UNIVERSIDADE FEDERAL DE PERNAMBUCO - UFPE
CENTRO DE CIÊNCIAS BIOLÓGICAS - CCB
MESTRADO EM BIOQUÍMICA E FISIOLOGIA

INFLUÊNCIA DA RESISTÊNCIA À INSULINA SOBRE ÍNDICES LIPÍDICOS E PROBABILIDADE DE EVENTO CORONARIANO

CARLOS RENATO FRANÇA DE CARVALHO MOTA

Orientadora: Profa. Dra. Vera Lúcia de Menezes Lima

Co-orientadora: Profa. Dra. Bianka Santana dos Santos

RECIFE (PE) – BRASIL
2011

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Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica e Fisiologia da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Mestre em Bioquímica e Fisiologia.

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Carlos Renato França de Carvalho Mota

"Influência da resistência à insulina sobre índices lipídicos e probabilidade de evento coronário"

Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco

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"A voz de Deus nos diz constantemente: uma falsa ciência faz um homem ateu, mas uma verdadeira ciência leva o homem a Deus."

Voltaire

A Deus, aos meus pais, às minhas orientadora e co-orientaodora, a todos que me ajudaram a concretizar este trabalho e especialmente aos voluntários que participaram generosamente desta pesquisa.

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SUMÁRIO

LISTA DE ABREVIATURAS

ADP - Adenosina Difosfato

AC – Circunferência Abdominal

ATP - Adenosina Trifosfato

ATP/ADP – Adenosina Trifosfato/Adenosina Difosfato

AHA/NHLBI – *American Heart Association/ National Heart, Lung, and Blood Institute*

BMI- Índice de Massa Corpórea

CHD – Doença Coronariana

CETP – Proteína Transferidora de Colesterol Éster

CT/TC – Colesterol Total

CT/HDL-c – Colesterol Total/HDL-colesterol

DBP – Pressão Arterial Diastólica

DCVs/CVDs – Doenças Cardiovasculares

ERF/FRS – Escore de Risco de Framingham

FPG – Glicose Plasmática de Jejum

FPI – Insulina Plasmática de Jejum

G – Glicose de Jejum

GLUT-1 – Transportador de Glicose 1

GLUT-4 – Transportador de Glicose 4

GLUT-12 – Transportador de Glicose 12

HDL – Lipoproteína de Alta Densidade

HDL-c – HDL-colesterol

HOMA-IR - Modelo Homeostático de Determinação da Resistência à Insulina

I – Insulina de Jejum

IMC – Índice de Massa Corpórea

IRS-1 – Substrato de Receptor de Insulina 1

IRS-2 – Substrato de Receptor de Insulina 2

LCAT – Lecitina:Colesterol Aciltransferase

LDL - Lipoproteína de Baixa Densidade

LDL-c – LDL-colesterol

LDL-c/HDL-c – LDL-colesterol/HDL-colesterol

LDL-sd – LDL pequena e densa

MEIA- Ensaio Imunoenzimático com Micropartículas

Não-HDL-c/non-HDL-c – Não-HDL-colesterol

Não-HDL-c/HDL-c – Não-HDL-colesterol/HDL-colesterol

NCEP-ATP III – *National Cholesterol Education Program-Adult Treatment Panel III*

OMS - Organização Mundial de Saúde

PIP3 – Fosfatidilinositol-(3,4,5)-trifosfato

PI3-cinase – Fosfoinositol-3-cinase

PKB – Proteína Cinase B

PKC – Proteína Cinase C

QUICKI – Índice Quantitativo de Checagem da Sensibilidade à Insulina

RI/IR – Resistência à Insulina

SEM – Erro Padrão da Média

SBC – Sociedade Brasileira de Cardiologia

SBP – Pressão Arterial Sistólica

TG – Triglicerídios

TG/HDL-c – Triglicerídios/HDL-colesterol

VLDL – Lipoproteína de Muito Baixa Densidade

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RESUMO

A Resistência à Insulina (RI), além de provocar alterações no metabolismo dos carboidratos, pode promover anormalidades no metabolismo lipídico, que apresentam uma forte relação com o desenvolvimento de doenças cardiovasculares (DCVs). Esses distúrbios lipídicos ainda não estão bem definidos, podendo elevar o risco de eventos coronarianos em uma determinada população. As razões triglicerídio (TG)/HDL-colesterol (HDL-c), não-HDL-colesterol (não-HDL-c)/HDL-c, Colesterol Total (CT)/HDL-c (Castelli I) e LDL-colesterol (LDL-c)/HDL-c (Castelli II) têm sido utilizadas como instrumentos para acessar o risco de surgimento de DCVs. O diagnóstico de RI é de alta complexidade para ser avaliado e, na região Nordeste do Brasil, atualmente não há levantamento epidemiológico nem indicações de sua contribuição para o risco de DCVs. Os objetivos deste trabalho consistem em pesquisar a prevalência de RI utilizando o método QUICKI (Índice para Checagem da Sensibilidade à Insulina), estabelecer o ponto de corte de QUICKI adequado à população do Nordeste do Brasil, avaliar a influência de RI sobre os índices lipídicos e sua relação com o risco de evento coronariano, acessado pelo clássico Escore de Risco de Framingham, além da relação com distúrbios da Síndrome Metabólica X. Foram coletadas amostras sanguíneas de 7128 voluntários (2124 homens e 5004 mulheres) normoglicêmicos, bem como foram aferidos seus níveis pressóricos, quantificados os parâmetros antropométricos e bioquímicos, tais como: Glicemia (G) e concentrações séricas de CT, HDL-c e TG, por metodologia enzimática; LDL-c e VLDL-colesterol (VLDL-c), através da equação de Friedewald; insulina plasmática (I), através de ensaio imunoenzimático de micropartículas (MEIA). O QUICKI foi construído para cada indivíduo e o ponto de corte adotado foi o do 25° percentil. Testes de χ^2, *t* de student, ANOVA, regressão logística, correlação de Pearson e testes z de comparação foram realizados ($p < 0,05$). Os dados foram apresentados como média ± erro padrão da média. Os pontos de corte encontrados foram 0,349 e 0,343 para homens e mulheres, respectivamente. A prevalência de RI foi 31,3% na população total, não havendo diferença significativa entre os sexos. Indivíduos que tiveram RI apresentaram níveis séricos de CT, LDL-c, VLDL-c, não-HDL-c e TG significativamente ($p<0,0001$) maiores que os indivíduos sem RI. Similarmente, foi observado aumento significativo ($p<0,0001$) nos índices de CT/HDL-c, LDL-c/HDL-c, TG/HDL-c e não-HDL-c/HDL-c. Contudo, observou-se redução significativa ($p<0,0001$) nos níveis de HDL-c nos indivíduos resistentes à insulina. Correlações negativas ($p<0,0001$) foram encontradas entre QUICKI e os índices lipídicos. A comparação entre os coeficientes de correlação demonstrou que VLDL-c (r = -0,279; $p<0,0001$), TG (r = -0,255; $p<0,0001$) e TG/HDL-c (r = -0,241; $p<0,0001$) foram os mais fortemente correlacionados com RI. Avaliação de regressão logística demonstrou uma razão de chance de 1,7 ($p<0,0001$) para alto risco de morte por evento coronariano em 10 anos em indivíduos com RI em comparação a insulino-sensíveis. Adicionalmente, foi observado que a razão de chance para a presença associada de quatro distúrbios metabólicos (hipertensão arterial sistêmica, obesidade, hipertrigliceridemia e baixos níveis de HDL-c) foi de 11,7 ($p<0,0001$). Estes resultados sugerem que um terço da população do Nordeste do Brasil apresenta RI e alto risco cardiovascular representado pela correlação de RI com anormalidades lipídicas.

Palavras-chave: Resistência à Insulina, Doença Cardiovascular, Anormalidades Lipídicas.

ABSTRACT

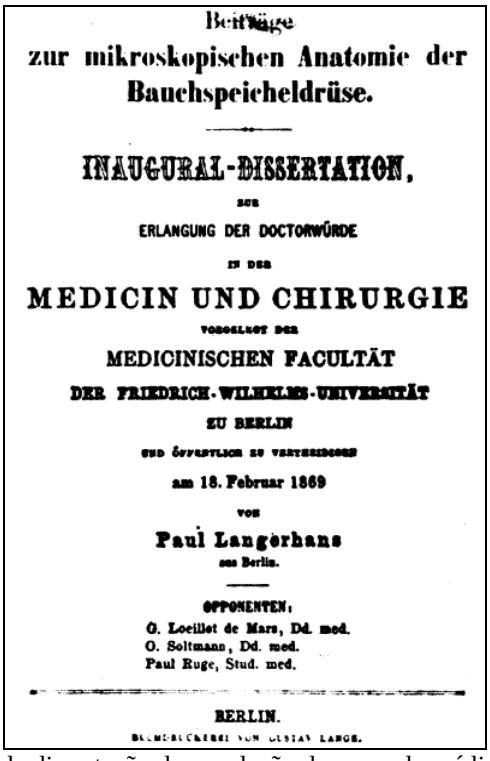
Insulin Resistance (IR) causes changes in carbohydrate metabolism, and it may promote abnormalities in lipids metabolism, which present a strong relationship with the development of cardiovascular diseases (CVDs). These lipid disorders are not well defined and may to increase the risk of coronary events in a determined population. The ratios between triglycerides (TG)/HDL-cholesterol (HDL-c), non-HDL-cholesterol (non-HDL-c)/HDL-c, Total Cholesterol (TC)/HDL-c (Castelli I) and LDL-cholesterol (LDL-c)/HDL-c (Castelli II) have been used as measurements to assess the risk for CVDs. The diagnosis of IR is very complex to be evaluated, and in Northeast region, of Brazil, nowadays, there is no epidemiological survey or something about its contribution to the risk of CVDs. The aims of these study were to investigate the prevalence of IR using QUICKI (Quantitative Insulin Sensitive Check Index) method, to establish the appropriate QUICKI's cut-off to the population of Brazilian Northeast, to evaluate the influence of IR on lipid indexes and its relationship with the risk of coronary events assessed by the classical Framingham Risk Score, besides the present study aimed to analyze the association between IR and the disturbances of Metabolic Syndrome X. Blood samples were collected from 7,128 normoglycemic volunteers (2,124 men and 5,004 women), as well as blood pressure levels were measured, and the anthropometrical and biochemical parameters were quantified, such as: glycemia(G), serum TC, HDL-c and TG, by enzymatic methods; LDL-c and VLDL-cholesterol (VLDL-c) through the Fridewald equation; plasma insulin (I) by the microparticle enzyme immunoassay (MEIA). QUICKI was assessed for each individual and the cut-off adopted was the 25^{th} percentile. Tests of χ^2, Student *t*, ANOVA, logistic regression, Pearson's correlation and z tests of comparison were performed ($p<0.05$). Data were presented as mean ± standard error of the mean. The cut-offs found were 0.349 and 0.343 for men and women, respectively. The prevalence of IR was 31.3% in total population, and there were not significant differences among the sexes. Subjects with IR presented the serum levels of TC, LDL-c, VLDL-c, non-HDL-c and TG significantly ($p<0.0001$) higher than those without IR. Similarly, it was observed a significant increase ($p<0.0001$) of the indexes of TC/HDL-c, LDL-c/HDL-c, TG/HDL-c and non-HDL-c/HDL-c. However, there was significant reduction ($p<0.0001$) in HDL-c levels in insulin resistant individuals. Negative correlations ($p<0.0001$) were found between QUICKI and lipid indexes. The comparison between the correlations coefficients showed that VLDL-c ($r = -0.279$, $p<0.0001$), TG ($r = -0.255$, $p<0.0001$), and TG/HDL-c ($r = -0.241$, $p<0.0001$) were the parameters that presented the strongest correlations with IR. Logistic regression evaluation showed an odds ratio of 1.7 ($p<0.0001$) for high risk of death from coronary event in 10 years in subjects with IR compared to insulin sensitive individuals. In addition, it was observed that the odds ratio for the associated presence of four metabolic disturbances (systemic arterial hypertension, obesity, hypertriglyceridemia and low HDL-c levels) was 11.7 ($p<0.0001$). These results suggest that a third of the Northeast population of Brazil presents IR and high cardiovascular risk represented by the correlation of IR and lipid abnormalities.

Keywords: Insulin Resistance, Cardiovascular Disease, Lipid Abnormalities.

I. INTRODUÇÃO

1. Histórico

O metabolismo da insulina é um dos principais temas abordados em diversas discussões científicas. Os primeiros estudos relacionados à regulação do metabolismo de carboidratos pela insulina datam do século XIX. Em 1869, a dissertação de conclusão do curso de medicina do alemão Paul Langerhans abordou os aspectos histológicos do pâncreas e descreveu a existência de células agrupadas que apresentavam características diferentes das encontradas no restante do pâncreas (**FIGURA 1**). Porém, Langerhans não conseguiu identificar nenhuma atividade específica ao grupo celular descoberto (LANGERHANS, 1869).


Beiträge
zur mikroskopischen Anatomie der Bauchspeicheldrüse.

INAUGURAL-DISSERTATION,

ERLANGUNG DER DOCTORWÜRDE

MEDICIN UND CHIRURGIE

MEDICINISCHEN FACULTÄT

DER FRIEDRICH-WILHELMS-UNIVERSITÄT

ZU BERLIN

am 18. Februar 1869

Paul Langerhans

OPPONENTEN:

G. Locillet de Mars, Dd. med.
O. Soltmann, Dd. med.
Paul Ruge, Stud. med.

BERLIN.

FIGURA 1 – Capa da dissertação de conclusão de curso do médico Paul Langerhans.
Fonte: SAKULA, 1988

Estudos visando compreender a função do pâncreas no metabolismo corpóreo foram desenvolvidos posteriormente e, em 1889, Joseph von Mering e Otto Minkowski observaram que quando uma pancreatectomia total era realizada, em um modelo experimental com cães, estes passavam a apresentar glicosúria, aumento da glicemia, polifagia, polidipsia, poliúria e emagrecimento, o que levou esses pesquisadores a concluírem que o pâncreas exercia algum papel ainda desconhecido relacionado com o metabolismo dos glicídios (VON MERING et al, 1890). Em 1893, o histologista francês Edouard Laguesse sugeriu que as ilhotas pancreáticas produziam uma secreção endócrina, denominando este agrupado de células com o nome do seu descobridor, Ilhotas de Langerhans (LAGUESSE, 1893), e, paralelamente às pesquisas supracitadas, a busca por algum composto que apresentasse ação hipoglicemiante a fim de tratar pacientes hiperglicêmicos era incessante. Verificou-se que extrato de pâncreas administrado via subcutânea apresentava ação hipoglicêmica de curta duração, porém, efeitos colaterais como necrose do local de aplicação do extrato inviabilizavam a utilização deste tratamento (BLUMENTHAL, 1893; RENNIE, 1907).

No início da segunda década do século XX, pesquisadores canadenses da Universidade de Toronto, sob a orientação de Fredrick G. Banting, otimizaram os protocolos de elaboração do extrato pancreático e os resultados agora obtidos tornaram-se bastante promissores com reversão da hiperglicemia e da glicosúria nos animais pancreatectomizados. Em seguida, iniciou-se o aprimoramento das técnicas de extração, isolamento e purificação do princípio ativo e, desse modo, foi descoberta a insulina (**FIGURA 2**) e devido aos avanços nos estudos sobre insulina, foi possível realizar pela primeira vez o tratamento com sucesso de um paciente com diabetes mellitus (BANTING et al, 1922).

FIGURA 2 – Recipiente contendo as primeiras unidades de insulina isoladas na Universidade de Toronto, Canadá.
Fonte: http://bioinsulina.blogspot.com/2009/11/embora-leonard-thompson-tenha-sido.html

A deficiência de insulina foi considerada a causa do diabetes mellitus. Entretanto, este conceito começou a ser modificado a partir de 1939, com a publicação dos resultados das pesquisas desenvolvidas por Harold Himsworth e seus colaboradores. Estes sugeriram que diabetes não estava apenas associado à redução ou à falta dos níveis de insulina, mas também à diminuição da sensibilidade a este hormônio. Diabetes mellitus foi então classificado em dois tipos: tipo 1 ou insulino-sensível; e tipo 2 ou insulino-insensível. Foi observado que os indivíduos diabéticos insulino-sensíveis eram mais jovens, magros, normotensos e apresentavam artérias saudáveis, enquanto que diabéticos insulino-insensíveis eram mais velhos, obesos, hipertensos e apresentavam arteriosclerose. Yalow e Berson em 1960 descreveram um método de quantificação da insulina plasmática e chegaram a conclusão de que tecidos provenientes de indivíduos mais velhos com diabetes mellitus não respondiam à insulina da mesma forma que tecidos de indivíduos normais e que, inclusive, os níveis de insulina encontrados eram maiores do que a média encontrada no grupo normal (YALOW & BERSON, 1960). Em 1988, Gerald Reaven estudando o

papel da resistência à insulina em anormalidades metabólicas prevalentes em humanos, observou que esta poderia apresentar associação causal não apenas com intolerância à glicose, mas também com obesidade, aumento de ácidos graxos livres na circulação e com hipertensão arterial, denominando este conjunto de anormalidades metabólicas de Síndrome X, o que posteriormente recebeu as terminologias de Síndrome de Resistência à Insulina ou Síndrome Metabólica X (REAVEN, 1988; REAVEN, 2005). Assim, estudos sobre a síntese e secreção da insulina, bem como sobre seus mecanismos de ação, além dos mecanismos de resistência a este hormônio, passaram a se tornar essenciais e começaram a ser realizados, haja vista Reaven ter proposto que um quarto da população mesmo enquanto normoglicêmica e não obesa pode apresentar sensibilidade reduzida à insulina, ou seja, pode apresentar um quadro de resistência à insulina (RI). É necessário então realizar estudos também sobre a prevalência de RI e sobre a sua associação com outras anormalidades metabólicas. Reaven ainda afirma que, com a população mundial se tornando cada vez menos ativa e mais obesa, é óbvio afirmar que os problemas associados com o quadro síndrômico de Resistência à Insulina são a praga do século XXI (REAVEN, 1988; REAVEN, 2005).

2. Síntese, Secreção e Ações da Insulina

As células β pancreáticas das ilhotas de Langerhans são as responsáveis pela produção da insulina. Inicialmente, este hormônio é sintetizado como uma proteína precursora, de cadeia única e de maior tamanho, denominada de preproinsulina, que rapidamente é convertida em proinsulina, no retículo endoplasmático rugoso, e armazenada no aparelho de Golgi. Posteriormente, as moléculas de proinsulina seguem para os grânulos secretores, nos quais são clivadas por enzimas proteolíticas, originando moléculas

de peptídeo C e insulina em quantidades equimolares (STEINER & OYEN, 1967; Kunt et al, 1999). A insulina, por fim, é um hormônio peptídeo biologicamente ativo, composto por duas cadeias, A e B, que se encontram ligadas por pontes dissulfídicas, com peso molecular de 5734 Da (**FIGURA 3**) (RYLE et al, 1955).

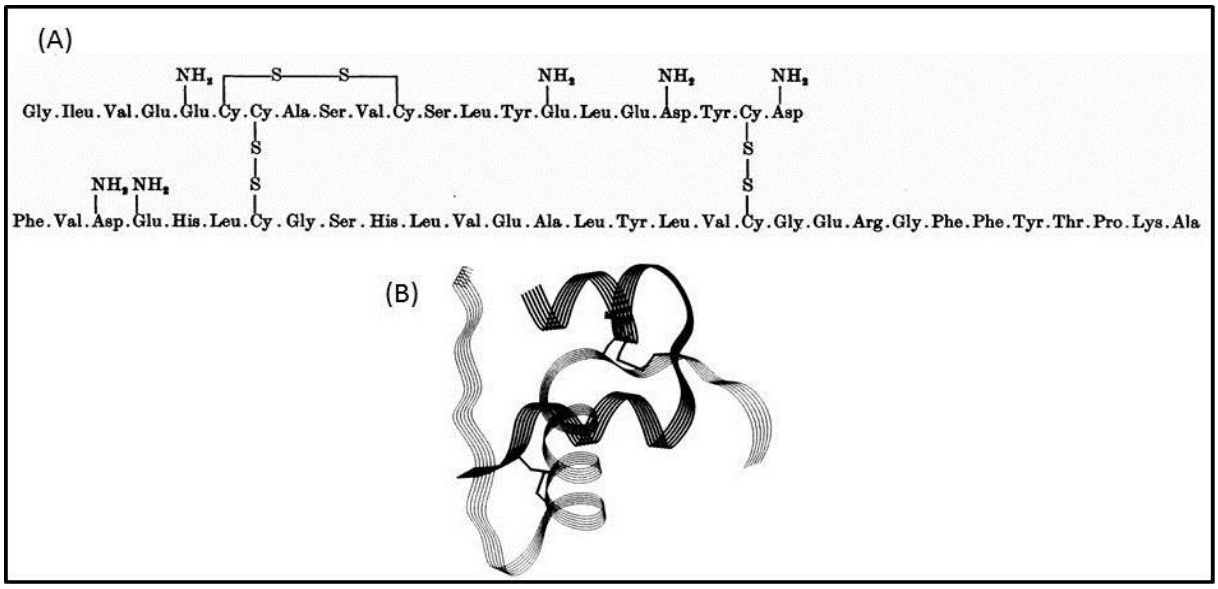


FIGURA 3 – A estrutura da Insulina. Estrutura primária (A) e quaternária (B).
Fonte: RYLE et al, 1955 (A); CONLON, 2001 (B)

O principal estímulo para a secreção de insulina é a presença de glicose no sangue, porém outras moléculas como neurotransmissores e hormônios também podem estimular ou inibir este mecanismo (TENGHOLMS & GYLFE, 2009). Quando moléculas de glicose são transportadas para o interior da célula β pancreática, ocorre fosforilação das mesmas pela ação catalítica da enzima glicocinase, levando à formação de glicose-6-fosfato. Esta, nas células β, destina-se preferencialmente à via glicolítica, para a produção de Adenosina Trifosfato (ATP) (MATSCHINSKY & COLLINS, 1997). O aumento intracelular de ATP e consequente elevação dos valores da razão ATP/ADP promovem o fechamento de canais de potássio-K^+ sensíveis à ATP, com retenção de K^+ no interior das células β e despolarização da membrana (RORSMAN & RENSTRÖM, 2003). Isto ocasiona a

abertura de canais de Cálcio-Ca^{++} voltagem-dependentes, com influxo de Ca^{++} e consequente aumento de sua concentração no citosol da célula β, o que, somado a outros sinais intracelulares, promovem a liberação da insulina, facilitando seu processo de exocitose (**FIGURA 4**) (LANG, 1999; HENQUIN, 2000).

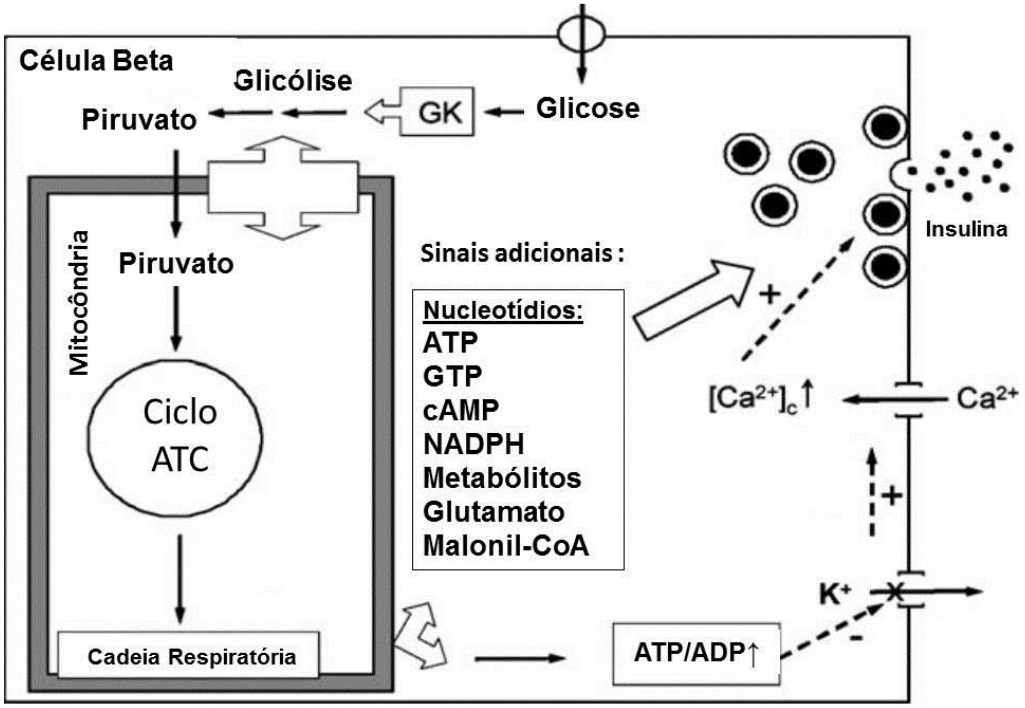


FIGURA 4 – Ação da glicose na secreção de insulina pelas células β pancreáticas.
Fonte: Maechler; Carobbio; Rubi, 2006.

A insulina exerce um papel central na regulação da homeostase da glicemia e atua de maneira coordenada em eventos de crescimento celular e regulação de rotas metabólicas, sendo capaz de ativar, em vários tecidos, o anabolismo protéico, lipídico e de carboidratos. Na grande maioria dos tecidos, é necessário que a insulina se ligue a seu receptor. Esta molécula receptora consiste em um heterodímero composto por duas subunidades α e duas subunidades β. A ligação da insulina à subunidade α promove alterações conformacionais e autofosforilação de resíduos de tirosina da subunidade β. O receptor de insulina fosforilado fica ativo e desencadeia o processo de fosforilação dos

Substratos de Receptor de Insulina 1 e 2 (IRS-1 e IRS-2), que, por sua vez, promovem a ativação da fosfoinositol-3-cinase de classe I (PI3-cinase), obtendo como produto de sua ação catalítica a molécula de fosfatidilinositol-(3,4,5)-trifosfato (PIP3) (**FIGURA 5**). Este serve como sítio de ligação e de ativação de proteínas como, por exemplo, a proteína cinase B (PKB), que sofre uma relocalização da membrana plasmática para o citosol e para o núcleo, onde interfere no mecanismo de transcrição de vários genes-alvo (LANGEVELD & AERTS, 2009).

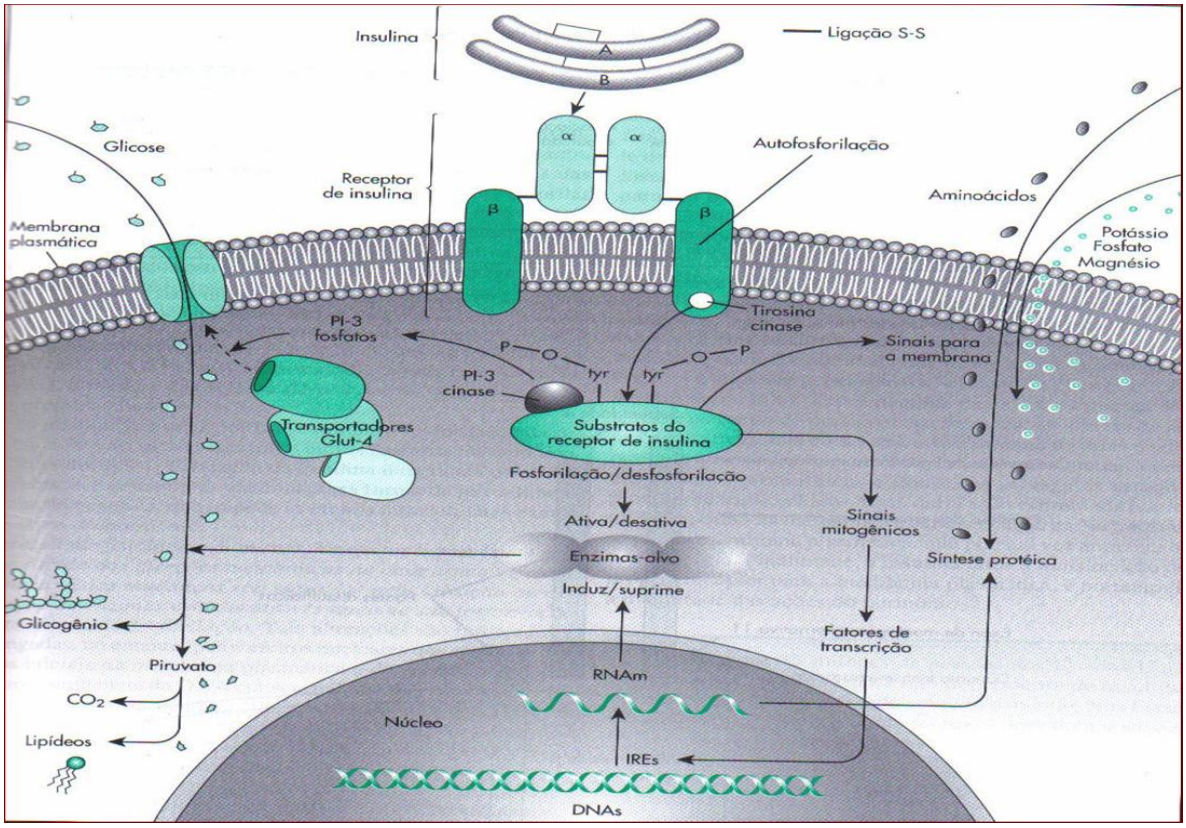


FIGURA 5 – Via de sinalização do Receptor de Insulina.
Fonte: Berne & Levy, 2004.

PKB em conjunto com proteína cinase C (PKC), ativada por PI3-cinase, promovem a translocação do transportador de glicose 4 (GLUT-4) para a membrana plasmática, permitindo captação imediata de glicose (NEDACHI & KANZAKI, 2006). GLUT-4 faz parte de uma família de proteínas transportadoras, na qual já foram identificados 12

membros (GLUT-1 a GLUT-12). GLUT-4 é um transportador sensível à insulina encontrado no coração, músculo esquelético, tecido adiposo e cérebro, e é o responsável pela redução da glicemia pós-prandial (WOOD & TRAYHURN, 2003). Adicionalmente, a ligação da insulina ao seu receptor induz uma outra via de sinalização relacionada com o fator de crescimento. Assim, alterações que prejudiquem a interação insulina-receptor podem gerar inúmeras anormalidades metabólicas (LANGEVELD & AERTS, 2009).

3. Resistência à Insulina e Anormalidades Metabólicas Associadas - O Papel dos Lipídios

RI consiste na redução da capacidade que a insulina tem de facilitar a captação da glicose para o interior das células. Os mecanismos moleculares envolvidos na fisiopatologia da RI ainda não foram completamente esclarecidos. Vários fatores são apontados como possíveis responsáveis pelo desenvolvimento de RI, incluindo mutações de receptores de insulina, antagonistas da insulina, produção anormal de insulina e alterações na cascata de sinalização do receptor da insulina, sendo este último um dos mais comuns (BURGERING et al, 1995; MESHKANI & ADELI, 2009).

Quando há o estabelecimento da RI, tecidos, tais como o hepático, o muscular e o adiposo, diminuem sua resposta à insulina. Este quadro de resistência pode evoluir ao longo do tempo para hiperglicemia franca, contribuindo para o estabelecimento de diabetes mellitus do tipo 2, bem como para o aparecimento de diversas outras anormalidades metabólicas, tais como hipertensão, obesidade e dislipidemias (REAVEN, 1995; REAVEN, 2002; REAVEN, 2005).

A hiperglicemia estimula as células neuronais, já que as mesmas não necessitam da ligação à insulina para captarem a glicose e, dessa forma, os neurônios associam a

hiperglicemia com uma diminuição da produção de insulina pelas células β pancreáticas. Então, de maneira compensatória, há um estímulo à produção e liberação da insulina, o que pode causar um quadro de hiperinsulinemia (MLINAR et al., 2007; SAVAGE; PETERSEN; SHULMAN, 2007). As alterações metabólicas que surgem no indivíduo com RI parecem estar ligadas à hiperinsulinemia compensatória necessária para prevenir a perda da tolerância à glicose (REAVEN, 1995). Quantidades elevadas de insulina no sangue podem estimular o sistema nervoso simpático causando vasoconstricção, retenção de sódio-Na^+ e água nos túbulos distais renais, aumento do débito cardíaco e do volume sanguíneo, contribuindo para o aparecimento de distúrbios hemodinâmicos, como a hipertensão arterial (HALL et al, 1994; ECKEL; GRUNDY; ZIMMET, 2005; POTENZA et al, 2005).

O tecido adiposo, principalmente o visceral, quando resistente à insulina, não responde ao efeito antilipolítico deste hormônio e aumenta a hidrólise dos triglicerídios armazenados, sob a ação catalítica de diversos tipos de lipases, com consequente liberação de quantidades excessivas de ácidos graxos livres na circulação, o que pode vir a desencadear uma série de distúrbios lipídicos. Estudos têm reportado, inclusive, que o excesso de lipídios no sangue apresentam um papel-chave na etiologia das anormalidades metabólicas induzidas pela RI (MLINAR et al, 2007; CHAPMAN & SPOSITO, 2008; LANGEVELD & AERTS, 2009). Maiores quantidades de ácidos graxos livres, devido à RI, podem provocar vasoconstricção por meio da ativação de α_1-adrenoceptores, atenuação da produção endotelial de óxido nítrico-NO com indução de estresse oxidativo, o que dá suporte ao papel direto de que os ácidos graxos livres podem apresentar na elevação da pressão arterial (SARAFIDIS & BAKRIS, 2007; CHAPMAN & SPOSITO, 2008).

Tem sido sugerido que a elevação na concentração de ácidos graxos circulantes nos indivíduos com RI provoca uma dislipidemia característica – hipertrigliceridemia e

diminuição de HDL-colesterol. A hipertrigliceridemia pode ser explicada pelo aumento do aporte de ácidos graxos para o fígado e ressíntese dos triglicerídios, o que leva a aumentar a produção das lipoproteínas de muito baixa densidade (VLDL). Os triglicerídios e VLDL são, então, liberados em excesso para a corrente sanguínea. Além da alteração quantitativa, a VLDL sofre uma alteração qualitativa na constituição de seus componentes, pois a mesma passa a apresentar maior quantidade de triglicerídios, passando a ser denominadas de VLDL ricas em triglicerídios (DEFRONZO et al., 1991; RIEMENS et al., 1999; NCEP-ATPIII, 2001; GAZI et al., 2006; GRUNDY, 2006).

Estudos têm sugerido que as modificações quantitativa e qualitativa da VLDL são o principal fator contributivo para as alterações na composição química e estrutural das lipoproteínas de baixa densidade (LDL) (KONTUSH & CHAPMAN, 2006; CHAPMAN & SPOSITO, 2008). A LDL que é formada a partir de VLDL rica em triglicerídios tem seu conteúdo de colesterol depletado e, assim como a VLDL que lhe deu origem, apresenta também alta quantidade de triglicerídios. Estes ao serem hidrolisados pela lipase lipoprotéica geram partículas de LDL pequenas e densas (GINSBERG, ZHANG; HERNANDEZ-ONO, 2005). A proteína transferidora de colesterol éster (CETP) também pode auxiliar na formação de LDL pequenas e densas, pois medeia a transferência de colesterol éster da lipoproteína de alta densidade (HDL) para as VLDL e LDL ricas em triglicerídios. A HDL, por sua vez, recebe, em troca do colesterol éster fornecido, conteúdo triglicerídico e como este provém de lipoproteínas que o apresentam em excesso, é transferido em maior quantidade também para a HDL. Isto torna a HDL uma lipoproteína instável, com perda de seu conteúdo protéico e consequente aumento de sua degradação, o que pode levar a redução de seus níveis (KONTUSH & CHAPMAN, 2006; CHAPMAN & SPOSITO, 2008).

Entretanto, como o grau de resistência à insulina pode variar de indivíduo a indivíduo, divergências podem ser encontradas quanto à predominância e à relevância de cada tipo de dislipidemia (REAVEN, 1995; REAVEN, 2005; BONORA et al, 1998; HOENIG & SELLKE, 2010). Muito se tem afirmado que a ocorrência de hipercolesterolemia é um distúrbio isolado e que não está acompanhado por RI, como se espera (BONORA et al, 1998). Uma dessas divergências é exatamente quanto aos níveis plasmáticos de colesterol total. Entretanto, estudos têm identificado uma associação entre síntese elevada de colesterol e RI, (PIHLAJAMÄKI et al, 2004; HOENIG & SELLKE, 2010). Estudos também têm reportado a necessidade de se investigar o chamado não-HDL-colesterol (não-HDL-c), que corresponde à concentração de colesterol total subtraída da quantidade de colesterol presente na HDL, e pouco se sabe ainda sobre sua relação com distúrbios metabólicos como a RI (GRUNDY, 2001; AL-DAGHRI; AL-ATTAS; AL-RUBEAAN, 2007)

As anormalidades lipídicas decorrentes da RI estão intimamente associadas ao desenvolvimento de DCVs, uma vez que um dos papéis mais reconhecidos das dislipidemias é a formação de um quadro favorável ao desenvolvimento de aterosclerose. A VLDL muito rica em triglicerídios, LDL pequena e densa e HDL enriquecida com triglicerídios são fatores predisponentes para a formação da aterosclerose (Gazi et al., 2006). Essas lipoproteínas modificadas podem ser fagocitadas por macrófagos, de maneira acentuada, de forma semelhante ao processo de fagocitose de substâncias não próprias ao organismo, o que desencadeia a conversão dos macrófagos normais em células espumosas, que recebem esta denominação por apresentarem muitos vacúolos de lipídios em seu interior. Estas células são consideradas as principais responsáveis pela ruptura endotelial, na placa aterosclerótica, devido à liberação de citocinas pró-inflamatórias, formação do trombo, o que aumenta a obstrução do vaso sanguíneo, fato este que é ocasionado

(BORGGREVE; VRIES; DULLAART, 2003). Modificações não apenas qualitativas na LDL, mas também o aumento das concentrações de LDL-c são considerados fatores de riso independente para DCVs, contudo a relação entre maiores níveis de LDL-c e RI ainda não foi estabelecida (REAVEN, 2002; MARUYAMA; IMAMURA; TERAMOTOL, 2003; BARRIOS et al, 2009). A diminuição dos níveis sanguíneos de HDL afeta o transporte reverso do colesterol, reduzindo-o e, com isso, aumentando a probabilidade do aparecimento do ateroma (BORGGREVE; VRIES; DULLAART, 2003; LIMA et al., 2004). Estudos têm sugerido que as concentrações de não-HDL-c apresentam uma melhor associação com DCVs que os marcadores tradicionais, e se tem indicações de que não-HDL-c elevado pode apresentar consequências desfavoráveis em indivíduos não diabéticos, podendo ser o principal preditor de DCVs nestes indivíduos, enquanto que em indivíduos diabéticos o colesterol total continuaria sendo o mais correlacionado com doenças cardíacas (CUI et al, 2001; AL-DAGHRI; AL-ATTAS; AL-RUBEAAN, 2007).

A relação observada entre as alterações no metabolismo lipídico e lipoprotéico e a geração de risco para DCVs, tem sido bastante estudada e índices já bem estabelecidos de risco cardiovascular foram criados, tomando como base fundamental, os distúrbios lipídicos e lipoprotéicos. Castelli sugeriu, em 1983, dois índices de risco para DCVs: um é o valor resultante da proporção entre os níveis plasmáticos de colesterol total e os de HDL-colesterol (CT/HDL-c), denominado de Índice de Castelli I; e o outro é o valor obtido da proporção LDL-colesterol e HDL-colesterol (LDL-c/HDL-c), denominado de Índice de Castelli II. Ambos os índices, quando elevados, são indicativos de DCVs e sugerem que não apenas o conteúdo plasmático individual, ou seja, os níveis plasmáticos de cada lipídio e de cada lipoproteína, de forma isolada, pode contribuir para um quadro cardiovascular, mas também relações existentes entre os níveis dessas moléculas (CASTELLI et al., 1983) (**TABELA 1**).

TABELA 1 – Risco Cardiovascular Estratificado pelos Índices de Castelli, para Homens e Mulheres.

ÍNDICES	RISCO	HOMENS	MULHERES
CT/HDL-c (Castelli I)	**Média**	**4,97**	**4,44**
	2 x Média	**9,55**	**7,05**
	3 x Média	**23,39**	**11,04**
LDL-c/HDL-c (Castelli II)	**Média**	**3,55**	**3,22**
	2 x Média	**6,25**	**5,03**
	3 x Média	**7,99**	**6,14**

Fonte: Bianka Santana dos SANTOS, 2009

Recentemente, novos índices de risco cardiovascular foram criados, tomando por base os distúrbios do metabolismo lipídico e lipoprotéico, tais como, a razão TG/HDL-c, que também é utilizado para indicar a presença de HDL pequena e densa, assim como avaliar RI. Dentre os índices utilizados para estimar o risco de doença cardiovascular, o mais novo é composto pela razão entre o conteúdo de colesterol não pertencente à HDL e HDL-c (não-HDL-c/HDL-c), apresentando uma forte relação com o desenvolvimento de DCVs (SHISHEBOR; HOOGWERF; LAUER, 2004; MCLAUGHLIN et al., 2005; HERMANS; AHN; ROUSSEAU, 2007; HADAEGH et al, 2009).

Colesterol alto e níveis diminuídos de HDL-c, em conjunto com hipertensão, diabetes mellitus, uma faixa etária mais avançada e o tabagismo são todos considerados fatores predisponentes de eventos coronarianos e são utilizados como base para a determinação do Escore de Risco de Frahmingham (ERF) (**TABELA 2**), que estima a probabilidade de ocorrer infarto do miocárdio ou morte por doenças coronarianas no período de 10 anos em indivíduos sem diagnóstico prévio. Por meio do ERF, é possível classificar o risco cardiovascular em: baixo, quando a probabilidade de infarto ou morte por doença coronariana em 10 anos for menor que 10%; intermediário, quando esta probabilidade se encontrar entre 10% e 20%; e alto, quando esta for maior que 20%

(DAWBER; MEADORS; MOORE, 1951; D'AGOSTINO et al, 2001; SBC, 2007; KLEIN, 2002; BITTON et al, 2010).

TABELA 2 – Escore de Risco de Framingham (ERF) para Cálculo do Risco Absoluto de Infarto e Morte em 10 Anos.

HOMENS		MULHERES	
Idade	Pontos	Idade	Pontos
20-34	-9	20-34	-7
35-39	-4	35-39	-3
40-44	0	40-44	0
45-49	3	45-49	3
50-54	6	50-54	6
55-59	8	55-59	8
60-64	10	60-64	10
65-69	11	65-69	12
70-74	12	70-74	14
75-79	13	75-79	16

Colesterol Total, mg/dL	idade 20-39	idade 40-49	idade 50-59	idade 60-69	idade 70-79	Colesterol Total, mg/dL	idade 20-39	idade 40-49	idade 50-59	idade 60-69	idade 70-79
< 160	0	0	0	0	0	< 160	0	0	0	0	0
160-199	4	3	2	1	0	160-199	4	3	2	1	1
200-239	7	5	3	1	0	200-239	8	6	4	2	1
240-279	9	6	4	2	1	240-279	11	8	5	3	2
≥280	11	8	5	3	1	≥280	13	10	7	4	2

Fumo	idade 20-39	idade 40-49	idade 50-59	idade 60-69	idade 70-79	Fumo	idade 20-39	idade 40-49	idade 50-59	idade 60-69	idade 70-79
Não	0	0	0	0	0	Não	0	0	0	0	0
Sim	8	5	3	1	1	Sim	9	7	4	2	1

HDL-colesterol (mg/dL)	Pontos	HDL-colesterol (mg/dL)	Pontos
≥ 60	-1	≥ 60	-1
50-59	0	50-59	0
40-49	1	40-49	1
< 40	2	< 40	2

PA (sistólica, mm Hg)	não tratada	tratada	PA (sistólica, mm Hg)	não tratada	tratada
< 120	0	0	< 120	0	0
120-129	0	1	120-129	1	3
130-139	1	2	130-139	2	4
140-159	1	2	140-159	3	5
≥ 160	2	3	≥ 160	4	6

Fonte: IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose do Departamento de Aterosclerose**. Sociedade Brasileira de Cardiologia. 2007.**

A Organização Mundial de Saúde (OMS) estima que um número cada vez maior de indivíduos morrem continuamente com complicações resultantes da desregulação da homeostase de seu metabolismo lipídico (OMS, 2009). Assim, as concentrações lipídicas no sangue apresentam um intrínseco papel na gênese de outros distúrbios metabólicos, diabetes mellitus, alterações hemodinâmicas e DCVs, em conjunto com a RI, podendo subsidiar o fator causal da RI sobre essas anormalidades (SAVAGE; PETERSEN; SHULMAN, 2007; CHAVEZ & SUMMERS, 2010).

No Brasil, existem raríssimos estudos populacionais sobre RI e sua prevalência ainda não está bem estabelecida neste país, principalmente na Região Nordeste. Isto é preocupante, devido ao maior número de mortes por doenças do aparelho circulatório nesta região verificado nos últimos anos (**FIGURA 6**) (SABRY; SAPAIO; SILVA, 2002; ISHITANI et al, 2006; Ministério da Saúde, 2008).

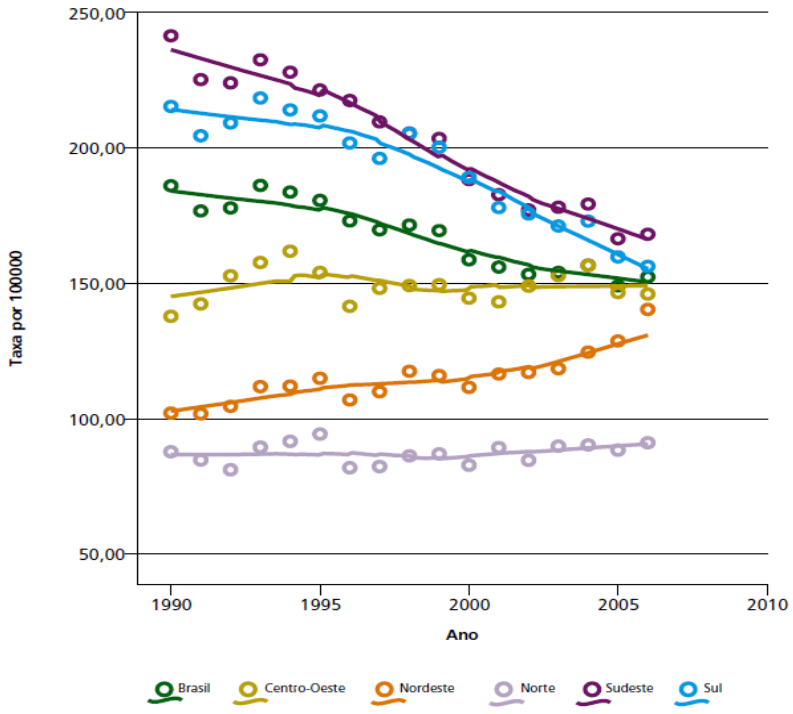


FIGURA 6 – Mortalidade por doenças do aparelho circulatório no Brasil e regiões entre 1990 e 2006
Fonte: Ministério da Saúde, 2009.

Outro dado epidemiológico importante da região Nordeste é referente ao aumento da taxa de mortalidade que tem o diabetes mellitus como fator causal direto. Entre 1990 e 2006, foi registrado um aumento, em todas as regiões do país, do número de causa *mortis* por diabetes. Porém, após o ano 2000, houve uma estabilização dessa taxa em todas as regiões, exceto no Nordeste, onde o crescimento foi contínuo ao longo de todos os anos (**FIGURA 7**) (Ministério da Saúde do Brasil, 2009).

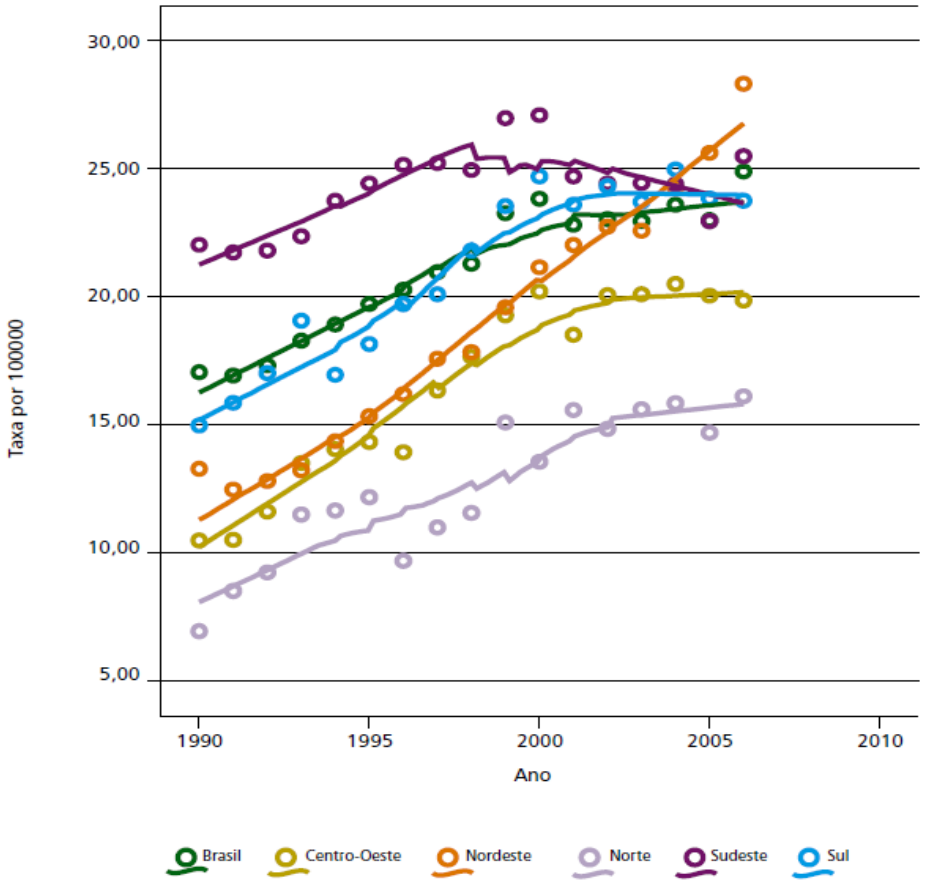


FIGURA 7 – Taxas ajustadas de mortalidade por Diabetes Mellitus para população adulta de 20 a 74 anos, Brasil e regiões, 1990 a 2006

Fonte: Ministério da Saúde, 2009.

Percebe-se a necessidade de estudos que realizem uma investigação sobre os mecanismos fisiopatológicos que unem RI, anormalidades lipídicas, lipoprotéicas e eventos coronarianos, fornecendo subsídios para a geração e aplicação de novas medidas

terapêuticas. Além disso, é de extrema relevância, para a saúde pública, dados que demonstrem a incidência de RI na população, haja vista que esta anormalidade é raramente determinada pela maioria dos estudos, sendo inédita ainda esta avaliação com um caráter de estudo populacional, na Região Nordeste. Outro dado bastante relevante é a investigação da probabilidade de eventos coronarianos na população, o que pode servir como base para a geração de políticas que possam melhorar o prognóstico desses indivíduos, melhorando a qualidade de vida de um modo geral e reduzindo, dessa forma, o número de óbitos nessa região do país. Entretanto, é de complexo método o diagnóstico considerado padrão-ouro de RI e estudos populacionais sobre este distúrbio tornam-se de difícil execução, havendo-se a necessidade do emprego de outros métodos, também consolidados, mas de execução adequada a grandes estudos epidemiológicos (MATTHEWS et al, 1985; KATZ et al, 2000).

4. Métodos de Diagnóstico da Resistência à Insulina

Numerosos métodos destinados à avaliação da sensibilidade tecidual à insulina foram desenvolvidos, no entanto, não existe um método de investigação laboratorial que preencha todos os parâmetros que compõem uma metodologia ideal para avaliação da sensibilidade à insulina. Dentre os critérios necessários, é possível citar: uma medida suficientemente precisa para comparar a RI entre indivíduos, possibilidade de aplicação clínica, baixo custo e valores obtidos com razoável esforço, em um tempo limitado e com um risco mínimo para o paciente. O *clamp* euglicêmico hiperinsulinêmico, adotado como padrão-ouro no diagnóstico da RI, fornece a mais pura e reprodutível informação sobre a ação tecidual da insulina, permitindo avaliar as contribuições individuais do fígado e tecidos periféricos no metabolismo da glicose induzido pela insulina. Entretanto, por se

tratar de uma técnica de complexa operacionalização, tornou-se um método de difícil aplicação tanto na rotina clínica quanto em levantamentos epidemiológicos (DEFRONZO et al, 1979; GELONEZE & TAMBASCIA, 2006).

Metodologias de estimativa indireta dos efeitos fisiológicos da insulina foram desenvolvidas em alternativa ao *clamp* euglicêmico hiperinsulinêmico. A técnica do modelo mínimo apresentada por Bergman e colaboradores mostrou-se um pouco mais viável que a técnica do *clamp*, porém é ainda dispendiosa, pois tem duração de aproximadamente quatro horas e para sua realização são necessárias 21 amostras sanguíneas (BERGMAN, 1989).

Modelos matemáticos preditores da sensibilidade à insulina surgiram como opção aos métodos sofisticados que requerem muito tempo tanto do paciente quanto do médico, além de possuírem altos custos para execução. O modelo homeostático de determinação da RI (HOMA-IR; glicemia de jejum [em mmol/L] × insulinimia de jejum [em µU/mL] / 22,5) é uma destas técnicas utilizadas com sucesso no diagnóstico da RI desde 1985 (MATTHEWS et al, 1985; LEONETTI et al, 2004). Entretanto, esta técnica estima que a sensibilidade à insulina é igual para o todo o corpo, de forma que a RI seria a mesma tanto no fígado quanto nos tecidos periféricos. Outro modelo, desenvolvido mais recentemente, que também se baseia na homeostasia e utiliza os níveis de glicose (G) e insulina de jejum (I) em sua avaliação é o índice quantitativo de checagem da sensibilidade à insulina – QUICKI, que utiliza a fórmula $1 / [\log(I) + \log(G)]$. Neste método, os valores de insulina e glicose sofrem uma transformação logarítmica para que a grande variabilidade dos valores de insulina seja normalizada. Estudos comparativos entre metodologias utilizadas para avaliar a ação da insulina mostraram que o QUICKI possui a maior reprodutibilidade em comparação à técnica padrão-ouro do *clamp*, pois apresenta o menor coeficiente de variação, fazendo com que o QUICKI seja uma preciosa ferramenta no diagnóstico da

resistência à insulina (KATZ et al, 2000; GELONEZE & TAMBASCIA, 2006; ANTUNA-PUENTE et al, 2008).

Portanto, estudos que avaliem a resistência à insulina através do QUICKI podem vir a contribuir de forma significativa para uma real estimativa de RI e risco de doenças cardiovasculares e outras anormalidades metabólicas relacionadas.

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II. JUSTIFICATIVA

As doenças cardiovasculares são responsáveis por aproximadamente 1/3 da mortalidade mundial. No Nordeste do Brasil, houve um drástico aumento no número de casos notificados de causa *mortis* por DCVs. Esta região brasileira ainda não apresenta estudos epidemiológicos significativos quanto à prevalência de RI nem sobre sua associação com distúrbios lipídicos e outras anormalidades metabólicas. O Nordeste também ainda carece de dados sobre a predição de eventos cardiovasculares em sua população e desde que RI em associação com dislipidemias pode ser um distúrbio causal de DCVs e de outras anormalidades, é válido que estudos com abordagem epidemiológica sobre RI e alterações bioquímicas decorrentes sejam realizados. Além disso, a avaliação de RI através de QUICKI com pontos de corte apropriados para a população do estudo pode refletir de forma mais específica seu real risco cardiovascular. Assim, o presente estudo teve este propósito e espera contribuir para a melhoria da qualidade de vida de uma população, haja vista a liberação de dados científicos que possam vir a auxiliar no diagnóstico da RI e elaboração de políticas públicas de intervenção, que possam resultar em redução do número de causa *mortis* por esses distúrbios e na melhora do prognóstico dos indivíduos com a redução do aparecimento de co-morbidades.

III. OBJETIVOS

3.1 Objetivo Geral

- Investigar a influência de Resistência à Insulina sobre os índices lipídicos de risco cardiovascular e a probabilidade de evento coronariano em indivíduos normoglicêmicos do Nordeste do Brasil.

3.2 Objetivos Específicos

- Identificar um valor de referência adequado para a população do Nordeste do Brasil para o diagnóstico de Resistência à Insulina;
- Investigar a prevalência de Resistência à Insulina diagnosticada pelo método QUICKI em homens e mulheres do Nordeste brasileiro, com os pontos de corte obtidos no presente estudo;
- Acessar a influência de resistência à insulina sobre distúrbios lipídicos, avaliando suas possíveis correlações com níveis lipídicos;
- Investigar a correlação entre resistência à insulina e índices lipídicos de risco cardiovascular em indivíduos normoglicêmicos, do Nordeste do Brasil;
- Analisar a influência de resistência à insulina sobre novos fatores lipídicos relacionados a risco cardiovascular;

- Avaliar a relação existente entre resistência à insulina e alto risco de infarto agudo do miocárdio ou morte por doença coronariana em 10 anos, bem como entre resistência à insulina e a presença simultânea em um mesmo indivíduo, de três ou quatro distúrbios da Síndrome Metabólica X.

IV. RESULTADOS E DISCUSSÃO

Influence of Insulin Resistance on Lipid Indexes for Cardiovascular Risk

O artigo será submetido ao periódico
Lipids in Health and Disease

Influence of Insulin Resistance on Lipid Indexes for Cardiovascular Risk: A Population-Based Survey

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Abstract

Background: The diagnosis of IR is very complex to be evaluated, and in Northeast region, of Brazil, nowadays, there is no epidemiological survey or something about its contribution to the risk of CVDs. The aims of these study were to investigate the prevalence of IR using QUICKI (Quantitative Insulin Sensitive Check Index) method, to establish the appropriate QUICKI's cut-off to the population of Brazilian Northeast, to evaluate the influence of IR on lipid indexes and its relationship with the risk of coronary events assessed by the classical Framingham Risk Score, besides the present study aimed to analyze the association between IR and the disturbances of Metabolic Syndrome X. **Results:** The prevalence of IR was 31.3% in total population, and there were not significant differences among the sexes. Subjects with IR presented the serum levels of TC, LDL-c, VLDL-c, non-HDL-c and TG significantly (p<0.0001) higher than those without IR. Similarly, it was observed a significant increase (p<0.0001) of the indexes of TC/HDL-c, LDL-c/HDL-c, TG/HDL-c and non-HDL-c/HDL-c. However, there was significant reduction (p<0.0001) in HDL-c levels in insulin resistant individuals. Negative correlations (p<0.0001) were found between QUICKI and lipid indexes. The comparison between the correlations coefficients showed that VLDL-c (r = -0.279, p<0.0001), TG (r = -0.255, p<0.0001), and TG/HDL-c (r = -0.241, p<0.0001) were the parameters that presented the strongest correlations with IR. Logistic regression evaluation showed an odds ratio of 1.7 (p<0.0001) for high risk of death from coronary event in 10 years in subjects with IR compared to insulin sensitive individuals. In addition, it was observed that the odds ratio for the associated presence of four metabolic disturbances (systemic arterial hypertension, obesity, hypertriglyceridemia and low HDL-c levels) was 11.7 (p<0.0001). **Conclusions:** These results suggest that a third of the Northeast population of Brazil presents IR and high cardiovascular risk represented by the correlation of IR and lipid abnormalities.

Introduction

Cardiovascular diseases (CVD) are the most common causes of death in the world. Global cardiovascular deaths were projected to increase from 17.1 million in 2004 to 23.4 million in 2030.[1] It has been reported an intrinsic association among CVD and lipid blood levels. Subjects with CVD are pointed as owner of an abnormal lipid profile, such as elevated levels of total cholesterol (TC), LDL-cholesterol and high levels of triglycerides (TG)[2,3]. Blood concentrations of HDL-cholesterol (HDL-c) are also established as an inverse predictor of CVD, and several components of HDL may to contribute to the antioxidant effect of this lipoprotein, including the enzyme lecithin:cholesterol acyltransferase (LCAT), that plays an important role in the process of normal intravascular lipoprotein metabolism.[4,5] However, not only the plasma levels of each lipid and each lipoprotein are related to cardiovascular risk, but an association among the type of lipids also be considered. Then, lipid indexes have been created and used to assess the risk of development of CVD. Traditional lipid indexes, such as Castelli I (TC/HDL-c), Castelli II (LDL-c/HDL-c), are used since 1983, as predictors of CVD.[6] In the last decade, new lipid indexes were reported as risk factors for CVD, sush as TG/HDL-c, and non-HDL-c/HDL-c. This first new candidate marker of CVD has also been interrelated to Insulin Resistance (IR). This disturbance of the homeostasis of the biochemistry metabolism might to present influence on the development of CVD and to contribute for a worse prognosis in individual with IR than in subjects with normal insulin sensitivity.[7,8,9,10] The relationship between IR and CVD might to be on basis of the plasma lipid changes, that may to occur in insulin resistants, the called atherogenic dyslipidemia: high levels of TG, lower concentrations of HDL-c, and presence of LDL small and dense (LDL-sd) particles.[11] However, it is not well established the association of IR with non-HDL-c/HDL-c ratio or even with only HDL-c

blood concentrations.[10] It is also conflicting the relationship between IR, and the traditional risk factors for CVD – Castelli I, and Castelli II, since TC and LDL-c levels, a despite of these lipids presented a relationship with CVD, have not been well established an association to IR. [12,13]. Another way used to evaluate CVD is the Framingham Risk Score, that estimates the probability of myocardial infarction or coronary heart disease (CHD) death in 10 years in individuals without previous diagnosis of clinical atherosclerosis, separately by sex, using age, smoking, levels of TC, HDL-c and systolic blood pressure,[14] and the influence of IR in this score has not been studied. But in spite of increasing knowledge about "traditional" risk factors, the pandemic associated with CVD not only continues unabated, and it continues also seen to be growing with tsunamic speeds.[15] Of this way, it is necessary to investigate the prevalence of IR, and its consequences on lipid and lipoprotein metabolism, that might to subscribe the pathophysiology of the CVD and to, be the greater contributors for this pandemia. Gerald Reaven, in 1988, already had suggested that IR would may to be distributed worldwide in approximately 25% of the people, [16] however scarce studies had investigated the prevalence of insulin resistance, and this investigation become necessary in regions from developing countries that nowadays are considered the greatest centers of new cases of CVD and of premature deaths for these causes.[17] In Brazil, 29.4% of the deaths registered in 2006 were caused by CVDs, and the Northeast of Brazil was the major region that contributes for this increase.[18] However, IR is not commonly assessed on clinical practice and its measurements in epidemiological survey studies by the gold standard test, the euglycemic hyperinsulinemic clamp, is infeasible.[19] A number of surrogate indexes have been derived from fasting plasma insulin and glucose levels to evaluate insulin resistance, such as QUICKI. This method for assess IR is a novel, accurate and reproducible tool for to determine insulin sensitivity in humans with lower coefficient of variation, when compared to the others methods in relation to the

clamp techniques.[20] So, the present study was a cross-sectional analytical and a survey research that aimed to provide information about IR prevalence in normoglycemic subject from a region with a growing number of deaths of CVD, with the use cut-offs for QUICKI values found in this study, and to investigate the influence of IR on lipid and lipoprotein metabolism, as well as to investigate the possible correlation among IR and classical and new lipid indexes of cardiovascular risk. It was also a proposal of this study to evaluate the relationship of IR and high risk of myocardial infarction or CHD death in 10 years, and to evaluate the association among the prevalence of IR and the prevalence of the disturbances of Metabolic Syndrome X.

Methodology

Study Population

A representative random sample of 7,128 volunteers, aging 20 to 79 years old, from a Westernized admixtured multi-ethnic population of Brazilian Northeast participated of this study. This number was greater than 7,128, however, pregnant women or individuals with known malignancy or individuals with endocrine, hepatic or renal diseases or individuals under therapies that present some influence over lipid and glucose metabolism were excluded of this cross-sectional analytical survey. Only normoglycemic subjects composed this sample (plasma glucose < 5,55 mmol/L). All participants were informed of the purposes and procedures of the study and provided a written consent term before their participation. This study was approved by a Ethical Committee, and this study followed up the recommendations of Declaration of Helsinki to study with humans.

Physical Examination and Anthropometric measures

Body Mass Index (BMI) and Abdominal Circumference (AC) were evaluated. Body weight was measured with a calibrated balance scale to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm. The subjects were wearing light clothes and no shoes. AC was measured by using a nonelastic tape with the individual standing, and it was determined in the midaxillary line midway between the lowest rib margin and the iliac crest.[21]

Blood Pressure Measurements

Three blood pressure measurements were determined from each participant, with the use of a sphygmomanometer (Littmann, USA), after at least twenty minutes at resting, under highly standardized conditions. Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were then assessed as the average of the second and third measurements.[22]

Blood Punctions and Laboratory Analysis

The blood punctions were made, in the morning, after fasting of 12 hours. Subjects were in a sitting position and all blood samples were drawn in appropriate tubes, by a vacuum system of blood punction (Beckton Dickinson and Company, USA) from the antecubital vein, and tourniquet was released before blood was drawn. Two tubes were used. One tube contained 1.8% dry potassium ethylenodyaminotetracetic acid in association with 3.0% sodium fluoride, to obtaining of the plasma, and the other tube had not any anticoagulant, which it was used to get the serum. The tubes were kept on ice and they were carried to Laboratory of Chemistry and Metabolism of Lipids and Lipoproteins of Federal University of Pernambuco, in a few hours. Blood samples were centrifuged at 2.500 *g* for 15 minutes at 4^{o}C (Sorvall RC6, NC, EUA). Plasma and serum were separated

into plastic cryotubes (500 µL in each) and stored at -80°C until the measurements, which occur in the same week of the collect. An exception from this, it was the determination of serum HDL-c levels, which were measured by the phosphotungstic acid/magnesium chloride precipitation method (MERCK, GE), in the same day of the blood punction. Plasma concentration of glucose, and serum concentrations of TC, and TG were assessed by enzymatic methods, according to recommendations of the manufacturers (MERCK, GE). LDL-c and VLDL-c levels were assessed by Friedewald equation.[23] While, a new marker for cardiovascular disease, the non-HDL-c values were calculated as levels of total cholesterol minus HDL-c.[24] Plasma insulin concentrations were determined by MEIA – Microparticles Enzyme Immunoassay (Abbott Laboratories, GE). This insulin assay shows a little cross-reactivity with human pro-insulin of only 0.016%.[25]

Insulin resistance

Insulin Resistance was defined by QUICKI (Quantitative Insulin Sensitive Check Index) determined by 1/(log insulinemia + log glycemia),[20] its values were lower than the cut-offs obtained to the 25th percentiles, for men and in women of the population of this study.

Cardiovascular Risk Indexes

Lipid indexes to evaluate cardiovascular risk were determined. The ratios suggested by Castelli, in 1983, the Castelli Index I (CT/HDL-c) and Castelli Index II (LDL-c/HDL-c), were assessed in this study. The new indixes of cardiovascular risk determined were the ratios between TG and HDL-c levels, and between non-HDL –c and HDL-c concentrations.[7,10,11,16] The traditional Framingham Risk Score (FRS), that estimates the

probability of myocardial infarction or coronary heart disease death in 10 years in individuals without previous diagnosis of clinical atherosclerosis, was also determined.[14]

Metabolic Disturbances

The AC, hypertriglyceridemia, low levels of HDL-c, and hypertension were the metabolic disturbances of Metabolic Syndrome X that were defined inthis study. All these metabolic disturbances followed up the criteria recommended by AHA/NHLBI, 2005. Abdominal obesity was identified when the men presented AC values ≥ 102 cm and women had AC ≥ 88 cm. Hypertriglyceridemia was diagnosed when TG levels ≥ 1.7 mmol/L or drug treatment for this dyslipidemia was reported when questioned. Reduced levels of HDL-c were presented when HDL-c < 1.03 mmol/L in men and < 1.30 mmol/L in women or when drug treatment for reduced HDL-c was registered). Hypertension was defined in subjects with Systolic Blood Pressure ≥ 130 mmHg or Diastolic Blood Pressure ≥ 85 mmHg or antihypertensive drug treatment in a patient with a history of hypertension.[27]

Statistical Analysis

The representative number of the individuals of obtained trough the estimated prevalence of 50%, with alpha error 5.0%, since the prevalence of IR was not known in the population of this study. The number of the volunteers still continued significant after the prevalences of IR, in men and women were identified. The cut-offs, for men and for women, of QUICKI were the values of the 25th percentiles. Men and women with QUICKI values under of their specifical 25th percentiles were considered insulin resistants, and the men and the women that had QUICKI above these percentiles were considered as individuals with normal sensitivity to insulin. Unpaired *t* test, ANOVA and tests of Pearson's correlation were used to the analysis of the continuous variables, while χ^2 test

was used to the investigation of statistical differences among categorical variables, i.e. for the comparisons among the prevalences of IR. The statistical differences between insulin sensitive and resistant individuals were obtained after adjustment for age, AC, BMI, SBP, and DBP values. Continues variables were expressed as the mean ± standard error of the mean (SEM), and the categorical variables were expressed in percent. A confidence interval of 95% (95% CI) or a level of significance lower than 0.05 ($p < 0.05$) was considered as a significant statistical difference. *z* test was used to compare the power of the correlations among QUICKI values and lipid levels, and among QUICKI and the traditional and the new lipid indexes of cardiovascular risk. Logistic regression analysis were applied to identify the odds ratio (OR) of IR by QUICKI for high risk of myocardial infarction or coronary death in 10 years, as well as to found the OR of IR for the presence of at least three metabolic disturbances and for the presence of four disorders of Metabolic Syndrome X. The statistical analysis were performed using computer softwares (Epi Info 3.5.1, 2008, StatView 5.0.1, 1998 and MedCalc 11.3, 2010).

Results

Baseline Chacteristics of Population

Overall data from 7,128 normoglicemic subjects were assessed. Of these, 2,124 (29.8%) were men, and 5,004 (70.2%) were women. Their baseline characteristics are summarized in Table 1. Men presented higher values of QUICKI, and higher levels of FPG, VLDL-cholesterol and triglycerides than women. Besides, the majority of the clinical parameters were higher in men than in women, with the exception of BMI. Conversely,

levels of FPI, HDL-cholesterol, total cholesterol, and LDL-cholesterol were lower in men. No differences were found regarding age, among the sexes.

QUICKI's Cut-off, and Prevalences of Insulin Resistance

Five percentiles of QUICKI values in both sexes are shown in Table 2. The cut-off (under of 25th percentile) was found, and it was equal to 0.345 in overall population, corresponding to 0.349 and 0.343, in men and women, respectively.

Assuming the cut-off mentioned above, the overall prevalence of insulin resistance assessed by QUICKI was 31.3% (95% CI: 30.0 – 32.6), as demonstrated in Figure 1. Men and women did not differ significantly each other regarding to the presence of insulin resistance (χ^2 = 0.521; p = 0.4705). Men presented a prevalence rate of 31.9% (95% CI: 29.6 – 34.4); and, in women, the prevalence of insulin resistance was 31.0% (95% CI: 29.5 – 32.6).

Influence of Insulin Resistance, by QUICKI, on Serum Lipid Concentrations, and on Lipid Indexes used to Assessment of Risk for CVDs

In overall, normoglycemic individuals that presented values of QUICKI under 25th percentile (men ≤ 0.349, and women ≤ 0.343), even after adjustment for age, abdominal circumference, BMI, SBP and DBP values, when compared to subjects with values of QUICKI up to these cut-offs, had significant by increased serum concentrations of all the lipids: TC (5.19 ± 0.03 *vs.* 4.96 ± 0.02 mmol/L; p<0.0001); LDL-c (3.38 ± 0.03 *vs.* 3.29 ± 0.02 mmol/L; p=0.0011); VLDL-c (0.75 ± 0.01 *vs.* 0.56 ± 0.01 mmol/L; p<0.0001); non-HDL-c (4.15 ± 0.03 *vs.* 3.86 ± 0.02 mmol/L; p<0.0001); and TG (1.76 ± 0.03 *vs.* 1.27 ± 0.01 mmol/L; p<0.0001), as reported in Figure 2. Insulin resistant also showed

significantly (p<0.0001) lower serum concentrations of HDL-c (1.05 ± 0.01) than non-insulin resistant individuals (1.10 ± 0.00).

Figure 3 shows that the lipid indexes used to assessment of the risk for the establishment of CVDs were significantly (p<0.0001) higher in insulin resistants versus the lipid indexes found in the individuals with normal insulin sensitivity (CT/HDL-c: 5.30 ± 0.05 *vs.* 4.76 ± 0.02; LDL-c/HDL-c: 3.46 ± 0.04 *vs.* 3.17 ± 0.02; non-HDL-c/HDL-c: 4.29 ± 0.05 *vs.* 3.76 ± 0.02; and TG/HDL-c: 1.86 ± 0.04 *vs.* 1.29 ± 0.02).

It was also observed, as demonstrated in Table 3, that QUICKI values correlated positively and significantly (p<0.0001) with HDL-c levels (r=0.149), while with TC (r=-0.104), LDL-c (r=-0.067), VLDL-c (r=-0.279), non-HDL-c (r=-0.142), TG (r=-0.255), TC/HDL-c (r=-0.186), LDL-c/HDL-c (r=-0.134), non-HDL-c/HDL-c (r=-0.186), and with TG/HDL-c (r=-0.241), QUICKI values correlated significantly of negative way. The serum lipid concentrations the strongest negative correlation with QUICKI, in this population, were, of similar way, VLDL-c and TG levels (Data not shown. p<0.0001, when comparing the others lipid concentrations). In second place, QUICKI values correlated stronger with non-HDL-c than TC or LDL-c levels (Data not shown. p<0.03). LDL-c levels presented the lowest correlation with levels of QUICKI (p<0.05). Regarding to the indexes to assessment risk of CVD, the TG/HDL-c ratio was the main index that presented correlation with QUICKI values (p<0.0001). The new non-HDL-c/HDL-c ratio divided with the renowned Castelli I index – TC/HDL-c ratio the second place of correlation with QUICKI (p<0.002); while Castelli II index – LDL-c/HDL-c ratio had the lowest correlation with QUICKI (p<0.002).

Influence of Insulin Resistance, by QUICKI, on High Risk of Myocardial Infarction and Coronary Death in 10 Years, and on The Cluster of Disturbances of The Metabolic Syndrome X

In individuals aging 20 to 79 years, insulin resistance, by QUICKI, presented an odds ratio equal to 1.7 (95% CI: 1.3 – 2.3; $p<0.0001$) for high risk (probability greater than 20%) of myocardial infarction or coronary death in 10 years defined by Framingham Cardiac Risk Score, as showed in Table 4.

It was found an odds ratio, after adjustment for age, of 4.9 (3.7 – 6.5; $p<0.0001$) of the presence of insulin resistance for the association of at least three disorders in the same subject, and an odds ratio of 11.7 (8.7 – 15.9; $p<0.0001$) of the insulin resistance for the presence of four other disorders (hypertension, hypertriglyceridemia, lower HDL-c levels, and abdominal obesity), as described in Table 4.

Discussion

In a normoglycemic population from Northeast of Brazil, a region with a great number of mortality by CVD, we have assessed the prevalence of IR and its influence on lipid abnormalities and lipid indexes for cardiovascular risk. IR was assessed through QUICKI method. The QUICKI's cut-off that was found for this total population was 0.345, being 0.349 for men and 0.343 for women. These cut-offs are on the average reported by others studies. Lee and cols.[28] (2006) have reported a 25th percentile value of QUICKI equal to 0.320 in South Korean, and Ascaso and cols.[29] (2003) found a cut-off of 0.330 in Spain. While Hrebicek and cols.[30] (2002), as well as Corrêa and cols.[31] (2007), identified a cut-off of 0.357, respectively, in the population from Czech Republic and from the Southeast region of Brazil. All these cut-offs were determined in normoglycemic

individuals, and the cut-off obtained in this survey study might represents the limit value of QUICKI in the population from Northeast of this developing country, that has inherited the high-fatty and high-carbohydrate diets from western culture, according to Ascherio and cols.[17] (1996). It is interesting to mention that the Northeast from Brazil is also one of the two latest regions of this developing country that is crossing an epidemiological transition, with increase of cases of chronic diseases, as reported by Batista Filho and Rissin[32] (2003). 31.3% of the normoglycemic subjects presented IR, being 31.9% in men and 31.0% in women. These data are alarming since this prevalence is greater than the prevalence of 25% suggested by Reaven[16] (1988) or higher than the prevalence found by Do and cols.[33] (2010) of 25.1% for Thai men and 21.5% for Thai women over 35 years, and many closer of the prevalence reported by Ioannou and cols.[34] (2007) also among normoglycemic individuals from USA, which was equal to 32.2%.

IR presented a significant influence on lipid metabolism, verified by the increase on serum concentrations of all lipids, with the exception of serum HDL-c concentrations, which were reduced, as well as by the negative and relevant correlations among QUICKI values and TG, VLDL-c, TC, and Non-HDL-c, and by positive and significant correlation of QUICKI and HDL-c values. The strongest correlations among IR and lipids were those between IR and VLDL-c levels, as well as, of similar way, between IR and TG levels. Its known insulin plays an inhibitory effect on VLDL secretion, and it stimulates the hepatic synthesis of HDL. So the reduced sensitivity to insulin stimulation might to promote an overproduction of VLDL, and lower production of HDL, according to Chapman and Spósito[35] (2008) and Bonora and cols.[36] (2008). However, the influence of insulin on TC and LDL-c is not well established. It has been suggested that the occurrence of hypercholesterolemia is an isolated disorder not accompanied by IR as expected and reported by Bonora and cols.[37] (1998). In this study, we had TC levels, and mainly LDL-c

concentrations, slightly correlated to IR, in comparison to the others lipids. Nevertheless, these correlations were significants, and recent studies, such as reported by Hoenig and Sellke[38] (2010) and Pihlajamäki and cols.[39] (2004), begin to demonstrate that IR is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic individuals.

Regardless of LDL-c concentrations have not been associated with a state of IR, the quantity of LDL-c is considered a strong predictor of CVD in diabetics, as identified by Howard and cols.[40] (2000), in The Strong Heart Study. So, even the pathophysiological mechanisms of LDL-c levels and IR have not been found, it is suggested that qualitative changes in the composition of LDL particles are interrelated as with IR as with CVD. Maruyama, Imamura and Teramoto[41] (2003) proposed that a novel lipid index, the TG/HDL-c ratio, is associated with the presence of LDL small, dense (LDLsd) particles, also called LDL subclass pattern B, which has a diameter lower than 25.5 nm. These researchers suggested that values of this ratio equal or lower than 0.9 implicate in the presence of LDLsd. Then, TG/HDL-c ratio is related a three-fold increase in the risk of myocardial infarction. In our results, we had an increase of the values of TG/HDL-c ratio in individuals with IR, and, of the lipid indexes studied, this ratio presented the greatest power of correlation with IR. In latest place, although of significant way, Castelli I – TC/HDL-c and mainly Castelli II – LDL-c/HDL-c were the lipid Indexes that presented the slightest correlations with IR, corroborating with our results obtained for TC and LDL-c concentrations. Nevertheless, several studies, such as the reported by Kinosian and Garland[42] (1994), Rader and cols.[43] (2003), and Ballantyne and Hoogeveen[44] (2003) have shown that these lipid ratios are stronger predictors of risk than TC or LDL-c alone. Walldius and Jungner[45] (2006) identified that increase of the apoB-lipoproteins and decrease of apoA-lipoprotein levels, especially when they are associated in a ratio, in

additive with the disorders that IR may to cause in a glucose metabolism, might to influence on high cardiovascular risk.

The other new lipid index was the ratio between the values of non-HDL-c and HDL-c. The non-HDL-c, regardless to have been highlighted as a key secondary goal of therapy ten years ago by the National Cholesterol Education Program Adult Treatment Panel III [46](2001), only recently its advantages among other lipid parameters are being emphasized. We found that non-HDL-c concentration and the values of non-HDL-c/HDL-c ratio increased in subjects with IR and were correlated to IR with stronger than TC or LDL-c and their respective indexes, corroborating to Blaha and cols.[13] (2008), and Hermans, Ahn and Rousseau[10] (2007).

We assessed a significant odds ratio of 1.6 for the influence of IR on the prevalence of high risk of myocardial infarction and death in 10 years. Hedblad and cols.[47] (2001) in a population-based prospective cohort study in 4,748 non-diabetic subjects, aged 46 to 68 years, in Sweden, found a risk relative of 2.2 for the influence of IR on the incidence of coronary events, and a risk relative of 1.6 for deaths for CVD. So, our results corroborate with the data obtained by these authors, showing that our estimative derived of this population survey in Northeast of Brazil can go to reflect a proximity with the reality that already occurred in other countries and in other places.

Reaven[16] (1988) suggested that IR was associated with Metabolic Syndrome X, which is composed by hyperglycemia, hypertriglyceridemia, lower levels of HDL-c, and abdominal obesity. We have assessed the odds ratio of IR for the presence, in a cluster, of at least three and four disturbances of this syndrome, with exception of hyperglycemia, since the individuals of this study were normoglycemics. The simultaneous presence of at least three metabolic disturbances was 4.9 times more prevalent in individuals with IR, and the presence of a cluster of the four abnormalities of the Metabolic Syndrome X, without

hyperglycemia, was 11.7 times greater in insulin resistants than in insulin sensitive subjects. These results are according to Undurti[48] (2007), and Meshkani and Adeli[49] (2009) that include IR in a set of pathologies that composes the Metabolic Syndrome X, and respect the pathophysiology proposed by Reaven[50] (1995), and Reaven[51] (2005) when called IR of syndrome and not of a simple disturbance – the Insulin Resistance Syndrome.

Our results suggest that predicting insulin sensitivity in normoglycemic individuals is very important, and QUICKI values obtained showed that one third of the population from a region of Brazil in epidemiological transition, the Northeast of Brazil, may to present itself in high risk of development of metabolic and cardiovascular diseases, represented by the correlations found with the lipid blood levels and with traditional and novel lipid indexes for assessment cardiovascular risk, besides of the relationship between IR and the Frahmingham Cardiovascular Risk Score. However, more studies are necessary to a better understanding about these relations, and this study has a pretension of becomes a prospective research.

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Table 1. Characteristics of Population, stratified by gender.

Data Analyzed	**Total**	**Men**	**Women**	***p****
N	7,128	2,124 (29.8%)	5,004 (70.2%)	
Age (years)	45.5 ± 0.20	45.5 ± 0.37	45.5 ± 0.23	0.8594
FPG (mmol/L)	4.54 ± 0.01	4.58 ± 0.01	4,53 ±0.01	<0.0001
FPI (µU/mL)	8.00 ± 0.10	7.50 ± 0.22	8.28 ± 0.11	0.0004
QUICKI	0.375 ± 0.00	0.385 ± 0.00	0.371 ± 0.00	<0.0001
Abdominal Circumference (cm)	91.2 ± 0.15	92.0 ± 0,25	90.9 ± 0.19	0.0012
BMI (Kg/m^2)	26.2 ± 0.06	25.4 ± 0.09	26.5 ± 0.08	<0.0001
Total Cholesterol (mmol/L)	5.01 ± 0.01	4.89 ± 0.03	5.06 ± 0.02	<0.0001
HDL-cholesterol (mmol/L)	1.10 ± 0.00	0.99 ± 0.01	1.14 ± 0.00	<0.0001
LDL- cholesterol (mmol/L)	3.29 ± 0.01	3.19 ± 0.02	3.33 ± 0.02	<0.0001
VLDL-cholesterol (mmol/L)	0.60 ± 0.00	0.67 ± 0.01	0.58 ± 0.00	<0.0001
Triglycerides (mmol/L)	1.40 ± 0.01	1.62 ± 0.03	1.30 ± 0.01	<0.0001
SBP (mmHg)	126.3 ± 0.29	127.6 ± 0.50	125.8 ± 0.35	0.0034
DBP (mmHg)	82.1 ± 0.16	83.3 ± 0.29	81.5 ± 0.19	<0.0001

Data are unadjusted means ± SEM or n (%). Statistical analysis by Student "*t*" test, *p* (significance level) < 0.05.

* *p* used to compare the differences among the values obtained in men and women.

Table 2. Percentiles of QUICKI in men and women from Northeast Region of Brazil

	Percentiles of QUICKI values				
	10^{th}	25^{th}	50^{th}	75^{th}	90^{th}
Total	0.323	0.345	0.369	0.398	0.433
Men	0.323	0.349	0.374	0.413	0.455
Women	0.322	0.343	0.366	0.393	0.426

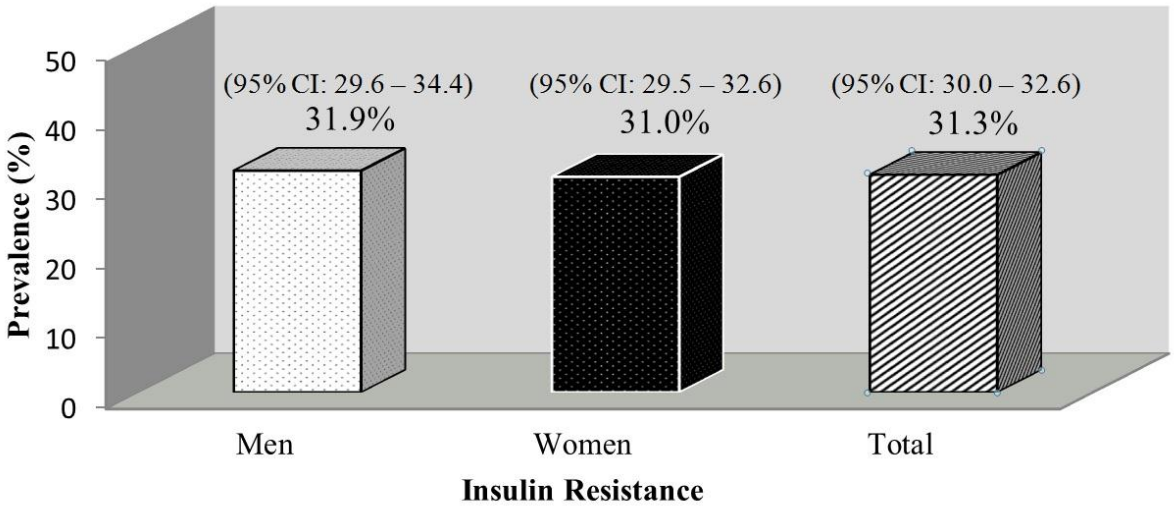

Figure 1. Prevalence of Insulin Resistance assessed by QUICKI in total population studied, in men and women.

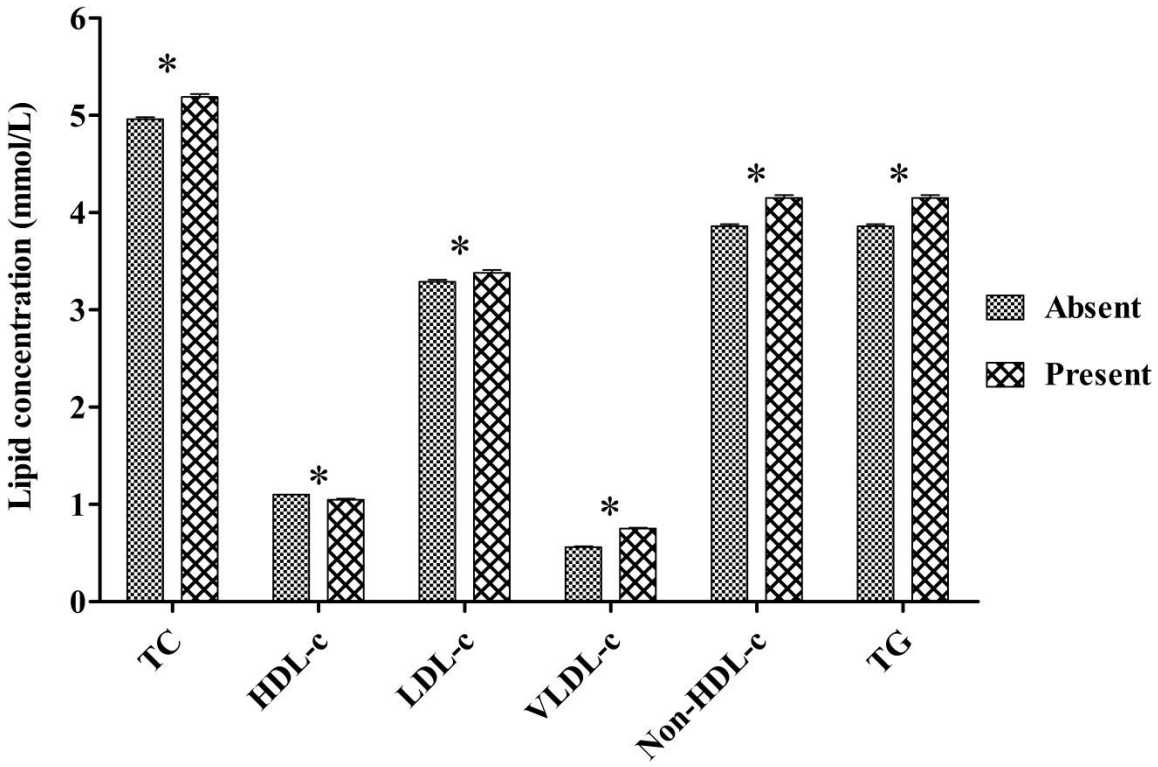

Figure 2. Insulin Resistance and serum Lipid concentration

* p <0.0001

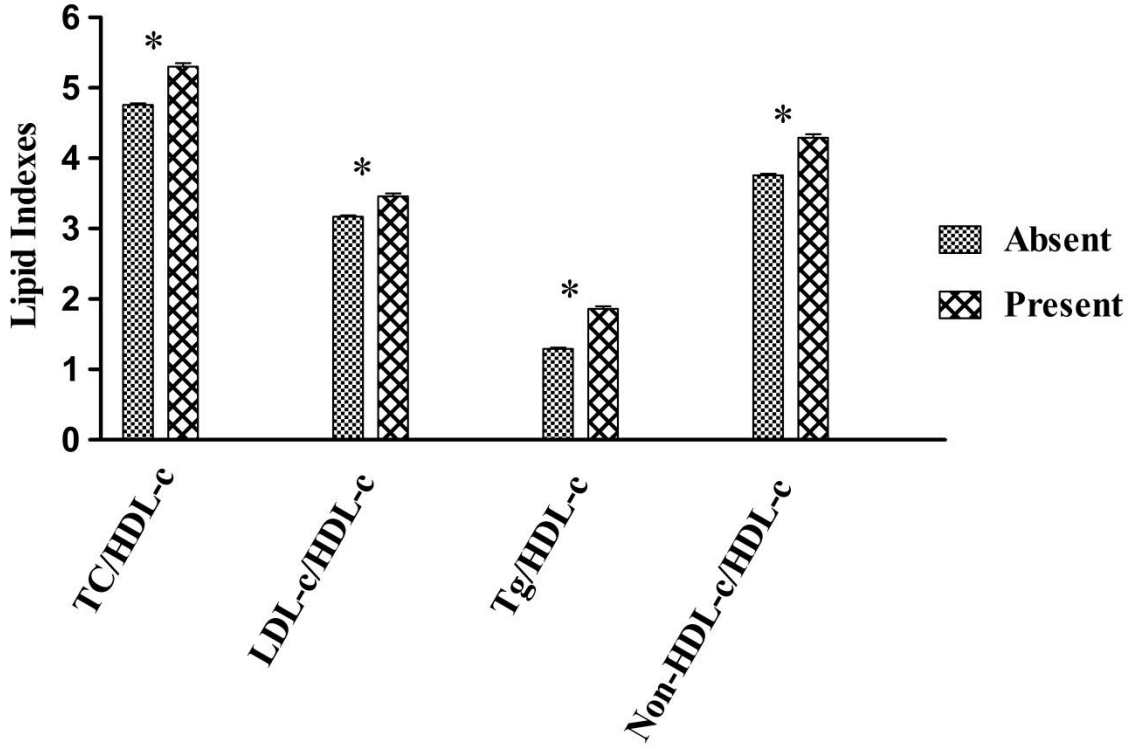

Figure 3. Insulin Resistance and lipid indexes of risk for the establishment of CVDs

* p<0.0001

Table 3. Correlation of Insulin Resistance (QUICKI) and lipid levels and indexes of CVDs.

	Correlation of Pearson		
	r	CI 95%	p*
TC	-0.104	-0.130 to -0.079	<0.0001
HDL-c	0.149	0.124 to 0.174	<0.0001
LDL-c	-0.067	-0.092 to -0.041	<0.0001
VLDL-c	-0.279	-0.303 to -0.256	<0.0001
Non-HDL-c	-0.142	-0.167 to -0.117	<0.0001
TG	-0.255	-0.279 to -0.231	<0.0001
TC/HDL-c	-0.186	-0.211 to -0.161	<0.0001
LDL-c/HDL-c	-0.134	-0.159 to -0.109	<0.0001
TG/HDL-c	-0.241	-0.265 to -0.217	<0.0001
Non-HDL-c/HDL-c	-0.186	-0.211 to -0.161	<0.0001

Statistical analysis by Pearson's Correlation

p: significance level

Table 4. Influence of Insulin Resistance on High Risk of Cardiovascular Disease and on The Cluster of Metabolic Syndrome X.

	Logistic Regression		
	Odds Ratio	95% CI	*p*
FCRS*	1.7	1.3 – 2.3	<0.0001
Three Disorders †	4.9	3.7 – 6.5	<0.0001
Four Disorders †	11.7	8.7 – 15.9	<0.0001

* Framingham Cardiac Risk Score (risk of myocardial infarction or coronary death in 10 years)

† Disorders of Metabolic Syndrome X: Hypertension, Hypertriglyceridemia, Lower HDL-c Levels, and/or Abdominal Obesity

V. CONCLUSÕES

- Os pontos de corte de QUICKI, de 0,349 para homens e 0,343 para mulheres, identificados na população do Nordeste do Brasil pelo presente estudo, encontram-se inseridos no intervalo de valores de QUICKI reportados por outros estudos, nacionais e internacionais, em populações também compostas de indivíduos adultos normoglicêmcios.
- Um terço da população nordestina encontra-se com resistência à insulina, necessitando, portanto, de maior atenção básica, devido à associação encontrada entre resistência à insulina e probabilidade de evento coronariano.
- Resistência á insulina apresentou influência relevante sobre o metabolismo lipídico, verificada pelo significativo aumento promovido nos níveis de Colesterol Total, de LDL-colesterol, VLDL-colesterol, e Triglicerídios, além da considerável redução das concentrações séricas de HDL-colesterol.
- Forte correlação entre resistência à insulina e dislipidemia do tipo aterogênica foi verificada na população do Nordeste do Brasil, haja vista que os níveis de triglicerídios e VLDL-colesterol foram os que melhor apresentaram correlação negativa com os valores de QUICKI.
- Na população nordestina do Brasil, resistência à insulina correlacionou-se fortemente com todos os índices lipídicos de risco cardiovascular, principalmente com a razão Triglicerídio/HDL-colesterol, a qual tem sido utilizada como indicativo da presença de partículas de LDL pequenas e densas, consideradas aterogênicas. Entretanto, a menos correlação, ainda que significativa, entre resistência à insulina e níveis lipídicos foi com a concentração sérica de LDL-colesterol, o que nos leva a sugerir que na população do Nordeste brasileiro, resistência à insulina correlaciona-se com alterações quantitativas

de LDL-colesterol, porém em menor grau quando comparado com as alterações qualitativas. Inclusive o índice de Castelli II, o qual inclui as concentrações de LDL-colesterol para acessar o risco cardiovascular, apresentou uma correlação positiva fraca em comparação com novos fatores lipídicos de risco cardiovascular, o não-HDL-colesterol e a razão não-HDL-colesterol/HDL-colesterol.

- Indivíduos do Nordeste do Brasil, mesmo normoglicêmicos, mas com resistência à insulina apresentaram um significativo risco de evento coronariano, com alto risco de infarto do miocárdio e morte por doenças coronarianas em 10 anos.
- A presença simultânea de, no mínimo, três distúrbios característicos das Síndrome Metabólica X, foi cerca de cinco vezes maior quando resistência à insulina também estava presente; e quando da presença de pelo menos quatro distúrbios da Síndrome Metabólica X foram identificados simultaneamente em um mesmo indivíduo, resistência à insulina apresentou uma relação de cerca de doze vezes com este cluster de distúrbios, enfatizando que um terço da população do Nordeste brasileiro pode se encontrar em alto risco de doenças metabólicas e cardiovasculares, representado pela correlação de RI com anormalidades lipídicas.

VI. ANEXO

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>
> R Smith: **Making progress with competing interests.** *BMJ* 2002, **325** :1375-1376
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> CD DeAngelis, PB Fontanarosa, A Flanagin: **Reporting financial conflicts of interest and relationships between investigators and research sponsors.** *JAMA* 2001, **286** :89-9
>
> K Morin, H Rakatansky, FA Riddick Jr, LJ Morse, JM O'Bannon 3rd, MS Goldrich, P Ray, M Weiss, RM Sade, MA Spillman: **Managing conflicts of interest in the conduct of clinical trials.** *JAMA* 2002, **287** :78-84

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3. Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

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If you wish to do so, you may submit an image which, in the event of publication, will be used to create a cover page for the PDF version of your article. The cover page will also display the journal logo, article title and citation details. The image may either be a figure from your manuscript or another relevant image. You must have permission from the copyright holder to reproduce the image. Images that do not meet our requirements will not be used.

Images must be 300dpi and 155mm square (1831 x 1831 pixels for a raster image).

Allowable formats - EPS, PDF (for line drawings), PNG, TIFF (for photographs and screen dumps), JPEG, BMP, DOC, PPT, CDX, TGF (ISIS/Draw).

Preparing tables

Each table should be numbered in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title that summarizes the whole table, maximum 15 words. Detailed legends may then follow, but should be concise.

Smaller tables considered to be integral to the manuscript can be pasted into the document text file. These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review; this will not always be the case if columns are generated by simply using tabs to separate text. Commas should not be used to indicate numerical values. Color and shading should not be used.

Larger datasets can be uploaded separately as additional files. Additional files will not be displayed in the final, published form of the article, but a link will be

provided to the files as supplied by the author.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). As with all files, please use the standard file extensions

Preparing additional files

Although *Lipids in Health and Disease* does not restrict the length and quantity of data in a paper, there may still be occasions where an author wishes to provide data sets, tables, movie files, or other information as additional information. These files can be uploaded using the 'Additional Material files' button in the manuscript submission process.

The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission.

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If additional material is provided, please list the following information in a separate section of the manuscript text, at the end of the document text file:

- File name
- File format (including name and a URL of an appropriate viewer if format is unusual)
- Title of data
- Description of data

Additional datafiles should be referenced explicitly by file name within the body of the article, e.g. 'See additional file 1: Movie1 for the original data used to perform this analysis'.

Formats and uploading

Ideally, file formats for additional files should not be platform-specific, and should be viewable using free or widely available tools. The following are examples of suitable formats.

- Additional documentation
    - PDF (Adobe Acrobat)
- Animations
    - SWF (Shockwave Flash)
- Movies
    - MOV (QuickTime)

    - MPG (MPEG)
- Tabular data
    - XLS (Excel spreadsheet)
    - CSV (Comma separated values)

As with figure files, files should be given the standard file extensions. This is especially important for Macintosh users, since the Mac OS does not enforce the use of standard extensions. Please also make sure that each additional file is a single table, figure or movie (please do not upload linked worksheets or PDF files larger than one sheet).

Mini-websites

Small self-contained websites can be submitted as additional files, in such a way that they will be browsable from within the full text HTML version of the article. In order to do this, please follow these instructions:

1. Create a folder containing a starting file called index.html (or index.htm) in the root
2. Put all files necessary for viewing the mini-website within the folder, or sub-folders
3. Ensure that all links are relative (ie "images/picture.jpg" rather than "/images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters
4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article

Style and language

General

Currently, *Lipids in Health and Disease* can only accept manuscripts written in English. Spelling should be US English or British English, but not a mixture.

Gene names should be in italic, but protein products should be in plain type.

There is no explicit limit on the length of articles submitted, but authors are encouraged to be concise. There is no restriction on the number of figures, tables or additional files that can be included with each article online. Figures and tables should be sequentially referenced. Authors should include all relevant supporting

data with each article.

Lipids in Health and Disease will not edit submitted manuscripts for style or language; reviewers may advise rejection of a manuscript if it is compromised by grammatical errors. Authors are advised to write clearly and simply, and to have their article checked by colleagues before submission. In-house copyediting will be minimal. Non-native speakers of English may choose to make use of a copyediting service.

Help and advice on scientific writing

The abstract is one of the most important parts of a manuscript. For guidance, please visit our page on "Writing titles and abstracts for scientific articles".

Tim Albert has produced for BioMed Central a list of tips for writing a scientific manuscript. MedBioWorld also provides a list of resources for science writing.

Abbreviations

Abbreviations should be used as sparingly as possible. They can be defined when first used or a list of abbreviations can be provided preceding the acknowledgements and references.

Typography

- Please use double line spacing.
- Type the text unjustified, without hyphenating words at line breaks.
- Use hard returns only to end headings and paragraphs, not to rearrange lines.
- Capitalize only the first word, and proper nouns, in the title.
- All pages should be numbered.
- Use the *Lipids in Health and Disease* reference format.
- Footnotes to text should not be used.
- Greek and other special characters may be included. If you are unable to reproduce a particular special character, please type out the name of the symbol in full.

  Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.
- Genes, mutations, genotypes, and alleles should be indicated in italics, and authors are required to use approved gene symbols, names, and formatting. Protein products should be in plain type.

Units

SI Units should be used throughout (liter and molar are permitted, however).