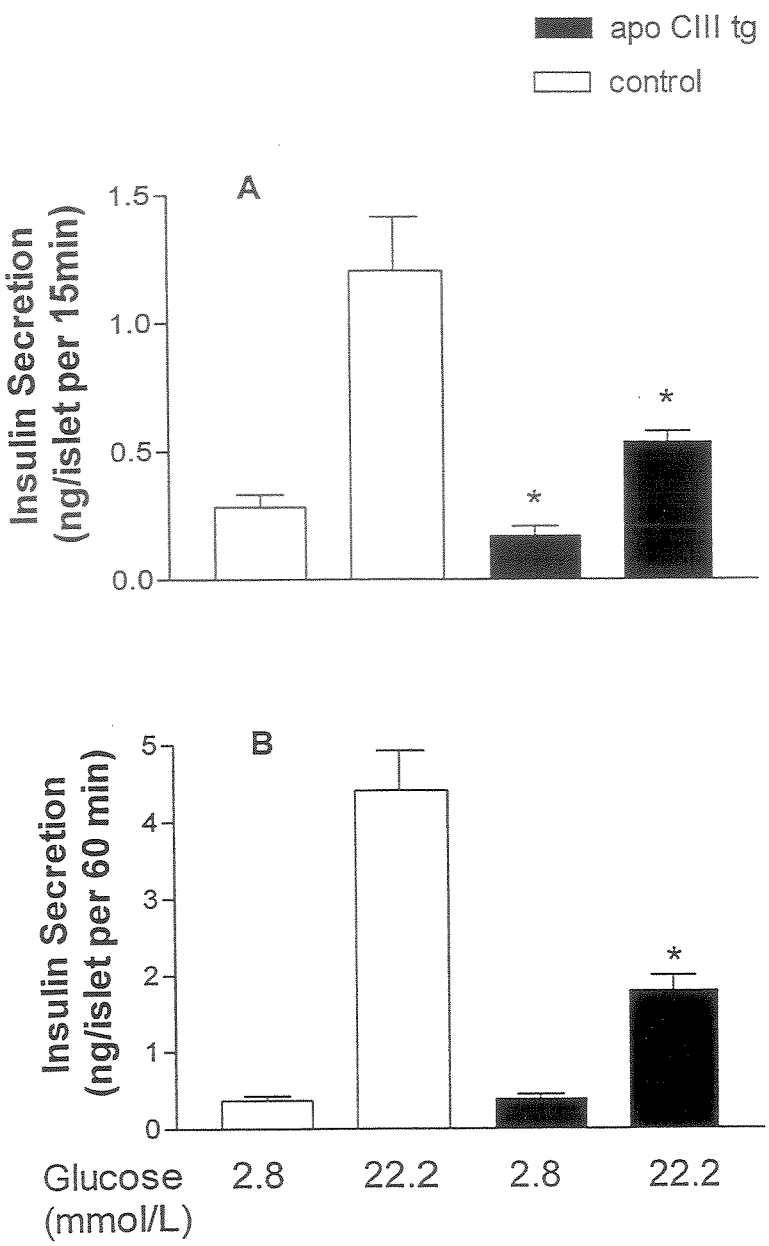

apo CIII tg
control
A
Insulin Secretion (ng/islet per 15min)
1.5
1.0
0.5
0.0
*
*
B
Insulin Secretion (ng/islet per 60 min)
5
4
3
2
1
0
*
Glucose (mmol/L)
2.8
22.2
2.8
22.2

Figure 5

II.2. Apresentação em Congresso

FeSBE

25 a 28 de Agosto / Caxambu - MG 99

Certificamos que

O resumo n° 08.021, intitulado "Hipertrigliceridemia não altera a homeostasia da glicose em camundongos transgênicos para apolipoproteína CIII (apoCIII Tg).", de autoria: Amaral, M. E. C.; Oliveira, H. C. F.; Carneiro, E. M.; Berti, J. A.; Vieira, E. C.; Crepaldi-Alves, S. C.; Boschero, A. C., foi apresentado na

XIV Reunião Anual da Federação de Sociedades de Biologia Experimental-FeSBE, realizada no Hotel Glória na cidade de Caxambu-MG, de 25 a 28 de agosto de 1999.

Comissão Organizadora

FeSBE

25 a 28 de Agosto / Caxambu - MG 99

Certificamos que

O resumo n° 08.022, intitulado "Aumento agudo de ácidos graxos livres (AGL) circulantes modifica o teste de tolerância à glicose (GTT) em camundongos transgênicos para apolipoproteína CIII (apoCII TG).", de autoria: Amaral, M. E. C.; Oliveira, H. .C. F.; Carneiro, E. M.; Berti, J. A.; Oliveira, E.C.; Delghingaro, A. V.; Bosqueiro, J. R.; Boschero, A. C., foi apresentado na

XIV Reunião Anual da Federação de Sociedades de Biologia Experimental-FeSBE, realizada no Hotel Glória na cidade de Caxambu-MG, de 25 a 28 de agosto de 1999.

Comissão Organizadora

FeSBE 99

25 a 28 de Agosto / Caxambu - MG

Certificamos que o trabalho Aumento agudo de ácidos graxos livres (AGl) circulantes modifica o teste de tolerância à glicose (GTT) em camundongos transgênicos para apolipoproteína CIII (apoCII TG) de autoria Amaral, Maria Esméria C., Oliveira, H. C. F., Carneiro, E. M., Berti, J. A., Vieira, E. C., Delghingaro, A. V., Bosqueiro, J. R., Boschero, A. C. – UNICAMP foi apresentado sob a forma de comunicação oral no módulo temático Doenças endócrino-metabólicas: modelos em animais transgênicos na

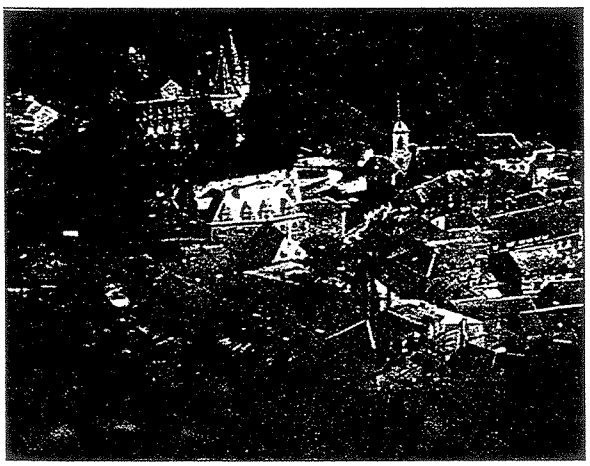
XIV Reunião Anual da Federação de Sociedades de Biologia Experimental-FeSBE, realizada no Hotel Glória na cidade de Caxambu-MG, de 25 a 28 de agosto de 1999.

Comissão Organizadora

Diabetes & Metabolism

1999 Annual Meeting of the EASD Islet Study Group

Durbuy, Belgium



October 2-4, 1999

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01 Regulation of plasma glucose and insulin secretion in hypertriglyceridemic mice.

M.E.C. AMARAL, H.C.F. OLIVEIRA, E.M. CARNEIRO, V. DELGHINGARO, E.C. VIEIRA, J.A. BERTI, A.C. BOSCHERO. Department of Physiology and Biophysics, Institute of Biology, State University of Campinas, Campinas, SP, Brazil.

Non insulin dependent diabetes mellitus is frequently associated with obesity and/or elevated plasma FFA. Hyperlipidemia with elevation of FFA decreases glucose tolerance and increases insulin resistance in humans and in animals. We have analyzed here the glucose homeostasis in transgenic mice overexpressing the human apo CIII gene (tg apo CIII). These animals have elevated plasma levels of triglycerides, FFA and cholesterol compared with control mice. Body weight, plasma glucose, plasma insulin, glucose disappearance rate and the area under *ip*GTT curve of adult tg apo CIII mice (4-8 mo old) were not different from values found in control animals. In addition, plasma glucose and insulin levels as well *ip*GTT values were similar in aged tg apo CIII and control mice (20-24 mo old). The differences in the cholesterol, FFA and triglyceride levels between tg apo CIII mice and controls were maintained in the old animals. Previous treatment with heparin for 4h significantly increased plasma FFA levels in control and tg apo CIII mice. The glucose disappearance rate in heparin-treated tg apo CIII mice (2.8 ± 0.49 %/min ; $n = 7$) was lower than controls (3.6 ± 0.33 %/min ; $n = 6$). In addition, the *ip*GTT performed in heparin-treated animals showed an increased area when compared to that found in saline-treated tg apo CIII mice (18.7 ± 1.0 mmol/L.120 min and 13.6 ± 1.2 mmol/L.120 min, respectively ; $n = 10$ for both groups). The *ip*GTT values of heparin-or saline-treated controls were not different from those of non- treated controls. Basal insulin secretion in islets derived from heparin-treated tg apo CIII mice were lower than controls (non transgenic and tg apo CIII saline-treated mice) after 10 (first phase) and 60 min incubation (second phase). In conclusion, hypertriglyceridemia *per se* did not affect insulin secretion or insulin resistance in adult or old tg apo CIII mice. However, additional elevation of FFA, induced by heparin administration in transgenic mice, impaired the *ip*GTT mainly by an increase in insulin resistance, probably associated with a mild reduction of insulin secretion.
Supported by the Brazilian foundations : FAPESP, CAPES, CNPq, FINEP-PRONEX.

02 pH dependence of β-cell granule insulin crystal dissolution.

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Stimulus-secretion coupling in the β-cell involves many steps : glucose influx, membrane depolarization, calcium influx, insulin granule docking and fusion with the plasma membrane. This last step is often considered the final event leading to insulin secretion ; however, the insulin crystal must be dissolved and the insulin transported across the endothelial cell layer before the hormone enters the circulation.
Pancreatic cells require the physiological buffer HCO_3/CO_2 for normal function. We have studied the effects of removal of HCO_3/CO_2 and changes of extracellular pH on various aspects of β-cell stimulus-secretion coupling, using patch clamp and intracellular voltage recording techniques, insulin secretion and electron microscopic studies in mouse β cells and islets of Langerhans.
Removal of HCO_3/CO_2 at pH 7.4 (using Hepes buffer) stimulates K_{ATP} potassium channels, hyperpolarizes the β-cell and inhibits insulin secretion. Acidification of the perifusion medium blocks K_{ATP} channels and restores bursting electrical activity but does not revert the inhibition of insulin secretion. In fact, secretion is further inhibited. Re-introduction of a HCO_3/CO_2-buffered medium immediately increases insulin secretion with a transient overshoot. Electron micrographs of the β-cells in intact islets taken under each condition were compared. Under acidic conditions without HCO_3/CO_2 buffer, multiple granule dense cores were observed enfolded within the β-cells, representing the undissolved insulin crystals in fused granules. This explains the overshoot in insulin secretion on return to pH 7.4 medium, as insulin from the already fused granules is dissolved. It is concluded that β-cell granules contain a pH sensitive matrix that shrinks or swells in response to changes in pH, much as has been described in chromaffin cells. It is proposed that one of the final steps in stimulus-secretion coupling is the dissolution of the insulin crystal upon exposure to the neutral pH of the extracellular medium.

03 p300/CBP is a transcriptional co-activator for hepatocyte nuclear factor- 1 alpha and enhances GLUT2 gene expression.

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Several mutations of hepatocyte nuclear factor-lalpha (HNF-1α) gene are found in MODY3 pedigrees, but the precise mechanisms by wich these mutations evoke diabetes are unknown. We have previously shown that human GLUT2 gene is closely regulated by HNF-1α via sequences downstream of transcriptional start site. Here, we examined the molecular mechanism of HNF-1α-mediated transcription. Mammalian two-hybrid study and immunoprecipitation study revealed that HNF-1α interacts with the N-terminal region of p300 (amino acids 180-662) and the C-terminal region of p300 (amino acids 1818- 2079). The transactivation domain of HNF-1α (amino acids 319-540) interact with N terminus and C terminus of p300, respectively. Immunohistochemical analyses were performed in pancreatic islets of 8-week-old rats using anti-HNF-1α antibody or anti-p300 antibody. Almost all pancreatic β-cells were detected by nuclear and cytoplasmic staining with the anti-HNF-1α antibody or anti-p300 antibody.
These findings demonstrate that p300 acts as a transcriptional co-activator for HNF-1α and provide new insights into the regulatory function of HNF-1α.

04 Caspase-3 activation is dependent of MAPK's pathways activation by nitric oxide in insulin secreting RINm5F cells.

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NO is known to participate in the apoptotic action of inflammatory cytokines in rodent pancreatic β-cells, but the chain of events that links the generation of NO with the apoptotic process is not well understood. The effect of chemical NO generation on DNA fragmentation, LDH release and caspases activation was studied in insulin producing RINm5F cells. The impact of NO generation on MAPK's pathways activation was also studied. The results indicate that NO actions on pancreatic β-cells apoptosis are associated with activation of MAPK's and JNK pathways and with phosphorylation of the anti-apoptotic protein Bcl-2 and with specific activation of caspase 3. Blockade of MAPK's pathways with inhibitors such as PD098059 (ERK inhibitor) or SB203580 (p38K inhibitor), or inhibition of JNK pathway following transfection with a dominant negative of PAK, had no effect on Bcl-2 phosphorylation. By contrast, all these manipulations blocked NO-induced caspase-3 activation. Thus, the data show that NO-dependent MAPKs activation controls apoptotic events in RINm5F cells such as caspase 3 activation. NO-induced phoshorylation of Bcl-2 was also observed that is controlled by hitherto unknown kinases. Studies on the role of phosphorylation of Bcl-2 in the control of his function in apoptosis and cell cycle control are in progress.

III. DISCUSSÃO

Diabetes Mellitus Tipo 2 é freqüentemente associado à obesidade e hiperlipidemia (DeFronzo, 1997). Esses dois fatores fazem parte da chamada Síndrome Metabólica. Porém, a relação causa/efeito entre elas permanece controversa. Dois aspectos principais desta Síndrome têm sido exaustivamente estudados, ou seja: resistência à insulina provocada por um aumento de AGL (Randle, 1998; Boden *et al*,1995) e mais recentemente a lipotoxicidade sobre a célula beta (Prentki et al, 1996).

Neste trabalho, estudamos a homeostase da glicose e secreção de insulina em animais apo CIII tg, um modelo onde o defeito primário é a hipertrigliceridemia. Camundongos apo CIII tg possuem peso corpóreo, concentração de insulina e glicose sangüínea semelhantes aos dos camundongos controles. Entretanto, os níveis de triglicérides, de colesterol e de ácidos graxos livres plasmáticos são significativamente mais elevados que aqueles registrados nos animais controles. Esses parâmetros caracterizam o modelo animal proposto neste trabalho (Aalto-Setala *et al*,1992 e 1996; Ito *et al*,1990). Confirmando resultados prévios (Reaven *et al*,1994), observamos que a glicemia e insulinemia de camundongos apo CIII tg entre 4-8 meses de idade foram similares às encontradas nos animais controles. Interessante notar que, em animais transgênicos com 20-24meses de idade, os parâmetros acima citados também não diferiram dos existentes em animais controles da mesma idade. Além disso, observamos que a secreção de insulina por ilhotas isoladas de animais transgênicos e controles, expostas a concentrações basais ou estimulatórias de glicose, foi semelhante nos dois grupos. Assim, a hipertrigliceridemia com aumento de AGL plasmático, pelo menos nos níveis observados, não afeta a homeostase da glicose e a secreção de insulina em animais adultos e idosos. Resistência à

insulina tem sido induzida em seres humanos pela administração conjunta de triglicérides e heparina (Roden *et al*,1996; Piatti *et al*,1995). Os valores plasmáticos de AGL encontrados no plasma dos indivíduos nesses estudos foram bem menores do que aqueles observados nos camundongos apo CIII tg (Reaven *et al*, 1994; Roden *et al*,1999). É possível que o aumento de AGL plasmático necessário para causar resistência à insulina seja espécie - específica. Outra alternativa é que a presença de altos níveis de AGL e triglicérides nesse modelo animal é necessária mas não suficiente para alterar a ação da insulina nos tecidos alvos. Então, induzimos, através da administração de heparina, um aumento suplementar de AGL plasmático nos animais apo CIII tg e controles. Esse aumento temporário dos níveis de AGL provocou uma menor velocidade de consumo de glicose (Kitt) durante o ITT e maior área sob a curva glicêmica durante o GTT quando comparado aos controles. O tratamento dos animais com heparina também reduziu a secreção de insulina após 15min de exposição a concentrações baixas e a altas de glicose nos apo CIII tg. Após 60min, a redução na secreção de insulina nos apo CIII tg foi observada somente quando as ilhotas foram submetidas a altas concentrações de glicose. O mecanismo preciso pelo qual o aumento de AGL pode induzir redução na sensibilidade à insulina ainda não é conhecido. De acordo com o ciclo glicose-AGL, é necessário que um aumento na oxidação de AGL provoque inibição na atividade da fosfofrutoquinase, acúmulo de G-6-P, inibição da atividade da hexoquinase e, finalmente, redução da captação de glicose (Randle, 1998; Boden *et al*,1994; Randle, 1963). Uma alternativa para a explicação acima descrita foi mais recentemente elaborada. Segundo esta, o aumento das concentrações plasmáticas de AGL provoca inibição do transporte e/ou da fosforilação de glicose, redução das taxas de oxidação da glicose e síntese de glicogênio e por último acúmulo de G-6-P (Roden *et*

al,1996). Portanto, o aumento dos níveis de triglicérides e AGL nos camundongos apo CIII tg não indica, necessariamente, aumento na oxidação de AGL. Além disso, se a oxidação alta de AGL estiver presente nesse modelo animal, esta não parece ser suficiente para alterar o metabolismo celular e provocar resistência à insulina em animais apo CIII tg.

CONCLUSÃO

A hipertrigliceridemia, *per se*, mesmo com aumento da concentração de AGL plasmático não modificou a homeostasia da glicose nos camundongos transgênicos para apolipoproteína CIII humana. No entanto, incremento adicional da concentração de AGL plasmático induzido pela administração de heparina, que sabidamente aumenta a atividade da lipoproteína lipase, prejudicou a homeostasia da glicose nos animais transgênicos. Esse efeito parece relacionar-se à redução na secreção de insulina, associada a um ligeiro aumento de resistência periférica ao hormônio

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