Universidade Estadual de Londrina

CHIARA CRISTINA BORTOLASCI

RELAÇÃO ENTRE O S*TATUS* DA PARAOXONASE-1, OS GENÓTIPOS QQ, QR E RR E O ESTRESSE OXIDATIVO EM PACIENTES COM TRANSTORNOS DO HUMOR E COMORBIDADES: TRANSTORNO POR USO DE TABACO E SÍNDROME METABÓLICA

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Estadual de Londrina, como requisito para a obtenção do título de Doutora.

Orientador: Prof. Dr. Décio Sabbatini Barbosa

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*"Life begins at
the end of your
comfort zone."*

(Neale Walsch)

BORTOLASCI, Chiara Cristina. **Relação entre o *status* da Paraoxonase-1, os genótipos QQ, QR e RR e o estresse oxidativo em pacientes com transtorno de humor e comorbidades:** transtorno por uso de tabaco e síndrome metabólica. 2015. 89f. Tese de Doutorado (Pós-Graduação em Ciências da Saúde) Universidade Estadual de Londrina, Londrina, 2015.

RESUMO

Introdução: Os transtornos do humor são as doenças mais incapacitantes atualmente. É uma doença crônica associada com o aumento do estresse oxidativo e de marcadores inflamatórios. Apresenta comorbidades como a síndrome metabólica (SM) e é acompanhada pelo aumento de espécies reativas de oxigênio/nitrogênio (ROS/RNS) e pela redução dos níveis de antioxidantes que podem ser mensurados perifericamente através do potencial antioxidante total plasmático (TRAP). Paraoxonase-1 (PON1) é um potente antioxidante ligado à lipoproteína de densidade alta (HDL) que protege da lipoperoxidação principalmente a lipoproteína de densidade baixa (LDL). Tem sido relatado uma diminuição nos níveis de PON1 em pacientes psiquiátricos e o mesmo acontece em indivíduos fumantes. Pouco se sabe ainda sobre a influência do polimorfismo da PON1 nessas condições supracitadas.
Objetivos: Delinear as associações da atividade da PON1 e os genótipos funcionais nos transtornos do humor e suas relações com tabaco, estresse oxidativo, resistência à insulina e SM.
Métodos: As análises foram realizadas em 191 indivíduos nunca fumantes e 144 fumantes; 91 com depressão e 45 com transtornos bipolares; 97 com SM. PON1, que compreende a atividade plasmática da PON1 e os 3 genótipos Q192R funcionais (QQ, QR e RR), foi avaliada em todas as amostras. Outros parâmetros, como TRAP, malondialdeído (MDA), ácido úrico, HDL, triacilgliceróis, índice de massa corporal, índice de HOMA, índice aterogênico do plasma e severidade da dependência da nicotina foram medidos.
Resultados: Os níveis de PON1 apresentaram-se diminuídos em pacientes depressivos, mas não bipolares. A atividade da PON1 mostrou-se reduzida pelo tabaco e em pacientes com depressão e genótipo QQ. Os genótipos QQ e QR mostraram-se protetores contra a SM, enquanto o tabaco aumentou o risco metabólico apenas no genótipo QQ. Os níveis de TRAP foram associados com um aumento na atividade da PON1, genótipo RR e não fumantes. O risco no subgrupo com baixos níveis de TRAP mostrou-se aumentado pelo tabaco e diminuído pelo genótipo RR e atividade da PON1. MDA está associado com os níveis de triacilgliceróis. Transtornos do humor e transtornos por uso de tabaco estão associados com aumento do potencial aterogênico.
Conclusão: Existem diferentes interações dos diferentes Q192R genótipos da PON1 e fatores ambientais nos transtornos do humor. Níveis reduzidos da atividade da PON1 e sua interação com outros fatores de risco podem contribuir para o advento das comorbidades depressão e SM. As interações dos genótipos e o tabaco nos transtornos do humor também contribuem para alterações nos níveis de antioxidantes. Interações entre os transtornos de humor, transtorno por uso de tabaco, altos níveis de ácido úrico e MDA contribuem para o aumento do potencial aterogênico e resistência à insulina.

Palavras-chave: Transtorno do humor. Paraoxonase-1. Síndrome metabólica. Estresse oxidativo. Perfil lipídico. Transtorno por uso de tabaco.

BORTOLASCI, Chiara Cristina**. Relationship among Paraoxonase-1 status, QQ, QR and RR genotypes and Oxidative Stress in patients with mood disorders and comorbidities:** tobacco use disorder and metabolic syndrome**.** 2015. 89p. PhD Thesis (Health Science Postgraduate Program) – State University of Londrina, Londrina, 2015.

ABSTRACT

Background: Mood disorder is one of the most disabling diseases nowadays. It is a chronic disease associated with the increase of oxidative stress and inflammatory markers. It's related to comorbities like metabolic syndrome (MS) and accompanied by an increase in reactive oxygen and nitrogen species and a reduction of antioxidant levels that can be measured peripherally by the total radical trapping antioxidant potential (TRAP). Paraoxonase-1 (PON1) is a potential antioxidant bound to high density lipoprotein (HDL) that protects from lipid peroxidation, specially the low density lipoprotein. It has been reported that PON1 activity is lower in psychiatric patients and the same happens in smokers. The influence of PON1 polymorphism in these conditions is not clear yet.
Aims: Define the associations of PON1 activity and functional PON1 Q192R genotypes in mood disorders and the relations with TRAP, tobacco and MS.
Methods: The analyses were performed in 191 non-smoking subjects and 144 smokers; 91 depressive and 45 bipolar disorder patients; 97 with MS. PON1 status was measured in all samples and comprehends plasma PON1 abundance and three functional PON1 Q192R genotypes (QQ, QR and RR). Others parameters like TRAP, malondialdehyde (MDA), uric acid, HDL, triglycerides, body mass index and severity of nicotine dependence were measured.
Results: PON1 levels were decreased in depressive patients but not in bipolar patients. The PON1 activity was reduced by smoking and in major depression with a QQ genotype. QQ and QR genotypes were protective against MS, while tobacco increased the metabolic risk only on QQ genotype. TRAP levels were associated with increased PON1 activity, RR genotype and non-smokers. The risk in the subgroup with low TRAP is increased by smoking and reduced by RR genotype and PON1 activity. MDA is associated with triglyceride levels. Comorbid mood disorders and TUD further increase AIP.
Conclusion: There are differential interactions between PON1 Q192R functional genotype x environment factors in mood disorder. Lowered levels of PON1 activity and it interactions with other risk factors may contribute to comorbidity between depression and MS. The interactions between genotypes and tobacco in mood disorders also contributed to changes in antioxidant levels. Interactions between mood disorders, tobacco user disorder, high levels of uric acid and MDA contribute to an increase in the atherogenic potencial and insulin resistance.

Keywords: Mood disorder. Paraoxonase-1. Metabolic syndrome. Oxidative stress. Lipid profile. Tobacco use disorder.

LISTA DE ABREVIATURAS

AHC	Ambulatório do Hospital de Clínicas
ALT	Alanina aminotransferase
ANCOVA	Análise de covariância
ANOVA	Análise de variância
AST	Aspartato aminotransferase
ASSIST	Teste de triagem do envolvimento com álcool, tabaco e outras substâncias
ATP	Adenosina trifosfato
BDNF	Fator neurotrófico derivado do cérebro
CID	Classificação Internacional de Doenças
CMPA	4-(clorometil)fenil acetato
CRATT	Centro de Referência de Abordagem e Tratamento do Tabagismo
DSM-IV	Manual Diagnóstico e Estatístico dos Transtornos Mentais - quarta edição
DSM-5	Manual Diagnóstico e Estatístico dos Transtornos Mentais - quinta edição
DNA	Ácido desoxirribonucleico
ERNs	Espécies reativas de nitrogênio
EROs	Espécies reativas de oxigênio
FTND	Teste de Fagerström para dependência de Nicotina
GABA	Ácido gama-amino butírico
HDL	Lipoproteínas de densidade alta
HDRS	Escala de Avaliação para Depressão de Hamilton
HOMA2IR	*Homeostasis model assessment* para medir resistência à insulina
HOMA2S%	*Homeostasis model assessment* para medir sensibilidade à insulina
HOMA2B%	*Homeostasis model assessment* para medir função da célula β
IL-1	Interleucina-1
IL-4	Interleucina-4

IL-6	Interleucina-6
IL-10	Interleucina-10
IMC	Índice de massa corporal
IFN-γ	Interferon gama
LDL	Lipoproteínas de densidade baixa
MEIA	Imunoensio enzimático de micropartículas
MDA	Malondialdeído
OMS	Organização Mundial da Saúde
PA	Fenil acetato
PCR	Proteína C reativa
PON1	Paraoxonase 1
QL	Quimiluminescência
SM	Síndrome metabólica
SNPs	Polimorfismos de um único nucleotídeo
TNF-α	Fator de necrose tumoral
TRAP	Potencial antioxidante total plasmático
TRYCATs	Metabólitos do triptofano
TUT	Transtorno por uso de tabaco
UEL	Universidade Estadual de Londrina

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1 INTRODUÇÃO

Os transtornos do humor se caracterizam pela alternância de estados de depressão, mania e eutmia. O transtorno depressivo maior compreende episódios depressivos únicos ou que se repetem ao longo da vida (2). Já no transtorno bipolar, ocorre a alternância de episódios de mania (ou hipomania), depressão e eutmia (3). Os transtornos do humor estão classificados, de acordo com a Classificação Internacional de Doenças – CID 10 da Organização Mundial da Saúde (OMS), nos grupos F30-F39 (1).

Essas são algumas das doenças mais incapacitantes no mundo, gerando altos custos econômicos e sociais para os governos, devido aos gastos com tratamentos para a população e às perdas de produtividade. A prevalência do transtorno depressivo maior, em um ano, nos Estados Unidos gira em torno de 7% na população geral (4). Já os transtornos bipolares I e II apresentam uma prevalência estimada de 0,6% a 0,8% (5). De acordo com a OMS, até o ano de 2030 os transtornos depressivos serão os maiores contribuintes para a carga global de doenças (6). No Brasil, cerca de 17% da população sofre depressão ao longo da vida. Considerando a doença nos cuidados primários, essa taxa eleva-se para 29,5% (7).

Vários mecanismos têm sido propostos pra explicar a fisiopatologia dos transtornos do humor, incluindo ações das monoaminases, eixo hipotálamo-hipófise-adrenal, glutamato e vias de sinalização secundárias (8). Quatro principais teorias são apresentadas, porém é importante ter em mente que os transtornos do humor são resultados das interações entre as alterações discutidas em todas as teorias, ao invés de alterações em apenas uma delas (9).

Na teoria das monoaminases, a depressão está relacionada com a redução nos níveis extracelulares de monoaminas (serotonina, dopamina e norepinefrina) e redução da neurotransmissão monoaminérgica. Estudos demonstram concentrações reduzidas de metabólitos das monoaminas no fluido cérebro-espinhal, sangue, urina e tecidos cerebrais de pacientes depressivos (9).

Na teoria do eixo hipotálamo-pituitária-adrenal, há uma desregulação e inúmeros estudos mostram uma associação entre transtornos do humor e níveis elevados de cortisol, corticotrofina e hormônio liberador de corticotrofina. Além disso, há um controle no *feedback* debilitado (9).

Na teoria inflamatória, há processos flogísticos envolvidos nos transtornos do humor tais como: aumento nos níveis de marcadores pró-inflamatórios, como fator de necrose tumoral (TNF-α), interleucina 6 (IL-6), interleucina 1 (IL-1), proteína C reativa (PCR) e interferon gama (IFN-γ) e redução nos níveis de marcadores anti-inflamatórios como a interleucina 4 (IL-4) e a interleucina 10 (IL-10) (9).

Na teoria da neurogênese e da neuroplasticidade há redução da neurogênese no hipocampo e os níveis de fatores neurotróficos podem estar relacionados aos transtornos do humor (no que diz respeito ao desenvolvimento e na persistência da doença). O fator neurotrófico derivado do cérebro (BDNF) é a neurotrofina mais estudada com relação aos transtornos de humor e meta-análises confirmam a correlação entre a redução dos níveis de BDNF e os transtornos do humor (9).

A baixa função do GABA também foi proposta como um marcador biológico herdado de susceptibilidade para o desenvolvimento de transtornos do humor (10). Com relação ao glutamato, evidências sugerem que pacientes com depressão apresentam níveis elevados de glutamato em algumas regiões do cérebro. Mediadores inflamatórios podem potencializar as ações do glutamato através de 3 vias diferentes: a) aumento da produção de ácido quinolínico (através da ativação da via da quinurenina), b) aumento da liberação de glutamato e c) inibição da remoção de aminoácidos excitatórios pela astroglia.(11). Além disso, tem sido relatado na literatura que a ativação de vias imune-inflamatórias e do estresse oxidativo estão relacionados com os sintomas e estágios da depressão e com processos neuroprogressivos (12).

Estresse oxidativo é o termo dado ao desequilíbrio causado pela produção excessiva de radicais livres, representados pelas espécies reativas de oxigênio/nitrogênio (EROs/ERNs) e/ou redução nos níveis de antioxidantes (13). Os radicais livres podem ser gerados de fontes exógenas como radiação, poluição e cigarro ou por fontes endógenas como inflamação e explosão (do inglês *burst*) respiratória. Os antioxidantes podem ser endógenos, como a glutationa peroxidase, ácido úrico e a catalase ou obtidos pela dieta, como os polifenóis, vitaminas A, C, E e carotenoides (14). As EROs/ERNs podem causar dano ao ácido desoxirribonucleico (DNA), proteínas e iniciar o processo de lipoperoxidação, daí a importância do sistema antioxidante na defesa do organismo (15). Considerando que a concentração de um único antioxidante não é representativa, surgiu o conceito de capacidade antioxidante total que auxilia na avaliação das condições que afetam o estresse oxidativo *in vivo.*

A capacidade antioxidante total considera a ação de todos os antioxidantes presentes no plasma e garante assim uma análise mais integrada quando comparada à soma de vários antioxidantes medidos separadamente. Isso, pois considera as interações sinérgicas além de considerar a ação de antioxidantes conhecidos ou não (16).

Indivíduos com depressão uni e bipolar apresentam desregulação na sinalização redox. EROs atuam como mensageiros secundários e afetam vias inflamatórias pela ativação do fator nuclear κB. Quando em excesso, as EROs/ERNs levam à produção de malondialdeído (MDA), 8-iso-prostano e óxido nítrico. Estudos demonstram também a

redução no sistema antioxidante, representado por baixos níveis de vitamina E, superóxido dismutase e glutationa peroxidase. Considerando que alguns antioxidantes possuem atividades anti-inflamatórias, a redução nos níveis desses compostos aumenta a inflamação (12).

Interações da inflamação com radicais livres e baixos níveis de antioxidantes podem ocorrer através de alterações na via dos catabólitos do triptofano (TRYCAT). Os TRYCATs são depressores, ativam vias oxidativas, causam disfunções mitocondriais e possuem efeitos neurotóxicos que podem levar à neurodegeneração (17). A inflamação e o estado redox regulam a utilização do triptofano, o que causa consequências em processos associados à depressão. Além disso, baixos níveis de triptofano e altos níveis de TRYCATs causam supressão na resposta imune-inflamatória (12).

Quando as células falham na adaptação a essas mudanças no sistema redox, isso causa morte celular e, em conjunto com o dano causado pelos mediadores inflamatórios, que são considerados as maiores causas da neuroprogressão e, consequentemente, da depressão (18).

Os transtornos do humor são doenças neurodegenerativas. A neuroprogressão, um desdobramento no processo da neurodegeneração, possui estágios presentes nos transtornos psiquiátricos, a saber: apoptose, diminuição da neurogênese, perda neuronal, resiliência celular e diminuição da plasticidade sináptica. A função e integridade mitocondrial são afetadas em pacientes com transtornos psiquiátricos, causados pelo dano ao DNA mitocondrial, aumento das EROs e de cálcio. O tabagismo também pode ser responsável pela disfunção mitocondrial (19).

A disfunção mitocondrial é caracterizada pela redução na atividade da cadeia transportadora de elétrons e de suas enzimas, diminuição na produção de adenosina trifosfato (ATP), alterações nas estruturas mitocondriais no cérebro (córtex pré-frontal e frontal) e deleções no DNA (20).

Existem evidências de que, além de fatores genéticos, há fatores de risco comportamentais e fisiopatológicos que contribuem para as doenças psiquiátricas (21). O estilo de vida é fator determinante nos níveis de inflamação: dietas ricas em antioxidantes, vitaminas, minerais e fibras são associadas com a redução na inflamação sistêmica. Da mesma forma, atividade física está associada com a redução nos marcadores inflamatórios. Já o hábito de fumar aumenta a inflamação e o estresse oxidativo (21). Outra consequência de um estilo de vida não saudável é a obesidade. Obesidade e depressão possuem uma relação bi-direcional: obesidade contribui com a depressão pelo aumento de marcadores pró-inflamatórias (TNF-α, IL-6, IL-1, PCR) e a depressão contribui com o acúmulo de tecido adiposo (21). A desregulação do eixo hipotálamo-pituitária-adrenal em pessoas com depressão pode resultar em níveis elevados de cortisol que, por sua vez, podem inibir a

ação da insulina na regulação dos níveis plasmáticos de glicose ou causar depósito de gordura no abdômen (adiposidade visceral), que é fator de risco no desenvolvimento de diabetes e componente da síndrome metabólica (SM) (22).

A prevalência de SM é alarmantemente alta em pacientes com transtorno do humor (23). Essas doenças apresentam um alto grau de comorbidade com condições crônicas como doença cardiovascular e diabetes, doenças diretamente ligadas ao aumento de morbidade e mortalidade (8,24).

A SM é uma condição multifatorial que leva à aterosclerose acelerada, aumento do risco de diabetes e que tem alcançado proporções epidêmicas na última década. Essa condição é caracterizada pela combinação de três ou mais fatores: obesidade abdominal, hipertensão, hiperglicemia e dislipidemia (HDL baixo e/ou triacilgliceróis aumentados) (25,26). Além disso, outras comorbidades têm sido relatadas, entre elas: esteatose hepática, liberação de substâncias proinflamatórias pelos adipócitos, aumento da atividade dos fatores de coagulação, disfunção endotelial, inflamação e estresse oxidativo (15).

Evidências sugerem uma estreita ligação entre a SM, onde há um processo inflamatório crônico em baixo nível e estresse oxidativo, sendo esse último um mecanismo importante também na hipertensão, diabetes e obesidade e suas complicações. A produção excessiva de radicais livres e danos oxidativos parecem explicar, pelo menos em parte, a perpetuação da resistência à insulina, alterações na produção de energia, a disfunção endotelial e o aparecimento de complicações vasculares na SM (27). Evidências também sugerem que EROs/ERNs podem estar envolvidas com eventos cardiovasculares e altas taxas de mortalidade (25).

Não se sabe ao certo se a depressão causa aumento no risco de desenvolvimento de diabetes independentemente ou age através de fatores de risco como obesidade, sedentarismo, hábitos alimentares e o cigarro (22).

O ácido úrico, produto final do metabolismo das purinas, é um importante removedor de EROs/ERNs (responsável por mais de 60% da atividade antioxidante do organismo). Além disso, é capaz de suprimir a cascata inflamatória, diminuir a permeabilidade da barreira hematoencefálica, diminuir dano nos tecidos do sistema nervoso central e morte neuronal (28). O ácido úrico, em altas concentrações, está associado à resistência a insulina, aterogenicidade e SM (29). Indivíduos com SM apresentam atividade aumentada da xantina oxidase e os níveis aumentados de ácido úrico têm sido relacionados com a redução da excreção renal (29).

Estudos relatam níveis reduzidos de ácido úrico em pacientes com depressão maior (28) e níveis aumentados em indivíduos com transtorno bipolar (30).

O uso do tabaco é altamente ligado com transtornos do humor. No *National Comorbidity* Survey, aproximadamente 59% os indivíduos com um histórico de depressão são ou foram fumantes, comparado a menos de 39% daqueles sem o histórico de depressão (31).

O "Transtorno por Uso de Tabaco" (TUT), antigamente chamado de "Síndrome da Dependência da Nicotina" é descrita na 5ª edição do *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) da *American Psychiatric Association* (2013). Para o diagnóstico do TUT segundo o DSM-5, o indivíduo precisa apresentar pelo menos 2 dos 11 seguintes critérios no último ano:

a) Crescente uso do tabaco ou aumento do período de tempo de consumo;
b) Desejo persistente ou falha no controle do uso do tabaco;
c) Gasto de tempo significativo na obtenção e consumo do tabaco;
d) Urgência ou forte desejo de consumo do tabaco;
e) Uso recorrente do tabaco gerando transtornos ou complicações nas outras atividades diárias sejam elas no trabalho, escola ou ambiente doméstico;
f) Ininterrupção do uso do tabaco, mesmo após o surgimento de problemas sociais ou interpessoais causados pelos efeitos do tabaco;
g) Abandono ou redução de atividades sejam elas recreativas, ocupacionais ou sociais por causa do consumo do tabaco;
h) Consumo do tabaco mesmo em situações de risco;
i) Uso contínuo do tabaco apesar da ciência das consequências físicas e psicológicas causadas pelo mesmo;
j) Tolerância, avaliada pelo aumento significativo das quantidades de tabaco necessárias para alcançar o efeito ou redução significativa do efeito mesmo utilizando a mesma quantidade de tabaco;
k) Abstinência, manifestada pela síndrome de abstinência típica ou pelo consumo do tabaco para aliviar/evitar os sintomas da abstinência.

A comorbidade entre o transtorno por uso de tabaco e transtornos depressivos pode ser explicada através de vias e fatores predisponentes (genéticos e ambientais) em comum. Os efeitos de diversos neurotransmissores como glutamato, serotonina e dopamina têm sido demonstrados tanto na dependência quanto na regulação do humor (32). O polimorfismo genético do transportador da serotonina está envolvido no TUT e na depressão. A função serotoninérgica está reduzida em indivíduos fumantes depressivos (33).

Há indícios de que as vias inflamatórias podem ser um elo entre o tabaco e a depressão. Fumantes depressivos possuem maiores níveis de citocinas pró-inflamatórias (TNF-α, IL-6, PCR) quando comparados a fumantes não depressivos (32). A

exposição ao tabaco afeta o sistema imune e neurotransmissor; causa disfunção mitocondrial e estresse oxidativo, todos relacionados com a etiologia dos transtornos de humor e ansiedade (34).

O cigarro contém vários componentes que são oxidantes e pró-oxidantes, capazes de produzirem EROs/ERNs. Esse aumento na produção destas espécies radicalares pelo cigarro está diretamente relacionado ao estresse oxidativo (35). Há relatos de que o tabaco aumenta o dano oxidativo ao DNA em 35-50% (36). A exposição ao cigarro está associada com baixos níveis de antioxidantes como catalase, vitaminas A, C, E, glutationa e superóxido dismutase (12).

Outra enzima, relacionada com a atividade antioxidante, é a Paraoxonase 1 (PON1). A PON1, membro da família das paraoxonases (que consiste em PON1, PON2 e PON3), é uma enzima sintetizada principalmente no fígado e localizada, em grande parte, em HDL. É composta de 355 aminoácidos e é capaz de hidrolisar organofosforados (como paraoxon e diazon) e ésteres aromáticos (como fenilacetato-PA) e por isso recebe esse nome (38,41). Além de sua atividade como esterase, também atua como lactonase, sendo capaz de hidrolisar uma variedade de lactonas entre elas algumas drogas e compostos endógenos (42,43).

A PON1 está relacionada com a redução na oxidação da LDL e na inativação dos fosfolipídeos oxidados derivados desta lipoproteína. A oxidação da LDL é um processo chave na fisiopatologia da aterosclerose e, por consequência, da doença cardiovascular. A atividade antioxidante da HDL e a sua susceptibilidade à modificações aterogênicas (oxidação e glicação) estão associadas com a atividade da PON-HDL (44).

O gene que codifica a PON1 está localizado no braço longo do cromossomo 7 e já foram relatados mais de 160 polimorfismos de um único nucleotídeo (do inglês, *SNPs – single nucleotide polimorphism),* sendo alguns na região codificadora e outros na região promotora (43,45).

Dentre esses polimorfismos, dois da região codificadora tem destaque na literatura: a) PON1 Q192R, rs 662: a substituição de uma glutamina (Q) por uma arginina (R) na posição 192 da cadeia polipeptídica e b) PON1 L55M, rs 854560: a substituição de uma leucina (L) por uma metionina (M) na posição 55. O polimorfismo Q → R é responsável por uma notável diferença na especificidade do substrato relacionada à atividade hidrolítica da enzima. Além disso, o polimorfismo do gene da PON1 também contribui para variações nos níveis plasmáticos de HDL, agindo como protetor da oxidação da mesma e, dessa forma, preservando a integridade desta lipoproteína. Estudos sugerem que o alelo Q (mais abundante que o alelo R), é responsável pelo efeito protetor da PON contra aterosclerose (41,46). As frequências genéticas para alta ou baixa atividade hidrolitica da enzima variam entre os grupos de diferentes etnias e áreas geográficas.

O consumo de álcool, de cigarro e uma dieta rica em gordura são os fatores ambientais que mais interferem nos níveis da enzima PON1 (37). Existem alguns estudos que relatam os que transtornos do humor podem estar associados com uma diminuição da atividade da PON1 (38–40).

Em um estudo recente, foi descoberto que o genótipo Q192R da PON1 está relacionado com um aumento dos índices de Castelli I, sugerindo um aumento no potencial aterogênico. Porém, os efeitos da atividade da PON1 na SM ainda não estão bem estabelecidos (47).

Grande parte dos pacientes com transtornos do humor são fumantes (a probabilidade de fumar é duas vezes maior em pacientes psiquiátricos). Além disso, indivíduos fumantes com níveis reduzidos de PON1 apresentam aumento no risco para o desenvolvimento de transtorno depressivo maior ou transtorno bipolar (48).

Adicionalmente, o cérebro consome grandes quantidades de oxigênio e, por consequência, produz grande quantidade de radicais livres comparados às defesas antioxidantes. Assim, o possível envolvimento do estresse oxidativo na patogênese da depressão e do transtorno bipolar ligados aos níveis reduzidos de PON1 justificam as análises das associações da atividade dessa enzima e dos seus genótipos com os principais fatores de risco relacionados a essas doenças psiquiátricas. Isto, para tentarmos entender melhor os mecanismos fisiopatológicos dos transtornos do humor.

Considerando que os transtornos do humor têm alta comorbidade com o TUT e com a SM, que contribuem para o aumento da inflamação e do estresse oxidativo, justifica-se a realização do presente trabalho para a avaliação das possíveis associações entre esses fatores e seu impacto nos transtornos psiquiátricos.

2 OBJETIVOS

2.1 GERAL

Avaliar as associações do *status* da PON1 nos transtornos do humor (bipolaridade e depressão) com comorbidade pelo transtorno por uso de tabaco e síndrome metabólica.

2.2 ESPECÍFICOS

- Avaliar a atividade plasmática da PON1 nos transtornos do humor.

- Avaliar os efeitos do polimorfismo da PON1 Q192R nos transtornos do humor.

- Avaliar a atividade da PON1 nos transtornos do humor, relacionado com o TUT.

- Avaliar a relação entre a capacidade antioxidante total plasmática (TRAP) e a atividade da PON1, os genótipos da PON1 Q192R e cigarro.

- Avaliar as interações dos genótipos da PON1 x tabaco e transtornos do humor.

- Avaliar se a atividade da PON1 e seus genótipos, tabaco e transtornos de humor podem elevar a chance de desenvolver SM.

- Avaliar a resistência à insulina e o potencial aterogênico nos transtornos de humor e nos transtornos pelo uso de tabaco.

- Avaliar os efeitos do MDA e ácido úrico na resistência à insulina e no potencial aterogênico de indivíduos com transtornos de humor, transtornos pelo uso de tabaco e SM.

3 CASUÍSTICA E MÉTODOS

3.1 DELINEAMENTO

Caso controle.

3.2 POPULAÇÃO

Todos os pacientes foram esclarecidos sobre os procedimentos do estudo e assinaram o Termo de Consentimento Livre e Esclarecido em duas vias. Este estudo foi aprovado pelo Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina (UEL), sob Parecer PF N.º 250/10 - Folha de Rosto N° 376220 (Anexo 1). A pesquisa fez parte do projeto 07517, cadastrado na Pró-Reitoria de Pesquisa e Pós-Graduação da UEL, intitulado Marcadores Biológicos em Fumantes de um Centro de Referência de Abordagem e Tratamento do Tabagismo.

Os participantes apresentaram idade entre 18 a 60 anos, de ambos os sexos com valores dentro do limite de referência para os seguintes exames: hemograma, aspartato aminotransferase (AST), alanina aminotransferase (ALT), ureia e creatinina. Os casos foram recrutados no Centro de Referência de Abordagem e Tratamento do Tabagismo (CRATT), localizado no Ambulatório do Hospital de Clínicas (AHC) da UEL. O grupo controles foi composto de trabalhadores da mesma universidade.

Indivíduos com delirium, demência, amnésia e outros transtornos cognitivos, doenças infecciosas como Hepatite B e C, HIV, doenças crônicas como insuficiência renal, doença pulmonar obstrutiva e autoimune, tratamento com intérferon, uso patológico de substâncias psicoativas e consumo de substâncias antioxidantes foram excluídos do estudo.

3.3 AMOSTRAGEM

O cálculo do tamanho da amostra foi baseado em indivíduos fumantes e nunca fumantes com ou sem depressão. Uma amostra de 34 indivíduos não fumantes e 27 fumantes (n total= 61) seria capaz de detectar 59% da prevalência de transtornos depressivos entre os fumantes, comparados com 17% de desordens depressivas entre não fumantes (OR= 7,03) com um intervalo de confiança com um nível de 0,95 e um poder de 95%. A razão entre casos e controles foi estimada em 1,27. Como obtivemos uma amostra de 191 indivíduos nunca fumantes e 144 dependentes de nicotina, o tamanho da amostra apresentava um poder adequado para detectar a prevalência de transtornos depressivos entre dependentes de nicotina e nunca fumantes.

O recrutamento aconteceu no período de março de 2011 a agosto de 2012. O estudo contou com um total de 335 participantes. Dentre esses, 191 indivíduos nunca fumantes e 144 fumantes; 91 com depressão e 45 com transtornos bipolares; 97 com SM.

3.4 INSTRUMENTOS

3.4.1 Questionários

Os participantes foram submetidos a um questionário para a coleta dos seguintes dados: sócio-demográficos, história de hospitalizações, história tabagística, história pregressa de doenças, capacidade de trabalho e para atividades domésticas, condicionamentos relacionados ao tabagismo, motivações para cessar o tabaco, história familiar para o tabagismo, tratamentos efetuados anteriores, além do registro de outras comorbidades médicas, psiquiátricas e tentativas de suicídio (Anexo 2).

3.4.2 Avaliação do Diagnóstico de Transtorno do humor e de Dependência de Nicotina

Os critérios diagnósticos para pesquisa de transtornos do humor, bem como de dependência de nicotina foram avaliados por um psiquiatra treinado de acordo com a Entrevista Clínica Estruturada para o DSM - IV" – versão clínica traduzida e validada para o português (49).

3.4.3 Avaliação da Gravidade da Depressão

A avaliação da severidade da depressão dos participantes do estudo foi realizada utilizando a Escala de Avaliação para Depressão de Hamilton (HDRS), traduzida e adaptada para a população brasileira (50). Uma pontuação de 20 ou mais indica depressão moderada a grave.

3.4.4 Avaliação do Uso de Substâncias Psicoativas

Para a triagem do uso de substâncias psicoativas, utilizou-se o teste de triagem do envolvimento com álcool, tabaco e outras substâncias (ASSIST), questionário desenvolvido pela OMS (51) e adaptado para o português (52) para o rastreamento do uso de: tabaco, álcool, canabinóides, cocaína, estimulantes do tipo anfetamina, sedativos, alucinógenos, inalantes, opióides e outras drogas. Para o rastreio do uso do álcool, utiliza-

se a seguinte pontuação: baixo risco (0-10), moderado risco (11-26) e alto risco (≥27). O ponto de corte utilizado neste trabalho foi o de alto risco.

3.4.5 Triagem da Gravidade da Dependência de Nicotina

O teste de Fagerström para Dependência da Nicotina (FTND), traduzido e adaptado para a população brasileira, foi utilizado para avaliar a gravidade da dependência de tabaco (53). Os escores para dependência de nicotina permitem a classificação em cinco níveis: muito baixo (0 a 2 pontos); baixo (3 a 4 pontos); moderado (5 pontos); alto (6 a 7 pontos); muito alto (8 a 10 pontos). O ponto de corte de FTND para a dependência de nicotina foi ≥ 5.

3.5 Avaliação do Diagnóstico de Síndrome Metabólica

O diagnóstico de SM foi determinado utilizando os critérios da Federação Internacional de Diabetes, os quais o indivíduo deve possuir pelo menos 3 dos 5 critérios listados abaixo (sendo um deles, obrigatoriamente a obesidade abdominal):

a) Obesidade abdominal (circumferência da cintura ≥ 90 cm para homens e ≥ 80 cm para mulheres em asiáticos e sul-americanos e ≥ 94 cm para homens e ≥ 80 cm para mulheres em caucasianos);

b) Baixos níveis de HDL-C (< 40 mg/dl em homens e < 50 mg/dl em mulheres ou em uso de medicamentos hipolipêmicos);

c) Hipertrigliceridemia (≥ 150 mg/dl ou em uso de medicamentos hipolipêmicos);

d) Glicose em jejum aumentada (≥ 100 mg/dl ou em uso de medicamentos hipoglicemiantes);

e) Pressão arterial aumentada (≥ 130/85 mmHg ou em uso de medicamentos antihipertensivos).

3.6 Cálculo do IMC

O Índice de Massa Corpórea (IMC) foi calculado dividindo-se o peso (kg) pela altura² (m²).

3.7 Determinação da Pressão Arterial

A pressão arterial foi aferida utilizando-se um esfigmomanômetro no braço direito e o valor utilizado foi a média de duas leituras com um intervalo de 5 minutos entre cada aferição.

3.8 Determinação da Circunferência da Cintura

A circunferência da cintura foi medida durante a expiração com o indivíduo em pé e com o abdome relaxado sobre o ponto médio entre a última costela e a crista ilíaca, paralelo ao chão.

3.9 Avaliações Laboratoriais

As coletas das amostras de sangue periférico para todos participantes foram realizadas pela manhã no AHC de Londrina após um jejum de 12 a 14 horas. As avaliações laboratoriais, tais como hemograma, lipidograma, glicose, insulina, creatinina, ácido úrico, AST, ALT, gama glutamil transferase, marcadores para hepatite C e B e sorologia para HIV foram realizados pelos setores de rotina do Laboratório de Análises Clínicas do Hospital Universitário de Londrina.

Para a dosagem da glicose foram coletados 5 mL de sangue em tubos com vácuo (vacutainer®) contendo EDTA como anticoagulante e fluoreto de sódio como inibidor da glicólise. Para o hemograma foram coletados 5 mL de sangue em tubos com vácuo (vacutainer®) contendo EDTA como anticoagulante. Também foram coletados um tubo com vácuo (vacutainer®) com 5 mL e um tubo com 20 mL de sangue sem anticoagulante para alguns parâmetros bioquímicos quantificados de rotina além da dosagem de TRAP, MDA e PON-1. Os tubos para dosagem de TRAP, MDA e PON-1 foram centrifugados por 10 minutos a 3000 rpm (EVLAB®, Londrina, PR, Brasil), sendo o soro aliquotado em criotubos e armazenado em freezer -70ºC (Kendro®, Asheville, NC, EUA) para a realização dos testes. As outras amostras foram processadas no mesmo dia da coleta.

O doseamento de HDL-C, triacilgliceróis e glicose foram realizados utilizando o sistema bioquímico automatizado Dimension® RxL (Siemens, Newark, EUA).

O doseamento de insulina foi realizado por imunoensaio enzimático de micropartículas (MEIA) utilizando o sistema automatizado AXSYM (Abbotts Laboratory, Germany).

O potencial aterogênico foi calculado através da fórmula: log10 (triacilgliceróis/HDL-C).

Os índices HOMA2IR (*Homeostasis model assessment* para medir resistência à insulina), HOMA2S% (*Homeostasis model assessment* para medir sensibilidade à insulina) e HOMA2B% (*Homeostasis model assessment* para medir função da célula β) foram calculados de acordo com Levy et al. (1998) (54).

3.9.1 Determinação do Malondialdeído

Para avaliar os níveis de MDA foi utilizado a metodologia descrita por Jentzsch et al. (1996) (55) (Anexo 3). A formação de MDA ocorre pela decomposição dos hidroperóxidos lipídicos e sua concentração tem sido utilizada para estimar a intensidade da peroxidação lipídica em sistemas biológicos, em células e tecidos. Este método consiste na medida de um cromógeno róseo formado pela reação do MDA com duas moléculas de ácido tiobarbitúrico, em meio ácido e alta temperatura. A quantificação do MDA é feita em espectrofotômetro nos comprimentos de onda de 535 e 572nm. Os valores encontrados são ajustados pelo valor de proteína e apresentados na unidade nmol MDA/g de proteína.

3.9.2 Determinação do Potencial Antioxidante Total Plasmático

O TRAP foi avaliado por quimiluminescência em uma adaptação do método da técnica descrita por Repetto e colaboradores, 1996 (56) (Anexo 4). Esta metodologia detecta antioxidantes hidro e/ou lipossolúveis presentes no plasma.

Ao meio de reação (1,8 mL de tampão glicina 0,1 M, pH 8,6) foram acrescidos 100 μL de luminol em solução aquosa 200 μM, 5 μL de plasma diluído 50% em tampão glicina e 100 μL de solução aquosa de 2,2' azo-bis (2-amidinopropano) 200 mM. O 2,2' azo-bis gera radicais peroxil rapidamente, via interação com radicais centrados em carbono e oxigênio molecular. Estes radicais livres reagem com o luminol (que atua como um amplificador de sinal), produzindo quimiluminescência (QL). Esta reação é inibida pela superóxido dismutase, catalase e análogos da vitamina E.

A adição de plasma diminui a QL em níveis basais por um período (tempo de indução t_i) proporcional à concentração plasmática de antioxidantes até que os radicais livres sejam regenerados, restituindo-se os níveis iniciais de QL. O sistema foi calibrado com análogo de vitamina E (Trolox), 100 μL na concentração de 20 μM em tampão glicina pH 8,6. Uma comparação do tempo de indução depois da adição de concentrações conhecidas de Trolox e plasma permite obter valores de TRAP em equivalentes de Trolox.

Este experimento foi conduzido em um contador β marca Beckman® LS 6000 (Fullerton, CA, EUA), em um modo de contagem não coincidente por 25 minutos e uma faixa de resposta entre 300 a 620nm. Todos os experimentos foram realizados em triplicata, sendo que as replicatas com variação > 10% foram repetidas.

3.9.3 Determinação do "Status" da Paraoxonase-1

A determinação da PON1 foi realizada segundo a metodologia descrita por Richter e colaboradores, 2008 (Anexo 5) (57). A hidrólise do 4-(clorometil)fenil acetato

(CMPA) foi medida em 280nm por 4 minutos a 25°C utilizando 20µL de plasma diluído 1:40 em um tampão de diluição (20 mmol/l Tris/HCl (pH 8.0), 1mmol/l $CaCl_2$). A hidrólise do PA em alta concentração salina foi medida em 270nm por 4 minutos a 25°C utilizando 20µL de plasma diluído 1:40 em tampão de diluição (2 mol/l NaCl, 20 mmol/l Tris/HCl (pH 8.0), 1mmol/l $CaCl_2$).

Os resultados obtidos foram plotados em um gráfico de atividade enzimática (Figura abaixo) que apresenta as taxas de atividade da arilesterase (hidrólise do PA em alta concentração salina) *versus* atividade da CMPase (hidrólise do CMPA). Considerando que o polimorfismo Q192 da PON1 confere diferentes atividades catalíticas frente a estes dois substratos, o gráfico abaixo divide a população em 3 genótipos (QQ, QR e RR):

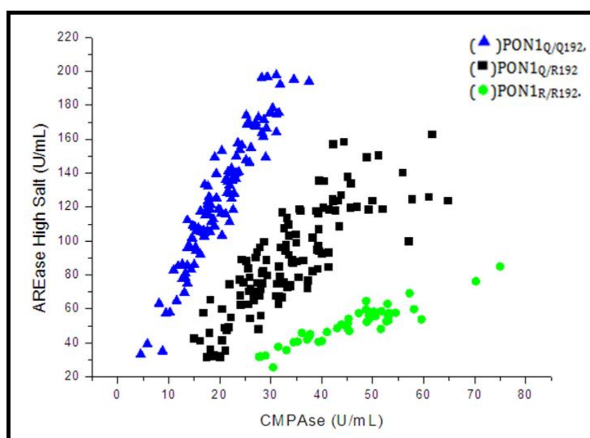

Figura. Genotipagem funcional para o polimorfismo Q192R da Paraoxonase 1 através da hidrólise do CMPA x PA em altas concentrações salina. Cada ponto indica um indivíduo.

A dosagem da hidrólise do PA em baixa concentração salina representa a atividade total plasmática da PON1, já que nessa condição o polimorfismo Q192R da PON1 não influencia a atividade catalítica da PON1 frente ao PA. Essa reação foi medida a 270nm por 4 minutos a 25°C utilizando 20µl de plasma diluído 1:80 em tampão.

Todas as reações foram realizadas em microplacas ultravioleta de 96 poços utilizando uma leitora de microplaca EnSpire (Perkin Elmer, NY, EUA). Todos os experimentos foram realizados em triplicata, sendo que as replicatas com variação > 10% foram repetidas.

3.10 ANÁLISE ESTATÍSTICA

Análises de variância (ANOVAs) e covariância (ANCOVAs) foram realizadas para examinar as diferenças entre os grupos, seguidos do teste Tukey. Análises

de contingência (Chi-quadrado) foram utilizadas para checar a distribuição das variáveis nas diferentes categorias.

As correlações entre as variáveis foram checadas utilizando correlações de Pearson e modelo linear generalizado.

As análises foram realizadas no SPSS para Windows, versões 15 e 19. O nível de significância foi fixado em $\alpha = 0,05$ (bicaudal).

Particularidades de cada análise serão apresentadas nos respectivos artigos apresentados a seguir.

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4 ARTIGOS

Os resultados e discussões serão apresentados na forma de artigos científicos publicados em periódicos internacionais indexados.

O artigo 1, intitulado "*Lowered plasma paraoxonase (PON1) activity is a trait marker of major depression and PON1 Q192R gene polymorphism-smoking interactions differentially predict the odds of major depression and bipolar disorder*" foi publicado no Journal of Affective Disorder (fator de impacto: 3.705), volume 159 de 2014, páginas 23 à 30.

O artigo 2, intitulado "*Paraoxonase 1 status and interactions between Q192R functional genotypes by smoking contribute significantly to total plasma radical trapping antioxidant potential*" foi publicado no Neuroscience Letters (fator de impacto: 2.055), volume 581 de 2014, páginas 46 à 51.

O artigo 3 intitulado "*Paraoxonase (PON)1 Q192R functional genotypes and PON1 Q192R genotype by smoking interactions are risk factors for the metabolic syndrome, but not overweight or obesity*" foi publicado no Redox Report (fator de impacto: 1.710), volume 19 de 2014, páginas 232 à 241.

O artigo 4 intitulado "*Factors influencing insulin resistance in relation to atherogenicity in mood disorders, the metabolic syndrome and tobacco use disorder*" foi publicado no Journal of Affective Disorder (fator de impacto: 3.705), volume 179 de 2015, páginas 148 à 155.

4.1 Lowered plasma paraoxonase (PON1) activity is a trait marker of major depression and PON1 Q192R gene polymorphism-smoking interactions differentially predict the odds of major depression and bipolar disorder.

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Research report

Lowered plasma paraoxonase (PON)1 activity is a trait marker of major depression and PON1 Q192R gene polymorphism–smoking interactions differentially predict the odds of major depression and bipolar disorder

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ABSTRACT

Background: Major depression and bipolar disorder are accompanied by the activation of immune-inflammatory and Oxidative and Nitrosative Stress (O&NS) pathways and lowered levels of antioxidants. Paraoxonase (PON)1 (EC 3.1.8.1) is an antioxidant bound to High Density Lipoprotein (HDL). Polymorphisms in the PON1 Q192R coding sequence determine three functional genotypes, i.e. 192QQ, 192QR and 192RR.
Aims: This study was carried out to delineate the associations of plasma PON1 activity and functional PON1 Q192R genotypes in major depression and bipolar disorder.
Methods: PON1 status that is plasma PON1 abundance and three functional PON1 Q192R genotypes were assayed in 91 major depressed and 45 bipolar patients and compared to 199 normal controls.
Results: Major depression, but not bipolar disorder, was accompanied by lowered PON1 activity. PON1 activity was decreased by smoking and a diagnosis by genotype interaction (i.e. lower PON1 in major depression with the QQ genotype). Logistic regression showed that smoking by QQ genotype significantly increased the odds of bipolar disorder and that major depression was predicted by plasma PON1 activity, serum HDL cholesterol and interactions between genotype × smoking.
Discussion: The results suggest that lowered plasma PON1 activity is a trait marker of major depression and that PONQ192R gene–environment (smoking) interactions differentially predict the odds of depression and bipolar disorder.
Limitations: Association studies are prone to a risk of false positive findings and replication is essential.
Conclusions: The findings suggest that there are differential PON1 Q192R functional genotype × environment interactions in major depression and bipolar disorder. The effects of lowered PON1 activity may contribute to increased O&NS and immune-inflammatory burden in depression. PON1 status may contribute to the comorbidity between depression and other immune- and O&NS-related disorders, e.g. cardiovascular disorder.

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1. Introduction

Growing evidence shows that activated immune-inflammatory (Maes et al., 1995; Berk et al., 2011; Leonard and Maes, 2012), Oxidative and Nitrosative Stress (O&NS) pathways (Maes, 2000; Maes et al., 2011a, 2011b, 2011c; Berk et al., 2011; Moylan et al., 2012) and lowered High Density Lipoprotein (HDL) cholesterol (Maes et al., 1997) play an important role in major depression and bipolar disorder. Activation of these pathways and lowered levels of key antioxidants, such as zinc, coenzyme Q10 and glutathione play a role in the neuroprogressive processes (that is increased neurodegeneration and apoptosis and lowered neurogenesis) and secondary autoimmune responses directed against oxidatively/nitrosatively modified epitopes that accompany depression and bipolar disorder (Berk et al., 2011; Leonard and Maes, 2012; Moylan et al., 2012). Increased translocation of gram-negative bacteria is another immune-inflammatory pathway noted in major depression (Maes et al., 2008). The strong comorbidity between depression and many (auto)immune disorders, including Cardiovascular Disorder (CVD), Chronic Obstructive Pulmonary Disorder (COPD) and Inflammatory Bowel Disease (IBD), may be explained by shared immune-inflammatory and O&NS pathways and lowered levels of antioxidants (Maes et al., 2011a, 2011b, 2011c).

There are a few reports that major depression and bipolar disorder may be associated with lowered activity of Paraoxonase 1 (PON1, EC 3.1.8.1), an enzyme with paraoxonase and arylesterase activity (Barim et al., 2009; Kotan et al., 2011a, 2011b; Ezzaher et al., 2010). PON1 is a hydrolytic antioxidant enzyme with a very wide range of substrates (Litvinov et al., 2012). PON1 is in the plasma almost exclusively bound to HDL and is responsible for most of the antioxidant activity of HDL (Razavi et al., 2012). HDL-associated PON hydrolyzes hydrogen peroxide and contributes to protective effects against lipid peroxidation and formation of oxidized Low Density Lipoproteins (LDL) and consequently CVD (Aviram et al., 1998). PON1 plays a role in the detoxification of Organophosphate Pesticides (OPs), has hydrolytic activity against some gram negative bacteria and is a strong anti-inflammatory and antioxidative agent (Furlong et al., 2010). The latter effects help explain the role of PON1 in immune- and O&NS-related disorders, e.g. CVD, COPD, lung cancer, IBD, etc. (Furlong et al., 2010; Isik et al., 2005; Rothem et al., 2007; Goswami et al., 2009).

PON1 is under genetic control and polymorphisms of the PON1 gene may determine the enzymatic activities, e.g. polymorphism Q→R at the 192 position (Q192R) (Liu et al., 2013). There is evidence that this Q192R polymorphism, i.e. RR genotype and the R allele, is associated with elevated risk for ischemic stroke (Liu et al., 2013). The Q isoform is associated with a lower paraoxon hydrolyzing activity and decreased protective activity against HDL and LDL oxidation (Mackness and Mackness, 2013). Individuals with a QQ genotype are more susceptible to genotoxicity, whereas the RR genotype may be more protective against oxidative stress (Singh et al., 2011; Kotani et al., 2012).

Barim et al. (2009) and Kotan et al. (2011a, 2011b) reported lower arylesterase activity in untreated patients with depression, while treatment with antidepressants increased arylesterase levels. Sarandol et al. (2006) and Kodydková et al. (2009), on the other hand, were unable to find any differences in PON activity between depressed patients and controls. There is also a report on lowered PON1 activity in bipolar disorder (Ezzaher et al., 2010). Lawlor et al. (2007) detected that in elderly women the R allele was significantly associated with depression (odds ratio 1.22). In two independent population-based studies no significant association was found between PON1 Q192R polymorphism and depression (Rice et al., 2009). Statins such as atorvastatin increase PON1 activity and statins are now thought to have a role in both the prevention and reduction of depressive symptoms (Samy and Hassanian, 2011; Pasco et al., 2008).

However, it is advocated that the assay of PON1 status should be based on the assays of both PON1 enzymatic activity and PON1 Q192R functional genotypes (Furlong et al., 2010). In addition, data on PON1 status should consider smoking effects because cigarette smoking decreases PON1 plasma activity (Haj Mouhamed et al., 2012) and genetic variation in O&NS and antioxidant genes and their interaction with smoking may affect inflammatory and oxidative burden (Stephens et al., 2008). Smoking and nicotine dependence are accompanied with the onset of depressive disorders and suicidal behavior (Vargas et al., 2013; Breslau et al., 2004; 2005; Jamal et al., 2012; Malone et al., 2003; Pasco et al., 2008).

The present study was carried out to examine PON1 status (both PON1 activity and functional PONQ192R polymorphism based on a 2-substrate assay) in major depression and bipolar disorder in relation to nicotine dependence.

2. Subjects and methods

2.1. Subjects

We recruited normal controls ($n=199$) and patients with major depression ($n=91$) or bipolar disorder ($n=45$) from staff at the Londrina State University (UEL) and an outpatient ambulatory of smoking cessation program, UEL, Parana, Brazil. Participants of Caucasian, Asian, African and mixed ethnicities, aged 18–60 years, were accepted in this study. Diagnoses of bipolar disorder and major depressive disorder were made by senior clinicians using the validated Portuguese version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) interview, Clinical Version (SCID-CV) (Del Ben et al., 2001). In this study, we combined patients in the acute phase of illness and patients in partial or total remission and separated these in two study groups, i.e. major depression versus bipolar disorder.

Based on a SCID-CV interview, normal controls with lifetime or current axis 1 and axis 2 diagnoses were excluded from participating in this study. In addition, we collected data on medical disorders and use of medication by means of a semi-structured interview performed by senior psychiatrists. Patients with axis 1 diagnoses other than major depression or bipolar disorder were excluded, e.g. schizophrenia, substance abuse disorders, psycho-organic syndromes, and anxiety disorders. Patients and controls were excluded (a) when they showed increased plasma levels of laboratory tests, such as hemogram, Aspartate Transaminase (AST), Alanine Transaminase (ALT), urea and creatinine; (b) when they suffered from any major medical illness, e.g. diabetes type 1 and 2, (auto)immune disorders, IBD, COPD, and neuroinflammatory disorders; (c) when they were treated with immunomodulatory drugs, including glucocorticoids; and (d) when they showed acute inflammatory or infective reactions the month prior to the study.

The severity of illness was measured using a validated Portuguese version of the 21-item Hamilton Depression Rating Scale (HDRS) which was adapted for use in the Brazilian population (Moreno and Moreno, 1998). Ethnicity (Caucasian, Asian, African, mixed ethnicities), years of education, marital status (single, stable relationship, separated or widowed), use of alcohol (no use, monthly, weekly, daily use), work disabled due to medical reasons, use of statins, and body weight and height were collected using a self-report questionnaire. A clinical diagnosis of nicotine dependence was made using DSM-IV criteria. Body Mass Index (BMI) was calculated as weight (kg)/body height (in meter)^2. The study was approved by the Research Ethics Committee at UEL (number

250/2010). All participants gave written informed consent prior to participating in this study.

2.2. Laboratory assessments

Blood for the assay of plasma PON1 enzyme activity and PON1 Q192R genotypes and serum HDL was sampled at 8.00 a.m. after an overnight fast. PON1 status (plasma activity levels and functional position 192 genotype) was determined using assays as described by Richter et al., 2010. The substrates were phenylacetate (PA, Sigma, USA) and 4-(chloromethyl)phenyl acetate (CMPA, Sigma, USA) and the analysis was conducted in a microplate spectrophotometer reader (EnSpire, Perkin Elmer, USA) using ultraviolet transparent 96-well microplates. All assays were carried out in triplicate and replicates that varied by > 10% were repeated. Briefly, CMPA hydrolysis was measured at 280 nm for 4 min at 25BC using 20 μL of plasma diluted 1:40 in dilution buffer [20 mmol/L Tris–HCl (pH 8.0), 1.0 mmol/L $CaCl_2$]. PA hydrolysis under high salt conditions was measured at 270 nm for 4 min at 25BC using 20 μL of plasma diluted 1:40 in dilution buffer. High salt media was composed by PA added to 2 mol/L NaCl, 20 mmol/L Tris–HCl (pH 8.0), 1.0 mmol/L $CaCl_2$. The results obtained with these two assays were used to plot a 2-dimensional enzyme activity graphic that displays rates of arylesterase activity (PA hydrolysis) under high salt conditions versus CMPase activity (CMPA hydrolysis). Since the polymorphism Q192R confers differential catalytic activity against these two substrates, the plot splits the population into the three functional position 192 genotypes (QQ, QR and RR). Measurement of PA hydrolysis at low salt concentrations reveals plasma PON1 abundance since at this condition PON1 Q192R polymorphism does not influence PON1 catalytic activity against PA (Richter et al., 2010). For this assay, rates of hydrolysis of PA under low salt conditions were measured at 270 nm for 4 min at 25BC using 20 μL of plasma diluted 1:80 in dilution buffer.

Serum HDL cholesterol was determined by an automated method in a clinical chemistry system, Dimension RXL (Siemens Healthcare Diagnostics Inc, Newark, DE, USA). This method measures HDL cholesterol levels directly, without the need for sample pretreatment or specialized centrifugation steps. HDL cholesterol was measured as a surrogate marker of HDL. The inter-assay coefficient of variation values were less than 4.0%.

2.3. Statistics

Analyses of contingence tables (Chi square test) were employed to check the distribution of variables in different study groups. Univariate analyses of variance (ANOVAs) were used to ascertain differences in PON activity, serum HDL and other continuous variables between study groups, e.g. controls versus major depression and bipolar disorder and QQ, QR and RR genotypes. Power analyses, performed using GPower 3.1, showed that for the examination of differences in PON1 between 3 groups at $\alpha=0.05$, power=0.80 and effect size of 0.20 the minimum sample size should be around 240. Factorial design analyses of covariance (ANCOVA) were employed to delineate the differences in continuous variables among groups taking into account the effects of different categories and their interactions and while adjusting for relevant covariates. When significant, the Tukey test or Bonferroni's test were used to examine planned comparisons among treatment means. Binary logistic regression analyses were used to examine the relationships between major depression or bipolar disorder (versus controls) as dependent variable and independent variables, including PON1 activity, PON1 genotypes (using additive, dominant and recessive models and fitting interaction models) and clinical/socio-demographic data. Relationships between variables were assessed by means of Pearson's correlation coefficients and linear multiple regression analyses. As PON1 is bound to HDL and contributes to the effects of HDL, the PON1 × HDL cholesterol product term was computed to denote possible (antioxidative) potentiating effects between both compounds. Transformations were used to normalize the distribution of PON1 activity, the PON1 × HDL cholesterol product term and years of education in Ln transformation. Data was analyzed using the SPSS windows version to analyze our data. Statistical significance was set at $\alpha=0.05$ (two tailed).

3. Results

3.1. Demographic data

Table 1 shows the demographic data of the 335 subjects in this study. There were no significant differences in age, ethnicity, marital status, work disabled, use of statins and years of education between the three diagnostic groups. There were more females in both mood disorder groups than in the normal control group. Smoking frequency was higher in bipolar participants than in normal controls ($\chi^2=12.48$, df=1, $p<0.001$) and depressed patients ($\chi^2=10.36$, df=1, $p=0.001$). BMI was significantly higher in bipolar participants than in depressed participants ($p=0.049$) and controls ($p=0.014$). Bipolar participants had a significantly increased HDRS score than controls ($p<0.001$) and depressed participants ($p<0.001$), while the latter had a higher HDRS score than controls ($p<0.001$).

Table 2 shows that there were no differences in age, gender, marital status, years of education, use of statins, BMI and HDRS between the PON1 Q192R genotypes. There was a significant association between ethnicity and PON1 Q192R genotypes. There was a marginally significant association between smoking and the three genotypes. Table 3 shows that in individuals with a QQ genotype but not in those with a QR+RR genotype there was an association between smoking and diagnosis.

3.2. PON1 activity and PON1 Q192R genotypes in depression

There were weak although significant correlations between PON1 and HDL ($r=0.189$, $p=0.001$, $n=331$), age ($r=-0.156$, $p=0.004$, $n=335$) and years of education ($r=0.282$, $p<0.001$, $n=335$), but not BMI ($r=-0.054$, $p=0.325$, $n=333$) and HDRS ($r=-0.094$, $p=0.085$, $n=334$). PON1 was significantly lower ($F=33.61$, df=1/333, $p<0.001$) in smokers (mean ± SD = 174.68 ± 50.63 U/mL) than in non smokers (mean=205.91 ± 53.48 U/mL). There were no significant differences in PON1 between men and women ($F=0.61$, df=1/333, $p=0.436$), ethnical groups ($F=1.11$, df=2/332, $p=0.331$), work disabled or not ($F=0.00$, df=1/333, $p=0.991$), marital status ($F=0.47$, df=2/332, $p=0.628$), use of statins ($F=2.47$, df=1/314, $p=0.12$) or use of alcohol ($F=0.44$, df=4/329, $p=0.781$).

Table 1 shows the mean PON1 activities in controls and depressed and bipolar participants. Simple ANOVA showed that there are significant differences between the groups, with lower levels in major depression than in controls (Tukey test: $p=0.028$), while no significant differences were found between bipolar disorder and depression ($p=0.722$) or controls ($p=0.500$). ANOVA showed no significant differences in HDL concentrations between the three study groups. Table 2 shows that there were no significant differences in PON1 activity and HDL cholesterol between the three PON1 Q192R genotypes.

Table 4 shows the outcome of univariate factorial design ANCOVA with PON1 activity as dependent variable and smoking, PON1 Q192R genotypes (QQ versus QR+RR), diagnosis (all entered as factors), age, gender, BMI, HDRS, and years of education as explanatory variables. We found that PON1 activity was significantly ($F=4.46$, df=16/315, $p<0.001$; $R^2=18.5\%$) predicted by age

Table 1
Demographic and clinical data of the controls and major depressed (MDD) and bipolar patients (BP) included in this study.

Variables	Controls	MDD	BP	F	df	p
Age (years)	46.4 (±8.3)	47.0 (±8.2)	44.5 (±8.9)	1.34	2/332	0.265
Gender				9.45	2	0.009
Female	117	69	33			
Male	82	22	12			
Ethnicity				7.38	4	0.117
Caucasians	136	67	26			
Asian+African	34	10	6			
Mixed	29	14	13			
Marital status				1.26	4	0.868
Single	27	15	8			
Stable	136	57	29			
Separated or widow	36	19	8			
Work disabled				4.52	2	0.105
No	184	81	37			
Yes	15	10	8			
Years of education	13.27 (±5.29)	14.60 (±7.71)	11.96 (±5.82)	1.98	2/332	0.14
Smoking				14.25	2	0.001
No	122	55	14			
Yes	77	36	31			
BMI (kg)/body height (in meter)^2	26.84 (±4.80)	26.27 (±4.35)	28.76 (±5.77)	4.16	2/330	0.017
HDRS	2.77 (±3.67)	8.96 (±7.11)	14.20 (±12.43)	69.53	2/331	<0.001
Plasma PON1 (U/mL)	197.16 (±50.64)	183.74 (±58.91)	189.52 (±59.99)	3.45	2/332	0.033
Serum HDL (mg/dL)	49.01 (±14.51)	45.86 (±14.95)	43.98 (±11.64)	2.99	2/328	0.051
PON1 × HDL	9871 (±4613)	8531 (±4250)	8109 ±(3437)	5.44	2/328	0.005

HDRS: total score on the Hamilton Depression Rating Scale.
BMI: body mass index.
PON1: paraoxonase 1 plasma activity.
HDL: high density lipoprotein cholesterol.

Table 2
Demographic and clinical data in subjects divided according to paraoxonase (PON)1 Q192R genotypes into QQ, QR and RR carriers.

Variables	QQ	QR	RR	F	df	p
Age (years)	46.1 (±8.5)	46.6 (±8.0)	46.2 (±9.0)	0.128	2/332	0.879
Gender				5.03	2	0.081
Female	89	94	36			
Male	62	39	15			
Ethnicity				22.63	4	<0.001
Caucasians	121	83	25			
Asian+African	11	27	12			
Mixed	19	23	14			
Marital status				6.13	4	0.189
Single	19	19	12			
Stable	102	93	27			
Separated or widow	30	21	12			
Years of education	14.38 (±7.19)	12.84 (±4.48)	12.29 (±6.21)	2.02	2/332	0.135
Smoking				7.67	2	0.022
No	97	72	22			
Yes	54	61	29			
BMI (kg)/body height (in meter)^2	26.47 (±4.71)	27.38 (±4.97)	27.20 (±5.04)	1.33	2/330	0.266
HDRS	5.77 (±7.64)	5.68 (±7.69)	7.49 (±8.04)	1.13	2/331	0.324
Plasma PON1 (U/mL)	201.7 (±61.4)	185.7 (±47.9)	183.1 (±44.5)	2.73	2/332	0.067
Serum HDL (mg/dL)	47.89 (±14.34)	47.48 (±14.18)	46.29 (±15.28)	0.228	2/328	0.796
PON1 × HDL	9838 (±4890)	8935 (±4101)	8456 (±3567)	1.95	2/328	0.144
Diagnosis				2.44	4	0.656
Controls	90	78	31			
MDD	45	34	12			
BD	16	21	8			

HDRS: total score on the Hamilton Depression Rating Scale.
BMI: body mass index.
PON1: paraoxonase 1 plasma activity.
HDL: high density lipoprotein cholesterol.
MDD: major depression.
BD: bipolar disorder.

Table 3
Differences in the association between smoking and diagnosis in paraoxonase (PON)192 QQ versus RR or QR carriers.

Genotype	Controls	MDD	BD	χ^2	df	p
QQ						
No smoking	63	30	4	12.14	2	0.002
Smoking	27	15	12			
QR+RR						
No smoking	59	25	10	3.80	2	0.150
Smoking	50	21	19			

MDD: major depression.
BD: bipolar disorder.

Table 4
Results of univariate analysis of covariance with plasma paraoxonase (PON)1 activity as dependent variable and diagnosis, PON1 Q192R genotypes, smoking, Body Mass Index (BMI), the Hamilton Depression Rating Scale (HDRS), years of education, gender and age as explanatory variables (entered as factors or covariates).

Explanatory variables	F	df	p
Diagnosis[a]	6.69	2	0.001
Genotype[a] (i.e. QQ versus QR+RR)	0.03	1	0.856
Smoking[a]	15.3	1	0
Diagnosis[a] × genotype[a]	3.35	2	0.036
Diagnosis[a] × smoking[a]	0.9	2	0.408
Genotype[a] × smoking[a]	0.2	1	0.655
Diagnosis[a] × genotype[a] × smoking[a]	2.08	2	0.126
BMI	0.03	1	0.86
HDRS	3.17	1	0.076
Years of education	3.75	1	0.054
Age	4.41	1	0.036
Gender	0.21	1	0.645

[a] These variables were entered as factors.

(negative), diagnosis, smoking, and a diagnosis × genotype interaction (i.e. lower PON1 in major depression with the QQ genotype). Bonferroni post hoc tests showed lower PON1 activity in major depression versus controls ($p=0.001$), while there were no significant differences between bipolar disorder and either controls ($p=0.99$) and major depression ($p=0.332$). After entering HDL cholesterol in this analysis, the effects of diagnosis remained significant while HDL was also a significant explanatory variable ($F=4.2$, df=1/314, $p=0.041$). Using the same predictor variables, we found that the product term POX × HDL was significantly predicted by diagnosis ($F=10.43$, df=1, $p<0.001$), smoking ($F=19.76$, df=1, $p<0.001$), gender ($F=18.65$, df=1, $p<0.001$) and BMI ($F=6.47$, df=1, $p=0.012$). Bonferroni's post hoc tests showed that the product ratio was significantly lower in depression than in controls ($p<0.001$), while there were no significant differences between bipolar participants and controls ($p=0.291$) or depressed subjects ($p=0.354$).

In the patient groups we were unable to find any significant difference in PON1 activity ($F=0.13$, df=1/132, $p=0.720$, results of factorial ANOVA with diagnosis as second factor) between patients who were drug free for at least 2 months (mean=184.44 ± 60.21 U/mL, $n=105$) and those who had taken psychotropic drugs (mean=189 ± 55.97 U/mL, $n=31$). In drug free depressed participants (mean=181.18 U/mL, $n=76$) PON activity was significantly lower ($F=8.05$, df=1/273, $p=0.005$) than in controls (mean=197.16 ± 49.01 U/mL, $n=199$).

3.3. *Logistic regression of odds of major depression compared to normal controls*

Table 5 shows the results of logistic regression analysis with depression versus controls as dependent variable. Entering PON1 Q192R genotypes (RR is used as reference group; Wald=0.54, df=2, $p=0.76$) and smoking (Wald=0.20, df=1, $p=0.888$) separately showed that both variables did not increase the odds of depression. We found that depression was significantly distinguished from controls ($\chi^2=105.42$, df=10, $p<0.001$; Nagelkerke=0.432); the number of correctly classified cases was 81.1% with a sensitivity of 53.8% and a specificity of 93.8%. The HDRS and an interaction between PONQ192R genotypes and smoking increased the odds of major depression, while PON1 activity decreased the odds of depression. The interaction pattern between PON1 Q192R genotypes and smoking shows that in non smokers the QQ and QR genotypes increase the odds of depression versus the RR phenotype. Logistic regression performed on the drug free depressed patients showed similar main and interaction effects. Ethnicity (Wald=0.80, df=2, $p=0.961$) or ethnic groups by smoking (Wald=0.301, df=2, $p=0.860$) did not show significant relationships with major depression.

Table 5
Results of logistic regression analysis with major depression versus controls as dependent variable and plasma paraoxonase (PON)1 activity, PON1 Q192R genotypes, Body Mass Index (BMI), the Hamilton Depression Rating Scale (HDRS), age, gender and High Density Lipoprotein (HDL) cholesterol as independent variables.

Variables	Wald	df	p	Odds ratio	Lower 95% CI	Upper 95% CI
Plasma PON1	6.4	1	0.011	0.216	0.066	0.708
Serum HDL	9.04	1	0.003	0.961	0.936	0.986
Age	0.026	1	0.871	1.003	0.966	1.041
Gender	2.95	1	0.086	0.535	0.262	1.092
BMI	0.862	1	0.353	0.968	0.903	1.037
HDRS	49.1	1	< 0.001	1.329	1.227	1.439
QQ genotype[a]	0.316	1	0.574	0.701	0.203	2.421
QR genotype[a]	0.4	1	0.527	0.68	0.206	2.245
Genotype[a] × smoking	15.27	2	< 0.001	–	–	–
QQ genotype[a] × smoking (0)	11.01	1	0.001	7.89	2.33	26.7
QR genotype[a] × smoking (0)	8.24	1	0.004	5.545	1.721	17.86

CI: confidence intervals.
[a] The RR genotype was used as the reference group.

3.4. *Logistic regression of odds of bipolar disorder compared to normal controls*

We performed a similar logistic regression analysis (without using HDRS as independent variable) with bipolar disorder and normal controls as dependent variables (Table 6). After adjusting for the effects of all other explanatory variables, we observed that bipolar participants significantly differed from controls ($\chi^2=26.53$ df=9, $p=0.002$) using female gender and smoking by genotype QQ as independent variables. PON1 activity and HDL were not significant in distinguishing the bipolar and control groups.

4. Discussion

The first major finding of this study is that plasma PON1 activity was significantly decreased in patients with major depression, but not in those with bipolar disorder. These results correspond with those of Barim et al. (2009) who found lowered arylesterase activity in patients with depression using assays with arylesterase with phenylacetate as substrates. Kotan et al. (2011a, 2011b) also reported lowered arylesterase activity of PON1 in depressed patients. Using a comparable method, two other studies were unable to find lowered PON1 activity in depression (Kodykova et al. 2009; Sarandol et al., 2006). Our study failed to

replicate the findings of Ezzaher et al. (2010) who reported decreased PON1 activity in bipolar disorder.

The reductions in PON1 activity may in theory contribute to the activated O&NS (including lipid peroxidation) and immune-inflammatory processes in depression. For example, PON1 hydrolyzes hydrogen peroxide and inhibits LDL oxidation and lipid peroxidation and the consequent inflammatory responses to LDL (Mackness and Mackness, 2013). PON −/− mice show a significant increase in vascular inflammation and aortic superoxide and consequently CVD (Ng et al., 2008; Shih et al., 1998). Inflammatory responses in depression, including increased interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF)α, may lower PON1 mRNA production and plasma PON1 activity thereby modulating the antiinflammatory and antioxidative properties of HDL (Kumon et al., 2003). Thus, both cytokines could play a role in the comorbidity between depression and artherosclerosis (Maes et al., 2011a, 2011b, 2011c) via alterations in PON1 mRNA (Kumon et al., 2003). Nevertheless, IL-6, another cytokine that is increased in depression (Maes et al., 1993), enhances PON1 gene expression in hepatocytes (Cheng et al., 2013).

The second major finding of this study is that PON1 Q192R functional genotypes may play a role in depression and bipolar disorder. As with Rice et al. (2009), we were unable to validate a previous report that the R allele is significantly associated with depression (Lawlor et al., 2007). We could similarly not validate the findings of Ezzaher et al. (2012) that there is an association between bipolar disorder and the QR and RR genotypes. Nevertheless, we found different interactions between PON1 Q192R genotypes and smoking and diagnosis. Firstly, major depressed subjects with a QQ genotype (in contrast to QR and RR) showed lowered PON1 activity. Secondly, the odds of major depression was significantly increased by an interaction between PON1 Q192R genotype and smoking, i.e. in non smokers the QQ and QR genotypes increased the odds to depression. The odds of bipolar disorder were increased by the QQ genotype in smokers, suggesting that the effects of smoking on bipolar disorder are mediated by PON1 Q192R polymorphisms.

This interaction may be explained by the effects of functional PON1 Q192R genotypes and smoking on immune-inflammatory and O&NS pathways. Firstly, smoking increases risk for mood disorders through activation of immune-inflammatory and O&NS pathways, including effects on proinflammatory cytokines, microglial activation, increased lipid peroxidation, decreased levels of antioxidants and neuroprogressive pathways (Nunes et al., 2013; Edirisinghe and Rahman, 2010; Vargas et al., 2013). Smoking-induced activation of these pathways including suppression of PON1 activity may explain the increased incidence of immune- and O&NS-related degenerative disorders in smokers, e.g. CVD, COPD, lung cancer, IBD, etc. (Minicucci et al., 2012; Milara and Cortijo, 2012; Leone, 2011). Secondly, individuals with the Q isoform have also less protective activity against oxidative stress, including HDL and LDL oxidation, lower paraoxon hydrolyzing activity and are more susceptible to genotoxicity (Mackness and Mackness, 2013).

Table 6
Results of logistic regression analysis with bipolar disorder versus controls as dependent variable and paraoxonase (PON)1 activity, PON1 Q192R genotypes, Body Mass Index (BMI), age, gender and serum High Density Lipoprotein (HDL) cholesterol as independent variables.

Variables	Wald	df	p	Odds ratio	Lower 95% CI	Upper 95% CI
Plasma PON1	0.558	1	0.455	0.566	0.127	2.518
Serum HDL	1.66	1	0.198	0.98	0.95	1.011
Age	2.95	1	0.086	0.964	0.925	1.005
Gender (female)	4.18	1	0.041	2.383	1.036	5.481
BMI	2.9	1	0.089	1.058	0.992	1.129
QQ genotype[a]	1.51	1	0.219	0.417	0.103	1.684
QR genotype[a]	0	1	0.973	0.98	0.31	3.101
Genotype[a] × smoking (1)	7.48	2	0.024	–	–	–
QQ genotype[a] × smoking (1)	6.86	1	0.009	5.647	1.547	20.619
QR genotype[a] × smoking (1)	0.828	1	0.363	1.631	0.569	4.675

CI: confidence intervals.

[a] The RR genotype was used as the reference group.

A third major finding of this study is that smoking not only interacts with the functional PON1 genotypes to predict bipolar disorder but also decreases plasma PON1 activity. We replicated previous findings that cigarette smoking lowers PON1 plasma activity in all three PON1 Q192R genotypes (Haj Mouhamed et al., 2012). In CVD patients and controls, smoking was associated with lowered PON activity an effect that was readily reversed upon cessation (James et al., 2000). In vitro, cigarette smoke extracts inhibit PON1 activity in a dose- and time dependent manner by modification of free thiols (Nishio and Watanabe, 1998). Evidently, the PON1 lowering effects of smoking may contribute to inflammatory and oxidative burden and thus to the increased incidence of degenerative disorders (Nishio and Watanabe, 1998; James et al., 2000). Likewise, the same pathways may in part account for the association between smoking and bipolar disorder. It is possible that the smoking status of patients and controls could explain some of the discrepancies in the literature on plasma PON1 in depression. Accordingly, some (e.g. Barim et al., 2009), but not all studies (e.g. Kodykova et al., 2009; Kotan et al., 2011a, 2011b) controlled their data for smoking.

Not all catalytic specifications and substrates of PON1 are known and therefore other putative pathways may explain the association between depression and functional PON1 Q192R genotypes and lowered PON1 activity. For example, PON1 alters bacterial colonization and displays lactonase activity hydrolyzing acyl homoserine lactones, which function as quorum sensing signals in gram negative bacteria (Pezzulo et al., 2012; Camps et al., 2011; Teiber and Draganov, 2011). Thus lowered PON1 activity could play a role in the immune effects directed against the increased translocation of gram negative bacteria in depression (Maes et al., 2008).

One limitation of our study is that we did not control for all possible dietary effects. PON1 activity is increased by polyphenol-rich diets and moderate alcohol consumption, while high fat diets may decrease PON1 activity (Thomas-Moya et al., 2007; Gouedard et al. 2004; Costa et al., 2011). Nevertheless, the subjects included in this study were largely on a traditional Brazilian diet and subjects with alcohol abuse were excluded from this study, while there was no association between moderate alcohol consumption and plasma PON1 activity. There were no effects of treatment with psychotropic drugs on our results because (a) there were no significant differences in PON1 activity between patients who were drug free for at least two months and those who took psychotropic drugs; and (b) drug free major depressed subjects showed significantly lowered PON1 activity than controls. In addition, Kotan et al. (2011a, 2011b) found that treatment with antidepressants in standard doses for 24 weeks normalized decreased baseline PON activity in depressed patients. Another limitation of the study is that reports from "association studies constitute tentative knowledge and must be interpreted with caution" (Sullivan, 2007). Strengths of our study are that we adjusted our results for many potential confounders, including age, sex, ethnicity, BMI, use of statins, education and excluded subjects with abnormal liver and kidney tests and with illnesses that affect plasma PON1 activity, e.g. liver cirrhosis, renal disease, increased uremia, diabetes, etc.

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None.

Conflict of interest

No specific funding was obtained for this specific study.

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4.2 PARAOXONASE 1 STATUS AND INTERACTIONS BETWEEN Q192R FUNCTIONAL GENOTYPES BY SMOKING CONTRIBUTE SIGNIFICANTLY TO TOTAL PLASMA RADICAL TRAPPING ANTIOXIDANT POTENTIAL

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Paraoxonase 1 status and interactions between Q192R functional genotypes by smoking contribute significantly to total plasma radical trapping antioxidant potential

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HIGHLIGHTS

- TRAP and PON1, 197 healthy, 91 with unipolar depression and 45 with bipolar disorder.
- PON1, male gender, RR genotype and body mass index are inversely correlated with TRAP.
- PON1 activity, interactions between PON1 Q192R genotypes and smoking influence TRAP.

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ABSTRACT

The measurement of the total radical trapping antioxidant potential (TRAP) is a general marker of peripheral blood antioxidant defenses. Paraoxonase 1 (PON1) is a potent antioxidant, which protects against lipid peroxidation. The study aimed to examine the relation between TRAP levels and PON1 activity, PON1 Q192R functional genotypes, smoking, interactions between PON1 genotypes and smoking, and mood disorders, while adjusting for effects of ethnicity, marital status, body mass index (BMI) and gender. The analyses were performed in 197 controls and 136 subjects with mood disorders. TRAP levels were significantly associated with higher plasma PON1 activity, the RR functional genotype, non smoking by RR carriers, male gender and a higher BMI. TRAP levels were significantly lower in patients with mood disorders than in controls, but this association was no longer significant after considering the effects of the above predictors. The risk in the subgroup with low TRAP levels is increased by a smoking X RR genotype interaction and decreased by male gender, the RR genotype, and higher BMI and PON1 activity. Plasma PON1 activity, the PON1 Q192R functional genotypes and specific interactions between this genotype and smoking contribute significantly to TRAP levels. Gender and BMI also appear to influence TRAP levels.

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Abbreviations: ANCOVAs, analysis of covariance; ANOVAs, analysis of variance; ASSIST, alcohol, smoking and substance involvement screening; BD, bipolar disorder; BMI, body mass index; CMPA, 4-chloromethyl phenol acetate; DSM-IV, diagnostic and statistical manual of mental disorder; GLM, general linear analysis; HDL, high-density lipoprotein; HDRS, Hamilton depression rating scale; LDL, low-density lipoprotein; OS, oxidative stress; PA, phenyl acetate; PON1, paraoxonase 1; ROS, reactive oxygen species; SEM, standard error of the mean; TRAP, total radical trapping antioxidant potential; TUD, tobacco use disorder.

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1. Introduction

Cigarette smoke contains hundreds of compounds which act as pro-oxidants and produce reactive oxygen species (ROS). Smoking enhances oxidative stress (OS) and decreases antioxidant defense mechanisms [1]. Mood disorders, including major depression and bipolar disorder (BD), are accompanied by increased OS and lowered levels of antioxidants [2].

One major measure of total plasma antioxidant defense is the total radical trapping antioxidant potential (TRAP) in plasma [3]. An important antioxidant enzyme is paraoxonase 1 (PON1), which is lowered in individuals with mood disorders and tobacco use disorder (TUD) [4]. PON1 is an enzyme synthesized in the liver, and secreted into plasma where it is bound to high-density lipoprotein (HDL) particles. These protect against lipoprotein peroxidation, a key process underpinning the pathophysiology of atherosclerosis [5]. Polymorphisms of the PON1 gene determine PON1 enzymatic activities, e.g. the polymorphism with Q/R substitution at position 192 [4,5]. This functional Q192R polymorphism determines PON1 enzyme activity which in turn protects low-density lipoprotein (LDL) from oxidation. In this regard the Q192 alloenzyme is more efficient than the R192 aloenzyme [5].

The aim of this study is to examine whether TRAP levels are associated with PON1 status, smoking, mood disorders, interactions between the PON1 Q192R genotypes and smoking, ethnicity, marital status, body mass index (BMI) and gender.

2. Subjects and methods

2.1. Subjects

The participants were 197 normal controls, 91 patients with major depression and 45 with BD recruited at the State University of Londrina. Participants of Caucasian, Asian, African and mixed ethnicities, aged 18–65 years, were enrolled in this study. The diagnoses of BD, depression and tobacco use disorder (TUD, current smokers) were made using a validated Portuguese version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [6]. All TUD subjects were current smokers who at the time of interview reported smoking every day or some days. Mood disorder patients in the acute phase of illness and in partial or total remission were included. Subjects with diagnoses other than depression and BD (e.g. schizophrenia, substance abuse disorders, and psycho-organic syndromes) were excluded. Normal controls were individuals without a lifetime or current diagnosis of axis I.

We excluded individuals with abnormal values of hemogram, aspartate transaminase, alanine transaminase, urea, creatinine; who suffered from diabetes, (auto)immune disorders, chronic obstructive pulmonary disorder, inflammatory bowel disease, and neuroinflammatory disorders; who were treated with immunomodulatory drugs or antioxidant supplements.

The severity of depression was measured using a validated Portuguese version of the Hamilton Depression Rating Scale (HDRS) [7]. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) was used to assess risk levels of alcohol use [8]. BMI was calculated as weight (kg)/body height (in meter)^2. The study was approved by the Research Ethics Committee (number 250/2010). All participants gave written informed consent prior to participating in this study.

2.2. Laboratory assessments

Blood was sampled after an overnight fast. TRAP was measured by chemiluminescence in an adaptation of the method described by Repetto et al. (1996) [9]. A comparison of the induction time after the addition of known concentrations of Trolox and plasma is used to measure TRAP values of Trolox equivalents.

PON1 status was determined as described by Richter et al. (1999) [10]. 4-Chloromethyl phenol acetate (CMPA) hydrolysis and phenyl acetate (PA) hydrolysis under high salt conditions was measured in a microplate spectrophotometer. The results obtained with these two assays were used to plot a 2-dimensional enzyme activity graphic that displays rates of arylesterase activity under high salt conditions versus CMPase activity. Since the polymorphism Q192R confers differential catalytic activity against these two substrates, the plot splits the population into the three functional positions 192 genotype (QQ, QR and RR). Measurement of PA hydrolysis at low salt concentrations reveals plasma PON1 activity since at this condition PON1 Q192R polymorphism does not influence PON1 catalytic activity against PA [10].

2.3. Statistics

Analyses of variance (ANOVAs) and covariance (ANCOVAs) were performed to examine differences between treatments means followed by Tukey's test. Analyses of contingency tables (χ^2 tests) were performed to ascertain differences in the distribution of variables between diagnostic categories. Relationships between variables were checked using Pearson's correlation coefficients and stepwise General linear model (GLM) analyses. We used automatic stepwise binary logistic regression analyses to examine the associations between patients with lower TRAP levels (versus higher TRAP levels, median-split dichotomized) as dependent variable and different explanatory variables, including PON1 status, smoking, BMI, gender, etc. The data were analyzed using SPSS and the statistical significance set at $\alpha = 0.05$ (two tailed).

3. Results

3.1. Plasma TRAP measurements

Table 1 shows that TRAP levels were significantly lower in women than in men. There were no significant associations between TRAP levels and ethnicity or marital status. Patients had significantly lower plasma TRAP levels than controls. TUD patients had significantly lower TRAP levels than non-smokers. There were no significant differences in TRAP values between QQ, QR and RR carriers.

In the total study group, there were no significant correlations between plasma TRAP levels and age ($r = -0.003$, $p = 0.952$, $n = 333$), years of education ($r = 0.059$, $p = 0.286$, $n = 333$), and alcohol use ($r = 0.082$, $p = 0.137$, $n = 332$). There were significant correlations between plasma TRAP and PON1 activity ($r = 0.125$, $p = 0.022$, $n = 333$), BMI ($r = 0.202$, $p < 0.001$, $n = 333$) and HDRS ($r = -0.200$, $p < 0.001$, $n = 332$).

Table 2 shows the results of GLM analyses with TRAP as dependent variable and age, BMI, years of education, plasma PON1 activity, HDRS, use of alcohol, gender, smoking, PON1 Q192R genotypes (entered as QQ+QR versus RR), diagnosis (entered as controls versus patients or as controls versus depression versus BP disorder), marital status, and ethnicity as explanatory variables. 28.4% of the variance in plasma TRAP levels was explained by PON1, the interaction smoking by PON1 Q192R genotypes, BMI and gender ($F = 16.03$, $df = 8/324$, $p < 0.001$). PON1, BMI, male gender and the RR genotype were significantly associated with increased TRAP levels. TRAP was significantly higher in RR than in QQ ($p = 0.009$) and QR ($p = 0.024$) carriers, but there were no significant differences between QQ and QR carriers ($p = 0.642$). The interaction pattern showed that non smoking in subjects with a RR and

Table 1
Measurements of total radical trapping antioxidant potential (µM Trolox) in 333 subjects.

Variables	Type	*n*	Mean (SD)	*F*	*df*	*p*
Gender	Men	115	901.4 (132.6)	60.43	1/331	<0.001
	Women	218	785.3 (138.2)			
Ethnicity	Caucasian	228	825.6 (138.6)	0.46	2/330	0.634
	African Asian	50	838.2 (126.6)			
	Mixed	55	812.8 (134.0)			
Marital status	Single	49	834.6 (119.5)	0.17	2/330	0.845
	Stable	221	825.0 (141.2)			
	Separated, widowed	63	819.7 (130.6)			
Diagnosis	Controls	197	839.6(136.5)	5.43	1/331	0.020
	Mood disorders	136	804.9 (132.9)			
Diagnosis	Controls	197	839.6 (136.5)	2.75	2/330	0.065
	Depression	91	808.2 (136.8)			
	Bipolar disorder	45	798.1 (125.8)			
TUD	No	190	837.7 (133.2)	4.05	1/331	0.045
	Yes	143	809.1 (138.2)			
PON1 Q192R Genotypes	QQ QR RR	151	822.0 (127.7)	1.36	2/330	0.258
		132	817.9 (140.9)			
		50	855.4 (144.9)			

Mood disorders: depression and bipolar disorder.
TUD: tobacco use disorder.

Table 2
Results of general linear model analysis with total radical trapping antioxidant potential as dependent variable and the listed variables as explanatory variables.

Variables	*F*	*df*	*p*	*η*
Plasma PON1	5.87	1/324	0.016	0.018
Smoking X PON1 Q192R genotypes	3.76	5/324	0.003	0.055
Body mass index	26.59	1/324	<0.001	0.076
Gender (male)	88.45	1/324	<0.001	0.214

PON1: paraoxonase 1.
PON1 Q192R genotypes: entered as QR and RR genotypes with QQ as reference group.
η: partial η^2.

QR genotype is associated with increased TRAP levels: TRAP levels were significantly higher in non-smokers than in smokers in QR (adjusted mean ± SEM = 875.0 ± 14.3 versus 806.7 ± 15.4 µM Trolox) and RR (906.3 ± 25.5 versus 863.6 ± 21.9 µM Trolox) carriers (the means were obtained by GLM analysis after adjusting for the abovementioned background variables). There were no differences in TRAP between QQ smokers (833.0 ± 16.0 µM Trolox) and QQ non smokers (835.2 ± 12.3 µM Trolox). All other variables were not significant in this regression analysis including diagnostic classifications. In addition, forced entry of the three diagnostic groups (depression, bipolar disorder and controls) showed that these diagnostic groups were not significant in predicting TRAP (F = 1.20, df = 2/322, p = 0.301), while PON1 (F = 6.11, df = 1/322, p = 0.014), smoking X PON1 Q192R genotypes (F = 3.60, df = 5/322, p = 0.004), BMI (F = 28.15, df = 1/322, $p < 0.001$) and gender (F = 84.30, df = 1/322, $p < 0.001$) remained significant in predicting TRAP. ANOVA showed that in TUD subjects, TRAP was not significantly different (F = 1.56, df = 2/140, p = 0.213) between QQ (mean ± SD = 822.9 ± 109.9 µM Trolox, n = 54), QR (785.4 ± 146.8 µM Trolox, n = 60) and RR individuals (832.2 ± 162.6 µM Trolox, n = 29).

We examined the effects of psychotropic drugs on our results. There were no significant differences (F = 0.08, df = 1/331, p = 0.775) in TRAP levels between subjects without (mean ± SD 824.6 ± 135.7 µM Trolox, n = 296) and with psychotropic drug use (831.4 ± 139.7 µM Trolox, n = 37). We re-ran the above GLM analysis in subjects who were drug free. 30% of the variance in TRAP was explained (F = 15.38, df = 8/287, $p < 0.001$) by PON1 (F = 4.63, df = 1/287, p = 0.016), smoking X PON1 Q192R genotypes (F = 3.05, df = 5/287, p = 0.011), BMI (F = 29.78, df = 1/287, $p < 0.001$) and gender (F = 81.61, df = 1/287, $p < 0.001$).

3.2. Characteristics of subjects with low versus high plasma TRAP levels

We dichotomized the study group into two equal groups using 818.80 µM Trolox as threshold value. Table 3 lists the characteristics of subjects with lowered versus higher TRAP levels. There were no significant differences in age, ethnicity, marital status, diagnosis, smoking, PON1 Q192R genotypic distribution, plasma PON1 activity and years of education between the two study groups. Subjects with lower TRAP levels showed a higher frequency of females and a lower BMI than subjects with higher TRAP levels.

Table 4 shows the results of an automatic stepwise logistic regression analysis with the low TRAP subgroup as dependent variable (<818.80 µM Trolox) and all variables listed in Table 3 and the interaction pattern smoking X PON1 Q192R genotypes as explanatory variables. We found that using five predictors, 67.3% of all cases were correctly classified (sensitivity = 70.7% and specificity = 63.9%; χ^2 = 72.61, df = 5, $p < 0.001$; Nagelkerke = 0.261). PON1, male gender, BMI and the RR genotype were inversely associated with the lower TRAP subgroup, whereas the interaction smoking by RR carriers increased the risk to belong to the low TRAP group.

4. Discussion

The findings of this study are that TRAP was significantly related to plasma PON1 activity, PON1 Q192R genotypes, an interaction between smoking and these genotypes, BMI and gender. In addition, subjects with mood disorders displayed lowered TRAP levels than controls, although these effects were not significant in the final regression analysis. TUD subjects showed lower levels of TRAP than controls, but these effects were no longer significant after

Table 3
Characteristics of subjects divided into those with lower versus higher (median-dichotomized) total radical trapping antioxidant potential (TRAP) levels.

Variables	Type	Lower TRAP (n = 167)	Higher TRAP (n = 166)	F or X2	df	p
TRAP (μM)Trolox)	-	718.3 (±76.6)	933.1(±89.4)	544.95	1/331	<0.001
Gender	Men Women	31136	8482	37.80	1	<0.001
Age (years)	-	46.3 (±8.2)	46.4 (±8.6)	0.00	1/331	0.946
Ethnicity	Caucasian African Asian Mixed	1132331	1152724	1.22	2	0.542
Marital status	Single Stable Separated + widowed	2410736	2511427	1.52	2	0.467
Diagnosis	Controls Mood disorders	9176	10660	30.2	1	0.082
Diagnosis	Controls Depression BD	914828	1064317	4.41	2	0.129
TUD	No Yes	9077	10066	1.37	1	0.242
PON1 Q192R Genotypes	QQ QR RR	787019	736231	3.53	2	0.171
Plasma PON1 (U/mL)		188.6 (±56.8)	196.1(±52.2)	1.55	1/331	0.213
BMI (kg/m^2)	-	26.03 (±4.84)	27.86(±4.74)	12.20	1/331	0.001
Education (years)	-	13.3 (±5.5)	13.6 (±6.8)	0.12	1/331	0.723

Results are shown as mean (±SD).
Results of analyses of variance (*F*) or analyses of contingence tables (X^2 tests).
BD: bipolar disorder.
TUD: tobacco use disorder.
BMI: body mass index.

considering the interaction between current smoking and PON1 Q192R genotypes. TRAP levels were significantly associated with higher plasma PON1 activity. PON1 is a HDL-associated enzyme with multifunctional activities including antioxidant properties [11]. Therefore, our findings confirm that this enzyme plays a role as one of the antioxidants contributing to total TRAP levels.

The RR functional genotype was associated with higher TRAP levels. These findings corroborate those of previous papers that the RR genotype is more protective against OS and that the QQ genotype increases susceptibility to genotoxicity [12]. The Q isoform is associated with a lowered protective activity against LDL and HDL oxidation and reduced paraoxon hydrolyzing activity [13]. Given the role of OS in aging, it is also interesting to note that QR and RR carriers have a better survival advantage than Q allele carriers and those patients with a QQ genotype have worse diabetes control than those with a RR genotype [14]. All in all, our data suggest that RR individuals are more protected than QQ carriers against OS.

We found that smoking decreased TRAP levels in RR and QR carriers. Thus, smoking has a stronger effect on antioxidant defenses in RR and QR individuals than in QQ individuals. Chronic smoking is related to depletion of antioxidant levels [15], total plasma antioxidant capacity and PON1 plasma activity [16]. Moreover, the acute effects of smoking also result in increased lipid peroxidation [17]. Our study however shows that the genotype-smoking interaction explained more of the variance in plasma TRAP levels than smoking alone, suggesting that the effects of smoking on total plasma TRAP levels are attributable to the effects in QR and RR carriers only. Future research should delineate whether the effects of smoking enhancing oxidative biomarkers and decreasing specific antioxidants are mediated by interactions between smoking and PON1 Q192R genotypes.

Our results showed that TRAP levels were significantly higher in males than in females. These results extend findings that women may show higher levels of OS than men [18]. For example, in a study sample of normal volunteers and patients with chronic fatigue, we found significantly higher plasma peroxide levels in women than in men [18]. Such differences may be associated with the lower plasma TRAP levels in women observed in our study. In preclinical experiments of peripheral tissue antioxidant defenses were higher in female than in male rats, while brain lipid peroxidation was higher in males than in females [19]. Higher levels of vitamin E and elevated activity of glutathione peroxidase were detected in females [20–22]. Estrogens have significant antioxidant properties, although the exact mechanisms by which estrogens exert this action remain unknown [23–25].

We found decreased TRAP levels in patients with mood disorders (either depression or BD) compared to controls. Moreover, there was an inverse correlation between TRAP and severity of depression. Those results corroborate previous data that mood disorders are accompanied by lowered antioxidant levels, either total plasma antioxidant activity or specific antioxidants and increased OS [2,26–28]. The differences between patients with mood

Table 4
Results of automatic stepwise logistic regression analysis with lower versus higher (median-dichotomized) TRAP levels (higher plasma TRAP as reference group) as dependent variable and the listed variables as predictors.

Variables	Wald	df	p	Odds ratio	CI lower	CI upper
Plasma PON1	4.61	1	0.032	0.39	0.16	0.92
PON1 Q192R genotypes	9.55	1	0.002	0.15	0.05	0.51
Smoking (1) X PON1Q192R genotype	5.62	1	0.018	5.42	1.34	21.91
Body mass index	18.39	1	<0.001	0.89	0.84	0.94
Gender (male)	41.78	1	<0.001	0.17	0.10	0.29

CI: 95% confidence intervals.
PON1 Q192R genotypes: paraoxonase1 Q192R genotypes, QQ + QR versus RR.

disorders and normal controls, however, were no longer significant after considering the effects of the other variables, suggesting that the association between mood disorders and TRAP levels are mediated by other variables.

Surprisingly, the results of this study show that plasma TRAP levels are significantly and positively correlated to BMI. In the ATTICA study, on the other hand, obese and overweight males and females showed lower total antioxidant capacity concentrations as compared to normal weight individuals [29]. In accordance with the ATTICA study, the Framingham offspring cohort, in which a series of laboratory assessments, obesity measures, OS and cardiovascular risk factors were measured, showed that obesity, particularly central obesity, was an independent positive predictor for systemic OS [30]. Obesity is known to increase OS in normal volunteers and clinical populations of all ages as indicated by increased lipid peroxidation [31]. Abdominal and hepatic fat increases lipid peroxidation through excess of free fatty acids, lipoprotein-bound lipids and cytokines [32]. Nevertheless, TRAP levels mainly reflect the antioxidant potential of urate (35–65%). There is evidence that uric acid is significantly related to the BMI [33]. Therefore, our findings that plasma TRAP levels are related to BMI may be explained by the effects of uric acid, which is positively associated with BMI.

This study has strengths and limitations which need to be noted in interpreting the results. In the present study, we controlled statistically for effects of ethnicity, drug state, including use of psychotropic medication and statins, and use of alcohol. A first limitation is that this study is cross sectional and therefore no deductions can be made on causal relationships. A follow-up study should be carried out to delineate the interrelationships between TRAP plasma levels and uric acid, on the one hand, and BMI and gender, on the other. Lastly, in this study we examined the effects of current TUD defined as patients with nicotine dependence who were current smokers. Thus, our study design does not allow examination of the chronic versus acute effects of smoking tobacco.

In conclusion, TRAP levels are significantly predicted by higher plasma PON1 activity, the RR functional genotype, non smoking by RR carriers, gender and BMI. Plasma PON1 activity, PON1 genotypes and an interaction between PON1 Q192R genotypes and TUD contribute significantly to plasma TRAP levels, independently of the effects of gender and BMI.

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4.3 PARAOXONASE (PON)1 Q192R FUNCTIONAL GENOTYPES AND PON1 Q192R GENOTYPE BY SMOKING INTERACTIONS ARE RISK FACTORS FOR THE METABOLIC SYNDROME, BUT NOT OVERWEIGHT OR OBESITY

Paraoxonase (PON)1 Q192R functional genotypes and PON1 Q192R genotype by smoking interactions are risk factors for the metabolic syndrome, but not overweight or obesity

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Background: The metabolic syndrome (MetS) is a complex of multiple risk factors that contribute to the onset of cardiovascular disorder, including lowered levels of high-density lipoprotein (HDL) and abdominal obesity. Smoking, mood disorders, and oxidative stress are associated with the MetS. Paraoxonase (PON)1 is an antioxidant bound to HDL, that is under genetic control by functional polymorphisms in the PON1 Q192R coding sequence.

Aims and methods: This study aimed to delineate the associations of the MetS with plasma PON1 activity, PON1 Q192R genotypes, smoking, and mood disorders (major depression and bipolar disorder), while adjusting for HDL cholesterol, body mass index, age, gender, and sociodemographic data. We measured plasma PON1 activity and serum HDL cholesterol and determined PON1 Q192R genotypes through functional analysis in 335 subjects, consisting of 97 with and 238 without MetS. The severity of nicotine dependence was measured using the Fagerström Nicotine Dependence Scale.

Results: PON1 Q192R functional genotypes and PON1 Q192R genotypes by smoking interactions were associated with the MetS. The QQ and QR genotypes were protective against MetS while smoking increased metabolic risk in QQ carriers only. There were no significant associations between PON1 Q192R genotypes and smoking by genotype interactions and obesity or overweight, while body mass index significantly increased MetS risk. Smoking and especially severe nicotine dependence are significantly associated with the MetS although these effects were no longer significant after considering the effects of the smoking by PON1 Q192R genotype interaction. The MetS was not associated with mood disorders, major depression or bipolar disorder.

Discussion: PON1 Q192R genotypes and genotypes by smoking interactions are risk factors for the MetS that together with lowered HDL and increased body mass and age contribute to the MetS.

Keywords: Paraoxonase 1, Metabolic syndrome, Depression, HDL cholesterol, Oxidative stress, Cardiovascular

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Introduction

The metabolic syndrome (MetS) is a combination of risk factors that are associated with an increased risk

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for cardiovascular disorder (CVD), cancer, type 2 diabetes mellitus, depression, and all-cause mortality.[1–3] There are different case definitions for the MetS (e.g. the criteria of the International Diabetes Foundation, the National Cholesterol Education Program, and the World Health Organization), which all consider similar risk factors, including increased central obesity as measured by means of waist circumference, increased fasting glucose (insulin resistance) and triglyceride levels, lowered high-density lipoprotein (HDL) levels, and increased blood pressure.[1,2,4,5] The MetS frequently coexists with obesity, as measured by an increased body mass index (BMI).[6] In some studies, both the MetS and BMI contribute to increased CVD risk, whereas in other studies the MetS, but not BMI, predicts CVD risk.[6,7]

The key components in the pathophysiology of the MetS syndrome are central obesity, insulin resistance and an increased flux of fatty acids stimulating elevated glucose production, and secretion of triglycerides and very low-density lipoproteins (LDLs).[2,8] Lowered levels of HDL cholesterol and increased levels of LDL and leptin are accompanying key components of the MetS.[4] Other theories, on the other hand, stress that central obesity is a compensatory mechanism and protects other non-adipose, lipid-sensitive tissues from the increased fatty acid spill over due to excess calories and dietary lipids in the presence of increased leptin resistance.[9]

New pathways that play a role in the MetS are activation of immune-inflammatory and oxidative and nitrosative stress (O&NS) pathways as indicated by increased levels of pro-inflammatory cytokines, acute phase proteins, and biomarkers of increased lipid peroxidation.[10] In accordance with the findings on activated O&NS pathways, the MetS is also accompanied by lowered antioxidant defenses, as shown by reduced total antioxidant potential in the plasma.[11]

Paraoxonase/arylesterase 1 (PON1, EC 3.1.8.1) is an antioxidant enzyme that is bound to plasma HDL and determines part of the antioxidant capacity of HDL.[12,13] PON1 associated with HDL attenuates the production of reactive oxygen species (hydrogen peroxide) thereby contributing to lowered production of oxidized LDL.[14] These antioxidant and anti-inflammatory effects of PON1 may explain that lowered levels are associated with an increased risk for CVD and insulin resistance.[15–17] There is now evidence that the MetS is accompanied by lowered plasma PON1 activity or PON1 concentrations.[18–23]

PON1 is under genetic control whereby functional polymorphisms at the 192 position Q→R determine the enzymatic activities and consequently increase risk to different diseases.[24] For example, the R allele and the RR genotype are associated with an increased risk for CVD, such as ischemic stroke.[24] The Q isoform confers lowered protective activity against oxidation of LDL and HDL.[25] In a recent study, we found that PON1 Q192R genotypes were associated with an increased Castelli 1 index, suggesting increased atherogenic potential (de Souza-Nogueira *et al.*, submitted). There is one study showing that PON1 QR and RR genotypes significantly increase the risk of MetS.[26]

Smoking is one of the environmental factors that may increase the risk of MetS. Thus, in a number of smaller studies an association between smoking and the MetS has been described.[27] A meta-analysis based on 13 studies and 56.691 subjects and 8.688 cases showed a significant positive relationship between smoking and the MetS.[28] Slagter *et al.*[29] in a large-scaled study showed that there was a positive association between smoking and the MetS, independent of BMI class and gender. Smoking not only affects the metabolism of fatty acids but also induces inflammatory reactions, increases lipid peroxidation, and lowers PON1 levels.[30,31] In addition, we recently observed that an interaction pattern between smoking and PON1 genotypes predicts an increased Castelli 1 index and therefore could be an important risk factor for the MetS (submitted).

Another factor that is related to an increased risk towards MetS is the presence of both unipolar and bipolar depression.[32,33] These mood disorders show a high degree of comorbidity with obesity, CVD, and diabetes type 2.[34] While increased rates of central obesity in mood disorders may be one factor explaining the comorbidity with the MetS, other shared processes are lowered HDL cholesterol and activated immune-inflammatory and O&NS pathways, and common environmental risk factors such as diet, smoking, and physical activity.[34–40] Moreover, patients with mood disorders have an increased rate of current smoking[41], while current smoking, interactions between PON1 Q192R genotypes and smoking, and reduced plasma PON1 levels are also risk factors for unipolar depression or bipolar disorder.[42] Possible interactions between smoking, mood disorders, and PON1 Q192R genotypes could therefore play a role in the MetS.

The aim of the present study was to examine whether lowered PON1 activity and PON1 functional Q192R polymorphisms, e.g. the RR genotype, current smoking, and mood disorders or their interactions may increase the odds to MetS, while controlling for other putative intervening factors, such as BMI, age, gender, and sociodemographic data.

Subjects and methods

Subjects

In this study, we included 335 subjects of Caucasian, African, Asian, and mixed ethnicities and aged

between 18 and 60 years old. The subjects were recruited from staff at the Londrina State University and an outpatient ambulatory of smoking cessation program, UEL, Parana, Brazil, and comprised 238 subjects with the MetS and 97 subjects without the MetS; 146 subjects with mood disorders, that is unipolar major depression ($n = 91$) or bipolar disorder ($n = 45$), versus 199 subjects without mood disorders, and 144 smokers versus 191 non-smokers. We have excluded subjects (a) with abnormal laboratory tests, including aspartate transaminase, alanine transaminase, hemogram, urea, and creatinine; (b) who had been taken immunomodulatory drugs, including glucocorticoids and antiviral medications; and (c) with other major medical illness, e.g. diabetes type 1 and 2, chronic obstructive pulmonary disease, (auto)-immune disorders, inflammatory bowel disease, CVD, and neuroinflammatory disorders. Patients with mood disorders were excluded if they had a lifetime history of other axis 1 Diagnostic and Statistical Manual of Mental Disorders (DSM) IV diagnoses, such as organic mental disorders, schizophrenia, substance abuse disorders, anxiety disorders, etc., while subjects without mood disorders were excluded for any axis I DSM-IV diagnosis. The study was approved by the Institutional Review Board of the UEL (number 250/2010) and all subjects gave written informed consent prior to participating in this study.

Methods

The diagnosis of MetS was made using the diagnostic criteria of the International Diabetes Federation, i.e. three of the five criteria should be present: (a) abdominal obesity, that is waist circumference ≥90 cm for men and ≥80 cm for women in South Asian and South Americans and ≥94.0 cm for men and ≥80.0 cm for women in Caucasians; (b) low HDL cholesterol, that is <40 mg/dl in men and <50 mg/dl in women, or on hypolipidemic drugs; (c) hypertriglyceridemia, that is ≥150 mg/dl, or on a hypolipidemic agent; (d) increased fasting glucose, that is ≥100 mg/dl, or on oral antidiabetic medication; (e) increased average blood pressure, that is ≥130/85 mmHg, or currently taking antihypertensive medication.[2,5,43] We measured waist circumference during expiration, in a standing and relaxed position, at the midline between the lower costal margins and the iliac crest parallel to the floor. We measured blood pressure using a mercury sphygmomanometer on the right arm and used the mean value of two measurements that were carried out 5 minutes apart. We calculated the BMI as weight (in kg) divided by square of height (in m^2).

The diagnosis of mood disorders was made by senior psychiatrists employing a validated Portuguese translation of the semi-structured interview of the DSM-IV.[44] We have examined the effects of the two mood disorders combined and or of each mood disorder, either major depression or bipolar disorder separately. We combined patients in the acute phase of illness and patients in partial or total remission. We measured the severity of depression using a validated Portuguese translation of the Hamilton Depression Rating Scale (HAM-D), which was adapted for use in the Brazilian population.[45] The diagnosis 'nicotine dependence' was made according to DSM-IV criteria. The severity of dependence was estimated using the Fagerström Nicotine Dependence Scale.[46] We used the six items of this scale to examine possible relationships between aspects of nicotine dependence with the MetS, while we used the total score as an index for the severity of nicotine dependence. We also divided the subjects into these with severe nicotine dependence (total score ≥5) versus those with moderate dependence (total score <4).[46] We used a self-report questionnaire to collect sociodemographic and clinical characteristics, including years of education, marital status (single, stable relationship, separated, or widowed), ethnicity (Caucasian, Asian, African, and mixed), use of alcohol (no use, monthly, weekly, daily use), and use of statins and any other medications.

Biomedical assays

Fasting blood (12–14 hours) was collected in all subjects for the assays of plasma PON1 activity, PON1 Q192R genotypes, and HDL cholesterol. PON1 status, that is PON1 plasma activity and PON1 Q192R functional genotypes or activity phenotypes, were measured as described before.[47] In short, the substrates were phenylacetate (PA, Sigma, St Louis, MO, USA) and 4-(chloromethyl)phenylacetate (CMPA, Sigma) and the analysis was conducted in a microplate spectrophotometer reader (EnSpire, Perkin Elmer, NY 14831, USA) using ultraviolet transparent 96-well microplates. All assays were carried out in triplicate and replicates that varied by >10% were repeated. Briefly, CMPA hydrolysis was measured at 280 nm for 4 minutes at 25°C using 20 μl of plasma diluted 1:40 in dilution buffer (20 mmol/l Tris-HCl (pH 8.0), 1.0 mmol/l $CaCl_2$). PA hydrolysis under high salt conditions was measured at 270 nm for 4 minutes at 25°C using 20 μl of plasma diluted 1:40 in dilution buffer. High salt media was composed by PA added to 2 mol/l NaCl, 20 mmol/l Tris-HCl (pH 8.0), 1.0 mmol/l $CaCl_2$. The results obtained with these two assays were used to plot a two-dimensional enzyme activity graphic that displays rates of arylesterase activity (PA hydrolysis) under high salt conditions versus CMPase activity (CMPA hydrolysis). Since the polymorphism Q192R confers differential catalytic activity

against these two substrates, the plot splits the population into the three functional position 192 genotypes (QQ, QR, and RR). Measurement of PA hydrolysis at low salt concentrations reveals plasma PON1 total activity, since at this condition PON1 Q192R polymorphism does not influence PON1 catalytic activity against PA.[47] For this assay, rates of hydrolysis of PA under low salt conditions were measured at 270 nm for 4 minutes at 25°C using 20 μl of plasma diluted 1:80 in dilution buffer.

Fig. 1 shows the division of the participants into the three functional PON1 Q192R polymorphisms: 151 individuals (45.1%) were homozygous for the PON1*192Q allele; 133 (39.7%) were heterozygous; and 51 (15.2%) were homozygous for the PON1*192R allele. Regarding PON1 plasmatic activity, which is determined by the hydrolysis of PA under low salt conditions, it varied from 47.67 to 414.60 U/ml (data not shown).

HDL cholesterol was assayed using an automated method in a clinical chemistry system, Dimension® RXL (Siemens Healthcare Diagnostics Inc., Newark, DE, USA). This assay measures HDL cholesterol without sample pretreatment or specialized centrifugation steps. HDL cholesterol was used as a surrogate marker of HDL. Triglycerides and glucose were determined using automated methods in a clinical chemistry system, Dimension® RXL (Siemens Healthcare Diagnostics Inc.). The inter-assay coefficients of variability for all analytes were <5.0%.

Statistics

Analysis of variance (ANOVA) was used to check the differences in continuous variables between different categories. The Mann–Whitney *U* test was employed to examine intergroup differences in the case variables

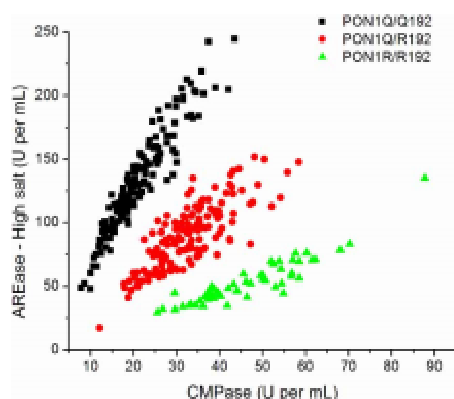

Figure 1 Functional genotyping for the PON1 Q192R polymorphism through the hydrolysis of CMPA versus PA under high salt condition. Each data point indicates one individual.

that were not normally distributed. Analyses of contingence tables were checked using χ^2 tests to assess differences in the distribution of variables among study groups. We used Pearson's correlation coefficients to assess the relationships between variables. Binary logistic regression analyses were employed to delineate the associations between a dichotomous dependent variable (the presence or absence of a characteristic), i.e. the diagnosis MetS versus no MetS, and a set of independent variables, e.g. PON1 status and smoking and their interactions, and clinical/sociodemographic data. The logistic regression coefficients are employed to estimate odds ratios (*B* with 95% upper and lower confidence intervals for each explanatory variable in the final model). Multinomial regression analysis was used to investigate the associations between a dependent variable with more than two categories, i.e. three groups divided according to the BMI, and a set of independent variables, e.g. PON1 status and smoking and their interactions, and clinical/sociodemographic data. We used transformations to normalize the distribution of PON1 activity and years of education (ln transformation). We analyzed the data using the SPSS Versions 15 and 19. Statistical significance was set at $\alpha = 0.05$ (two-tailed).

Results

Demographic data

Table 1 displays the demographic data of the 335 subjects subdivided into those with and without MetS. We did not use a *P*-correction to examine the multiple contrasts as these univariate analyses were used a priori to delineate the possible relevant variables to be consecutively employed as determinants of independent association with the MetS or obesity/overweight in ultimate multivariate analyses. There were no significant differences in age and gender between both groups. There was a significant association between smoking and MetS with more smokers in the MetS group. Likewise, there was a significant association between MetS and groups divided according to non-smoking and moderate and severe nicotine dependence. There was no significant association between PON1 Q192R genotypes and MetS. There were similarly no significant differences in PON1 activity between subjects with and without the MetS. Subjects with a MetS had significantly lower levels of HDL cholesterol and higher levels of triglycerides and glucose, waist circumference, systolic blood pressure, and BMI than those without MetS. Likewise, there was a significant association between MetS and groups divided based on BMI values. There were no associations between MetS and mood disorders, unipolar depression and bipolar disorder, HAM-D, education, ethnicity, marital status, and use of statins or

Table 1 Demographic data of the 355 subjects in the study divided into those with ($n = 97$) and without ($n = 238$) the MetS

Variables	No MetS ($n = 238$)	MetS ($n = 97$)	F or χ^{2*}	df	P
Age (years)	45.8 (8.1)	47.6 (8.9)	3.08	1/333	0.080
Gender (male/female)	78/160	38/59	1.25	1	0.264
Current smoking (no/yes)	146/92	45/52	6.29	1	0.012
Nicotine dependence: no/moderate/significant	146/27/65	45/15/37	6.29	2	0.043
Fagerström Nicotine Dependence Scale in smokers	5.53 (2.09) $n = 92$	5.92 (2.47) $n = 52$	1.02	1/142	0.315
QQ/QR/RR genotypes	115/92/v31	36/41/20	4.76	2	0.093
PON1 activity (U/ml)	195.5 (55.3)	185.2 (51.9)	2.46	1/333	0.117
HDL-cholesterol (mg/dl)	51.1 (14.6) $n = 234$	38.9 (9.6) $n = 97$	57.42	1/329	<0.001
Triglycerides (mg/dl)	105.5 (55.4)	192.9 (97.1)	107.42	1/333	<0.001
Glucose (mg/dl)	86.3 (8.1)	100.3 (25.5)	57.60	1/330	<0.001
Waist circumference (cm)	87.3 (13.2)	98.0 (11.3)	45.35	1/330	<0.001
Systolic blood pressure (mmHg)	117.2 (16.4)	134.2 (18.7)	66.8	1/321	<0.001
BMI (kg/m^2)	25.8 (4.4) $n = 236$	29.6 (5.0) $n = 96$	47.69	1/330	<0.001
BMI < 25, 25–30, ≥30 kg/m^2	117/88/31	12/47/37	47.93	2	<0.001
No mood – versus mood disorders	145/93	54/43	0.79	1	0.374
No mood – UP – BP	145/66/27	54/25/18	3.09	2	0.214
HAM-D	5.8 (7.9)	6.3 (7.4)	0.21	1/332	0.648
Education < 12 versus ≥ 12 years	94/144	51/46	4.80	1	0.028
Ethnicity C/A + A/M	168/34/36	61/16/20	2.07	2	0.356
Marital St/single/S + W	162/31/45	60/19/18	2.39	2	0.302
Statin use (yes/no)	18/207	8/83	0.054	1	0.817
Use of alcohol (no, 1, 2, 3, 4)	105/39/27/60/6	49/14/9/21/4	2.00	4	0.735
Use of any medication (yes/no)	119/117	66/31	8.64	1	0.003

Data are shown as mean (±SD).
*Results of ANOVA or contingency tables with χ^2 test.
No mood – UP – BP, UP, unipolar depression; BP, bipolar disorder; HAM-D, Hamilton Depression Rating Scale; Ethnicity: C, Caucasian; A + A, Asian and African; M, mixed; Marital: St, stable relationship or married; S + W, separated and widowed; Use of alcohol (alcohol abuse is excluded): no; 1, sporadically; 2, monthly; 3, weekly; 4, daily.

alcohol. The use of any medication was significantly greater in subjects without MetS than in those with MetS.

The MetS and the Fagerström Nicotine Dependence Scale in smokers

Table 1 shows that in smokers there was no significant association between the MetS and moderate versus heavy nicotine dependence ($\chi^2 = 0.00$, df = 1, $P =$ 0.949). Table 1 also shows that in smokers the Fagerström Nicotine Dependence Scale score did not differ significantly between those with and without the MetS. There was a significant relationship between MetS and the fourth item of the Fagerstrom scale, i.e. 'how many cigarettes per day do you smoke?', i.e. 10 or less (8/11), 11–20 (16/49), 21–30 (11/20), and 31 or more (17/12) ($\chi^2 = 10.39$, df = 3, $P = 0.016$). There were no significant associations between MetS and any of the other items of the Fagerström Nicotine Dependence Scale.

The MetS, smoking, and PON1 functional genotypes

Table 2 shows the stratification of the study population on the basis of PON1 Q192R genotypes and smoking while indicating for each subgroup the prevalence of MetS. The prevalence of MetS was significantly different in subgroups formed on the basis of smoking and QQ genotype but not QR and RR genotypes. In non-smokers, there were significantly more individuals with a MetS in QR carriers ($\chi^2 = 4.89$, df = 1, $P =$ 0.027) and especially RR carriers ($\chi^2 = 11.3$, df = 1, $P < 0.001$) than in QQ carriers. In smokers, no such differences were observed.

Table 3 shows the results of two bivariate logistic regression analyses with MetS versus no MetS as a dichotomous dependent variable. Entering current smoking as the only explanatory variable showed that smoking was significantly associated with the MetS (Wald = 6.21, df = 1, $P = 0.013$). Entering moderate and severe nicotine dependence versus non-

Table 2 Stratification of the study population on the basis of PON1 Q192R genotypes and smoking and indicating for each subgroup the (relative) prevalence of the MetS

PON1 Q192R	Smoking	No MetS	MetS	χ^2	df	P
QQ	No	82 (84.5%)	15 (15.5%)	10.48	1	0.001
	Yes	33 (61.1%)	21 (38.9%)			
QR	No	52 (72.2%)	20 (27.8%)	0.68	1	0.408
	Yes	40 (65.6%)	21 (34.4%)			
RR	No	12 (54.5%)	10 (45.5%)	0.63	1	0.427
	Yes	19 (65.5%)	10 (34.5%)			

Table 3 Results of bivariate logistic regression analyses with MetS versus no MetS as dependent variable and the listed variables as explanatory variables

Variables	Wald	df	*P*	Odds ratio	95% Lower CI	95% Upper CI
Smoking	0.63	1	0.428	0.632	0.203	1.967
PON1 Q192R genotype	9.43	2	0.009	–	–	–
QQ	8.77	1	0.003	0.220	0.080	0.599
QR	2.37	1	0.124	0.462	0.172	1.236
Smoking × PON1 Q192R genotype	6.53	2	0.038	–	–	–
QQ × smoking	5.91	1	0.015	5.508	1.392	21.803
QR × smoking	1.24	1	0.265	2.161	0.558	8.377
Smoking	3.55	1	0.060	0.272	0.702	1.054
PON1 Q192R genotype	10.47	2	0.005	–	–	–
QQ	10.36	1	0.001	0.145	0.045	0.470
QR	4.68	1	0.030	0.277	0.087	0.886
Smoking × PON1 Q192R genotype	6.59	2	0.037	–	–	–
QQ × smoking	6.59	1	0.010	8.091	1.642	39.879
QR × smoking	3.56	1	0.059	4.673	0.942	23.193
Age	4.98	1	0.026	1.038	1.005	1.073
Gender (female)	1.61	1	0.205	1.481	0.807	2.718
Mood versus no mood disorders	0.046	1	0.829	0.939	0.533	1.657
Plasma PON1 activity	2.06	1	0.151	1.004	0.998	1.010
Serum HDL cholesterol	40.33	1	<0.001	0.906	0.879	0.934

smoking as explanatory variables showed that severe (Wald = 5.27, df = 1, $P = 0.022$), but not moderate (Wald = 2.61, df = 1, $P = 0.11$) nicotine dependence predicted the MetS. Entering smoking 10 or less, 11–20, 21–30, or 31 or more cigarettes per day versus non-smoking as explanatory variables showed that only 31 or more cigarettes per day significantly predicted the MetS (Wald = 13.59, df = 1, $P < 0.001$).

The first logistic regression analysis in Table 3 shows that PON1 Q192R genotype (QQ is inversely associated with MetS) and the smoking by PON1 Q192R interaction pattern, but not smoking *per se*, increased the odds of belonging to the MetS group (χ^2 = 16.14.42, df = 5, $P = 0.006$; Nagelkerke = 0.067; the number of correctly classified cases was 71.0%). The interaction showed that smoking by QQ carriers increased the odds to MetS.

The second logistic regression in Table 3 shows that the effects of PON1 Q192R genotypes (QQ and QR are inversely associated with MetS) and the interaction smoking by PON1 Q192R genotypes remained significant after considering the effects of HDL, age, and sex ($\chi^2 = 80.81$, df = 10, $P < 0.001$; Nagelkerke = 0.309; the number of correctly classified cases was 76.7%). Smoking *per se*, plasma PON1 activity, and mood disorders were not significant in this analysis. Entering unipolar depression and bipolar disorder versus no mood disorders as an explanatory variable (instead of mood versus no mood disorders) showed that these mood disorders had no significant impact (Wald = 3.42, df = 2, $P = 0.181$). Entering use of any medications as an additional explanatory variable showed that the use of medications was inversely associated with MetS (Wald = 5.38, df = 1, P = 0.020, $B = 0.494$, 95% lower and upper confidence interval: 0.272 and 0.897, respectively) and that the effects of PON1 Q192R genotypes and the interaction PON1 Q192R genotype by smoking remained significant. Adjusting for other putative predictors showed that these were not significant and that the effects of PON1 genotype and the smoking by genotype interaction remained significant, i.e. ethnicity (Wald = 0.29, df = 1, $P = 0.587$); use of alcohol (Wald = 0.79, df = 1, $P = 0.375$); marital status (Wald = 1.95, df = 1, $P = 0.162$); years of education (Wald = 0.09, df = 1, $P = 0.770$), and use of statins (Wald = 0.26, df = 1, $P = 0.609$).

Interaction smoking by PON1 Q192R genotypes

In order to further examine the interaction between smoking and PON1 Q192R genotype, we performed additional logistic regression analyses in QQ, QR, and RR carriers and in smokers and non-smokers separately. We found that smoking was a significant explanatory variable increasing the odds of the MetS in QQ carriers (Wald = 9.91, df = 1, $P = 0.002$), but not in QR (Wald = 0.68, df = 1, $P = 0.409$) or RR carriers (Wald = 0.63, df = 1, $P = 0.428$). Table 4 shows the outcome of logistic regression analyses with the same variables as the second regression in Table 3 but performed in non-smokers and smokers separately. In non-smokers, we found that the PON1 genotypes QQ and QR and HDL cholesterol were inversely associated with MetS ($\chi^2 = 57.84$, df = 7, $P < 0.001$; Nagelkerke = 0.397; the number of correctly classified cases was 83.0%). In smokers, we found that HDL cholesterol was inversely associated with MetS, while age showed a positive relationship ($\chi^2 = 23.41$, df = 7, $P = 0.001$; Nagelkerke = 0.207; the number of correctly classified cases was 72.0%).

Table 4 Results of bivariate logistic regression analyses with MetS as dependent variable and the listed variables as explanatory variables and performed in non-smokers and smokers separately

	Wald	df	P	Odds ratio	95% Lower CI	95% Upper CI
Non-smokers						
PON1 Q192R genotype	10.84	2	0.004	–	–	–
QQ	10.79	1	0.001	0.114	0.031	0.416
QR	5.43	1	0.020	0.221	0.062	0.786
Age	0.63	1	0.429	1.021	0.970	1.075
Gender	2.24	1	0.134	0.488	0.191	1.248
Mood versus no mood disorders	0.68	1	0.408	0.685	0.279	1.679
Plasma PON1 activity	3.62	1	0.057	1.009	1.000	1.017
Serum HDL cholesterol	28.01	1	<0.001	0.871	0.827	0.917
Smokers						
PON1 Q192R genotype	0.063	2	0.969	–	–	–
QQ	0.045	1	0.833	1.120	0.392	3.202
QR	0.055	1	0.808	1.138	0.401	3.227
Age	4.64	1	0.030	1.049	1.004	1.096
Gender	0.32	1	0.573	0.793	0.353	1.779
Mood versus no mood disorders	0.12	1	0.730	1.143	0.534	2.446
Plasma PON1 activity	0.11	1	0.740	1.001	0.994	1.009
Serum HDL cholesterol	14.67	1	<0.001	0.929	0.895	0.965

Shown is the odds ratio *B* and the 95% upper and lower confidence interval (CI) values.

BMI, obesity, and overweight versus the MetS

Entering BMI as an additional explanatory variable in the full logistic regression depicted in Table 3 showed that BMI was significantly associated with MetS (Wald = 27.43, df = 1, $P < 0.001$, $B = 1.187$, 95% lower and upper confidence interval: 1.113 and 1.265, respectively) and that the effects of PON1 Q192R genotypes and the interaction PON1 genotype by smoking remained significant after adjusting for BMI. In order to delineate whether the PON1 Q192R genotypes may predict BMI, we carried out a multinomial regression analysis with obesity (BMI ≥ 30) and overweight (BMI between 25 and 30) as dependent variables (reference group: subjects with BMI < 25). Table 5 shows that two variables were significant in predicting obesity or overweight ($\chi^2 = 45.48$, df = 20, $P = 0.001$; Nagelkerke = 0.147), i.e. HDL cholesterol was significantly and inversely associated with overweight (Wald = 14.11, df = 1, $P < 0.001$, $B = 0.961$) and obesity (Wald = 9.53, df = 1, $P = 0.002$, $B = 0.961$) and male gender was significantly and negatively associated with obesity (Wald = 5.89, df = 1, $P = 0.015$, $B = 0.400$), but not overweight (Wald = 0.98, df = 1, $P = 0.322$).

Table 5 Multinomial regression analysis with obesity, i.e. BMI ≥30 and overweight, i.e. BMI between 25 and 30, as dependent variables and the listed variables as explanatory variables

Variables	χ^2	df	P
Smoking*	0.00	0	–
PON1 Q192R genotype*	0.00	0	–
Smoking × PON1 Q192R genotype	3.94	4	0.415
Age	3.073	2	0.215
Gender	6.21	2	0.045
Mood versus no mood disorders	2.11	2	0.349
Plasma PON1 activity	1.81	2	0.405
Serum HDL cholesterol	18.97	2	<0.001

*This reduced model is equivalent to the final model because omitting the effects does not increase the degrees of freedom.

Discussion

The first major finding of this study is that PON1 Q192R functional genotypes were significantly associated with the MetS. We observed that the QQ and QR genotypes were protective and decreased the odds of having the MetS and thus that RR carriers are more likely to be diagnosed with the MetS. These findings are in accordance with a previous report that the RR allele is significantly associated with an increased risk of the MetS[26] and with the a priori hypothesis that the RR genotype may confer an increased risk of MetS. For example, a significant association between the R allele or the RR genotype and increased risk for CVD has been established by Liu *et al.*[24] PON1 RR/QR genotypes and especially the RR genotype are associated with vascular disease, such as stroke and myocardial infarction.[48] In hypertensive individuals, the 192 R allele augments the risk of stroke.[49] In another study, carriers of the RR (odds ratio 16.24 (6.41–41.14)), but also the QR (odds ratio 2.73 (1.57–4.72)) genotypes had a significant higher risk of coronary artery disease.[50] Also in an Iranian study, the PON1 R allele was found to be an independent risk factor for coronary artery disease.[51] The PON1 R allele and RR genotype are increased in patients with coronary artery disease.[52] In other studies, however, the PON1 Q allele was associated with significantly higher LDL and a worse outcome of CVD.[53] Individuals with the QQ genotype have a higher risk of all-cause mortality and cardiac events than RR and QR individuals.[54]

Our findings that plasma PON1 activity is not lowered in individuals with MetS as compared to those without MetS is not in agreement with previous findings showing lowered PON1 activity in individuals with the MetS.[18–23] However, these discrepancies may be explained by differences in PON1 assays. Q192R polymorphism is known to affect PON1 catalytic activity in a substrate-dependent manner.[47,55] For example, R192 alloforms have a greater catalytic activity for hydrolysis of paraoxon and chlorpyrifos oxon than Q192 alloforms, Q192 alloforms show a higher activity against some nerve agents, and both alloforms show the same activity against PA and diazoxon.[47,55] In our study, we measured PON1 total catalytic (hydrolysis) activity against PA, which at the low salt concentrations used here is not affected by PON1Q192R polymorphism.[47,55] Previous studies in the MetS measured paraoxonase or arylesterase activity or PON1 concentrations using enzyme-linked immunosorbent assay methods.[20–22] Moreover, PONs are primarily lactonases/lactonizing enzymes, with overlapping substrates and distinctive substrate specificities, although their natural substrates are not well characterized.[56] A recent report showed that – until more specific PON1 activity assays employing natural substrates are developed – results of clinical studies may differ depending on the assays that were employed.[57]

We found that current smoking and especially severe nicotine dependence (according to scores on the Fagerström Nicotine Dependence Scale) and use of more than 31 cigarettes per day were significantly associated with the MetS. These findings are in accordance with previous studies showing a significant association between smoking and the MetS.[27–29] Slagter *et al.*[29] additionally found a dose-dependent association between current tobacco use and the MetS. In our study, however, the relationship between tobacco consumption and the MetS was present for heavy tobacco use only, defined as more than 31 cigarettes per day.

The second major finding is that the abovementioned effects of smoking predicting the MetS could be attributed to the interaction between smoking and the PON1 genotypes. Thus, the effects of smoking increasing the odds of MetS were confined to QQ individuals and could not be detected in QR and RR individuals. These results corroborate those of a previous study showing that smoking was significantly associated with a higher risk for myocardial infarction only among QQ homozygotes.[58] In another study, smoking increased the risk of ischemic stroke in QQ homozygotes slightly more than in QR + RR individuals, i.e. odds ratio = 2.84 versus 2.33, respectively.[49] Such effects may be explained by pathophysiological factors, such as findings that the Q isoform confers reduced protection against oxidation of LDL and HDL.[25] Thus, smoking could enhance lipid peroxidation especially in QQ carriers thereby increasing the odds of the MetS and thus CVD in those individuals. Interestingly, a significant interaction between smoking and PON1 genotypes on coronary heart disease risk was also established for the PON1 L55M polymorphism.[59] Interestingly, when MetS and diabetes are present PON1-192RR is associated with an increased risk of coronary stenosis especially in smokers.[60] All in all, the results show that MetS risk associated with smoking may be attributable to interactions with PON1 Q192R polymorphisms.

In this study, we found no significant association between mood disorders, including unipolar depression and bipolar disorder, and the MetS. A recent meta-analysis performed on 29 cross-sectional studies showed that depression and the MetS are significantly associated with a pooled estimate of 1.42 (95% confidence intervals of 1.28–1.57).[3] These discrepancies may perhaps be explained by diagnostic issues, i.e. we used formal DSM-IV diagnosis of mood disorders, while Pan *et al.*[3] showed that the association between depression and the MetS was stronger for self-rated depression (including also subsyndromal depression) than for diagnosis of major depression based on structural interviews. Nevertheless, cohort studies also showed that MetS significantly predicts future clinical depression and that depression may predict future MetS.[3] In line with this, reciprocal relationships appear to exist between depression and CVD, depression and diabetes, and depression and obesity.[61,62] The bidirectional relationships between mood disorders and the MetS may be explained by shared environmental risk as well as disorders in lipid metabolism, including lowered HDL cholesterol, and activation of immune-inflammatory and O&NS pathways (see the Introduction section).

Limitations of the study are the relatively small sample size of the study, the lack of a more complete PON1 genotyping, and the lack of a more complete evaluation of PON1 activity by means of assays on other substrates, including paraoxon and the newer substrate 5-thiobutyl butyrolactone. Another limitation of the study is that reports derived from 'association studies constitute tentative knowledge and must be interpreted with caution'.[63]

In conclusion, this study suggests that the MetS is associated with PON1 Q192R genotypes and a smoking by genotype interaction, but not with lowered PON1 total catalytic activity against PA. The QQ and QR genotypes appear to be protective while smoking by QQ genotype interactions increases the risk of MetS. Severe nicotine dependence and heavy smoking are significantly associated with the MetS, but these effects are no longer significant after

considering the effects of PON1 Q192R genotypes and smoking by genotype interactions. The effects of smoking increasing the risk of the MetS appear to be confined to QQ carriers.

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4.4 Factors influencing insulin resistance in relation to atherogenicity in mood disorders, the metabolic syndrome and tobacco use disorder

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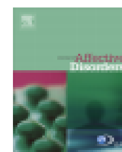

Research report

Factors influencing insulin resistance in relation to atherogenicity in mood disorders, the metabolic syndrome and tobacco use disorder

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ABSTRACT

Objective: This study examines the effects of malondialdehyde (MDA) and uric acid on insulin resistance and atherogenicity in subjects with and without mood disorders, the metabolic syndrome (MetS) and tobacco use disorder (TUD).

Methods: We included 314 subjects with depression and bipolar depression, with and without the MetS and TUD and computed insulin resistance using the updated homeostasis model assessment (HOMA2IR) and atherogenicity using the atherogenic index of plasma (AIP), that is log10 (triglycerides/high density lipoprotein (HDL) cholesterol.

Results: HOMA2IR is correlated with body mass index (BMI) and uric acid levels, but not with mood disorders and TUD, while the AIP is positively associated with BMI, mood disorders, TUD, uric acid, MDA and male sex. Uric acid is positively associated with insulin and triglycerides and negatively with HDL cholesterol. MDA is positively associated with triglyceride levels. Comorbid mood disorders and TUD further increase AIP but not insulin resistance. Glucose is positively associated with increasing age, male gender and BMI.

Discussion: The results show that mood disorders, TUD and BMI together with elevated levels of uric acid and MDA independently contribute to increased atherogenic potential, while BMI and uric acid are risk factors for insulin resistance. The findings show that mood disorders and TUD are closely related to an increased atherogenic potential but not to insulin resistance or the MetS. Increased uric acid is a highly significant risk factor for insulin resistance and increased atherogenic potential. MDA, a marker of lipid peroxidation, further contributes to different aspects of the atherogenic potential. Mood disorders and TUD increase triglyceride levels, lower HDL cholesterol and are strongly associated with the atherogenic, but not insulin resistance, component of the MetS.

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List of abbreviations: AIP, atherogenic index of plasma; BMI, body mass index; CVD, cardiovascular disease; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; ELISA, enzyme-linked immunosorbent assay; HDL-c, high-density lipoprotein; HDRS, Hamilton Depression Rating Scale; HOMA2B%, homeostasis model assessment of β-cell function; HOMA2IR, homeostasis model assessment of insulin resistance; HOMA2S%, homeostasis model assessment of insulin sensitivity; LDL-c, low-density lipoprotein; MDA, malondialdehyde; MEIA, microparticle enzyme immunoassay; MetS, metabolic syndrome; OS, oxidative stress; TG, triglycerides; TUD, tobacco use disorder

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1. Introduction

There is a significant comorbidity between mood disorders, either bipolar disorder or depression, the metabolic syndrome (MetS) and tobacco use disorder (TUD) (Vargas et al., 2014). The MetS is the clustering of an increased atherogenic lipid profile [e.g. hypertriglyceridemia and decreased high-density lipoprotein cholesterol (HDL-c)], insulin resistance, abdominal obesity and elevated blood pressure (Jamshidi et al., 2014). The MetS and related atherogenicity and insulin resistance are strongly related to an increased risk of diabetes type 2 and cardiovascular disease (CVD) (Jamshidi et al., 2014). Disorders of lipoprotein metabolism may account for around 50% of the population-attributable risk of developing CVD (Millan et al., 2009). Insulin resistance is defined as a condition in which insulinsensitive target tissues, including adipose tissue, pancreas, skeletal muscles and liver, do not respond adequately to the physiological activities of insulin (Laakso and Kuusisto, 2014). The homeostasis model assessment (HOMA) and the updated HOMA2 model offer methods to measure insulin resistance (HOMA2IR), insulin sensitivity (HOMA2S%) and beta-cell function (HOMA2B%) based on fasting plasma levels of glucose and insulin (Matthews et al., 1985). The use of the atherogenic index of plasma (AIP) and Castelli risk index 1 and 2 significantly predict vascular risk with a predictive value greater than the isolated lipid variables (Millan et al., 2009; Nunes et al., 2013). The AIP is computed as log10 triglyceride/HDL-c (Vargas et al., 2014) and reflects the presence of atherogenic small low-density lipoprotein cholesterol (LDL-c) and HDL-c particles in plasma and is a sensitive predictor of coronary atherosclerosis and CVD risk (Onyedum et al., 2014).

Patients with bipolar disorder and major depressive disorder show an increased mortality and morbidity due to CVD (Assies et al., 2014). A review, systematic review and meta-analysis showed a small but significant association between insulin resistance and depressive symptoms (pooled effect size 0.19, 95% CI 0.11–0.27) (Silva et al., 2012; Kan et al., 2013). There is a robust comorbidity between mood disorders and TUD and evidence for a bidirectional relationship between both disorders (Nunes et al., 2013). TUD increases risk of the MetS and insulin resistance and causes an atherogenic lipid profile (Bortolasci et al., 2014; Jamshidi et al., 2014). TUD leads to increased mortality (Ezzati and Lopez, 2003) and is one of the major risk factors for multiple chronic diseases, including CVD (Gellert and Scho, 2014).

Activated immune-inflammatory, oxidative and nitrosative stress (IO&NS) pathways and a pro-atherogenic lipid profile are found in mood disorders (either depression or bipolar disorder), the MetS, and TUD. Thus, these three conditions are accompanied by increased levels of pro-inflammatory cytokines, including interleukin (IL)-1β and IL-6, lipid peroxidation biomarkers, including malondialdehyde (MDA), and lowered levels of HDL-c and increased atherogenic indexes, including Castelli risk indexes 1 and 2 and AIP (Maes et al., 1994, 1997, 2011; Vargas et al., 2013; Nunes et al., 2014). Cigarettes contain a significant number of compounds that produce oxidative stress generating MDA through lipid peroxidation (Berger et al., 2014). Recently, we have reviewed that the comorbidities between mood disorders and TUD (Nunes et al., 2013), mood disorders and the MetS/CVD (Maes et al., 2011; Vargas et al., 2013) and TUD and the MetS/CVD (Nunes et al., 2013) may be explained by shared IO&NS pathways and atherogenic changes in lipid profile. Shared metabolic risk factors between mood disorders and the MetS/CVD are lowered serum levels of HDL-c and disorders in the reverse transport of cholesteryl esters (Maes et al., 1994; 2011; Maes and Smith 1998; Nunes et al., 2014). Oxidative stress is related to the development of hyperlipidemia (Ibrahim et al., 1997) and treatment with antioxidants may decrease MDA levels in association with improving triglyceride and total cholesterol levels (Zhao et al., 2014). Oxidative stress may oxidize HDL-c which may have adverse consequences because HDL-c has anti-inflammatory, antioxidant and antithrombotic properties and additionally transports excess cholesterol to the liver (the reverse cholesterol transport) thus decreasing cholesterol load and causing vascular inflammation (Linsel-Nitschke and Tall, 2005; He et al., 2013). This is concordant with the fact that MDA levels are associated with increased atherogenic lipid risk factors (Manohar et al., 2013). The exact relationships between mood disorders, insulin resistance and atherogenic indexes (after controlling for the effects of body mass index (BMI), gender and age) have remained elusive.

Another metabolic biomarker that is associated with the MetS/CVD, insulin resistance, and atherogenecity is uric acid. Epidemiological studies show a significant association between uric acid, insulin resistance and the MetS (Feoli et al., 2014), while the MetS is associated with a very high incidence of hyperuricemia (Facchini et al., 1991; Choi and Ford 2014). Uric acid elevation may be a risk factor for the onset phase of type 2 diabetes (Miyake et al., 2014). Some authors also suggest a relationship between elevated BMI, obesity and insulin resistance and high levels of serum uric acid (Fabbrini et al., 2014). Previous studies found significant positive correlations between uric acid levels and an increased AIP index (Lippi et al., 2010; Baliarsingh et al., 2012) and inverse relationships between uric acid and HDL-c levels (Chu et al., 2000; Lin et al., 2006; Onat et al., 2006a, 2006b). Previous studies showed increased uric acid levels in bipolar disorder (Albert et al., 2015) and lower uric acid in depression (Chaudhari et al., 2010; Wen et al., 2012). Allopurinol, the prototypical uric acid lowering agent, appears to have equivocal effects in mood disorders, with positive and negative trials published (Machado-Vieira et al., 2008; Weiser et al., 2014; Jahangard et al., 2015).

The aims of this study are: (a) to examine insulin resistance, as measured by homeostasis model assessment (HOMA2IR), and the AIP in mood disorders, the MetS and TUD; and (b) to examine the effects of MDA and uric acid on HOMAIR (and insulin and glucose levels) and AIP (and triglyceride and HDL-c levels) in subjects with and without mood disorders, the MetS and TUD. The primary hypothesis is that mood disorders and TUD are significantly associated with the MetS and its components, i.e. insulin resistance and atherogenicity, and that MDA and uric acid are related to both components of the MetS.

2. Subjects and methods

2.1. Subjects

314 subjects, aged 18–65 years and of Caucasian, African, Asian and mixed ethnicity, were enrolled in this study. They were recruited from staff at the State University of Londrina and an outpatient ambulatory of smoking cessation from the same institution, and comprised 120 individuals with mood disorders versus 194 without mood disorders, 224 individuals with MetS and 90 individuals without MetS; and 128 smokers versus 186 non-smokers. The diagnosis of the MetS was made using the diagnostic criteria of the International Diabetes Federation, i.e. 3 of the follow criteria should be present: (a) abdominal obesity (waist circumference ≥ 90 cm for men and ≥ 80 cm for women in South Asian and South Americans and ≥ 94.0 cm for men and ≥ 80.0 cm for women in Caucasians); (b) low HDL-c (< 40 mg/dL in men and < 50 mg/dL in women) or on hypolipidemic drugs; (c) hypertriglyceridemia (triglycerides > 150 mg/dL) or on a hypolipidemic agent; (d) increased fasting glucose (> 100 mg/dL) or on oral antidiabetic medication; (e) increased average blood pressure (130/85 mm Hg) or currently taking antihypertensive medication (Alberti et al., 2009). We measured waist circumference during expiration, in a standing and relaxed position, at the midline between the lower costal margins and the iliac crest parallel to the floor. We measured systolic and diastolic blood pressure using a mercury sphygmomanometer on the right arm and used the mean value of two measurements carried

out 5 min apart. We calculated the BMI as weight (in kg) divided by square of height (in m^2).

The diagnosis of mood disorders, either major depression or bipolar disorder, was made by senior psychiatrists using a validated Portuguese translation of the semi-structured interview of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (Del-ben et al., 2001). We measured the severity of depression using a validated Portuguese translation of the Hamilton Depression Rating Scale (HDRS) which was adapted for use in the Brazilian population (Moreno and Moreno, 1998). Mood disorder patients were included in the acute phase of illness and in partial or total remission. Subjects with diagnoses other than depression and bipolar disorder were excluded (e.g. schizophrenia, substance abuse disorders, and psycho-organic syndromes). The diagnosis of TUD/nicotine dependence was made according to DSM-IV criteria. The severity of dependence was estimated using the Fagerström Nicotine Dependence Scale (Heatherton et al., 1991). All TUD subjects were current smokers who at the time of interview reported smoking every day or some days.

We excluded individuals with abnormal values of hemogram, aspartate transaminase, alanine transaminase, urea, creatinine; who suffered from diabetes, (auto)immune disorders, chronic obstructive pulmonary disorder, inflammatory bowel disease, and neuroinflammatory disorders; who were treated with immunomodulatory drugs or antioxidant supplements, including omega-3 polyunsaturated fatty acid. The study was approved by the Research Ethics Committee (number 250/2010). All participants gave written informed consent prior to participating in this study.

2.2. Laboratory assessments

Blood was sampled after an overnight fast. MDA was determined using a method described by Jentzsch et al. (1996). Plasma insulin levels were determined by microparticle enzyme immunoassay (MEIA) (AXSYM, Abbotts Laboratory, Germany). HDL-c, triglycerides, uric acid and glucose were assayed using an automated method in a clinical chemistry system, Dimension® RXL (Siemens Healthcare Diagnostics Inc, USA). The interassay CV values for all analytes were $< 10\%$. The AIP was calculated as log10 (triglycerides/HDL-c). The HOMA2IR, HOMA2S% and HOMA2B% indexes were calculated according to Levy et al. (1998).

2.3. Statistics

Analyses of variance were employed to assess differences in continuous variables between study groups. We used analyses of contingence tables (X^2 test) to check the distribution of variables among categories. Automatic binary logistic regression analysis was used to delineate the significant predictors of the MetS (dependent variable) with mood disorders, AIP, HOMA2B%, HOMA2S%, HOMA2IR, TUD, age, gender, BMI, biomarkers, etc, as explanatory variables. Multivariate general linear model (GLM) analyses were employed to delineate the effects of explanatory variables on the HOMA2IR and AIP or their components, i.e. insulin, glucose, HDL-c and triglyceride levels. Univariate GLM analysis was used to examine the effects of a number of predictor variables on one dependent variable. In the case that data were not normally distributed we have log-transformed the variables. We used the SPSS (Windows version 19) to analyze the data. Statistical significance was set at $p=0.05$, two tailed.

3. Results

3.1. Metabolic syndrome, HOMA indexes, AIP and oxidative stress

Table 1 shows the socio-demographic and clinical data, glucose, insulin, HOMA indexes, lipids, AIP, uric acid and MDA in subjects with and without MetS. We did not use p-corrections to check the many analyses on clinical, socio-demographic and biochemical data in Table 1 because a) some of these variables are strongly intercorrelated and are thus interrelated characteristics of the dependent variable (the MetS), and b) these univariate statistical results were used to explore which explanatory variables should be used as determinants of independent associations with insulin resistance and atherogenicity in the ultimate GLM analyses (see Tables 3 and 4). There were no significant differences in age, gender, ethnicity, marital status, education level, HDRS score, use of psychotropic drugs or statins and HOMA2B% between both groups. The incidence of TUD, BMI, glucose, insulin, total cholesterol, triglycerides, HOM2IR, AIP, uric acid and MDA were significantly higher in subjects with MetS than in those without. HDL-c and HOMA2S% were significantly lower in those with MetS.

Table 2 shows the results of an automatic binary logistic regression analysis with MetS as dependent variable and all variables shown in Table 1 as possible explanatory variables. MetS was significantly and positively ($X^2=141.86$, df=3, $p<0.001$; Nagelkerke=0.52) associated with HOMA2IR, AIP and BMI. Forced entry of age (Wald=2.85, df=1, $p=0.092$), gender (Wald=2.06, df=1, $p=0.151$), mood disorders (Wald=0.53, df=1, $p=0.810$), TUD (Wald=0.20, df=1, $p=0.655$), uric acid (Wald=2.59, df=1, $p=0.107$) and MDA (Wald=1.69, df=1, $p=0.194$) showed that these variables did not have any significant effect. Since HOMA2IR and AIP were the significant metabolic predictors of interest we entered these two variables as dependent variables in multivariate GLM analyses.

3.2. Effects of different predictors on HOMA2IR index and AIP

Table 3 shows the outcome of multivariate GLM analysis with HOMA2IR and AIP as dependent variables and age, gender, BMI, the diagnostic groups, TUD, uric acid and MDA as explanatory variables. Gender, BMI, mood disorders, TUD, uric acid and MDA were significantly associated with HOMA2IR and AIP. The same table shows the effects of the explanatory variables on the 2 dependent variables separately (tests of between-subjects effects). The explanatory variables explained 24.4% of the variance in HOMA2IR ($F=13.82$, df=7/299, $p<0.001$) and 33.4% of the variance in AIP ($F=21.42$, df=7/299, $p<0.001$). HOMA2IR did not differ significantly between men and women, while AIP was significantly higher in men (estimated marginal mean $\pm$ SE=0109 $\pm$ 0.030) than in women (0.017 $\pm$ 0.020). There were significant positive associations between BMI and HOMA2IR ($B=+0.043$, SE=0.006) and AIP ($B=+0.009$, SE=0.003). Mood disorders were significantly and positively associated with AIP ($B=+0.107$, SE=0.031), but not with HOMA2IR. TUD was significantly and positively associated with AIP ($B=+1.61$, SE=0.030), but not with HOMA2IR. MDA was significantly associated with AIP ($B=+0.007$, SE=0.003), but not with HOMA2IR. Univariate GLM analysis showed that 36.8% of the variance in uric acid ($F=89.51$, df=2/308, $p<0.001$) was explained by BMI ($F=47.97$, df=1/308, $p<0.001$) and gender ($F=144.87$, df=1/308, $p<0.001$). Men (estimated marginal mean $\pm$ SE=5.61 $\pm$ 0.10 mg/dL) had significantly higher uric acid levels than women (4.05 $\pm$ 0.08 mg/dL).

We also examined the differences between depression and bipolar disorder by entering HOMA2IR and AIP as dependent variables in a multivariate GLM analysis with diagnosis (controls, depression and bipolar disorders as three groups), TUD, the MetS, gender, age and BMI as explanatory variables. We found a significant effect of diagnostic classification on both dependent variables ($F=3.96$, df=4/608, $p=0.004$) which was attributable to significant effects on AIP ($F=5.66$, df=2/304, $p=0.004$), but not HOMA2IR ($F=2.06$, df=2/304, $p=0.129$). There were no significant differences in AIP between depression and bipolar disorder ($p=0.707$). Univariate GLM analyses showed that there were no significant differences in MDA ($F=2.52$,

Table 1
Socio-demographic, clinical, HOMA indexes, AIP and MDA in subjects with and without the metabolic syndrome (MetS).

Variables	No MetS (n=224)	MetS (n=90)	F	df	p-Value
Age (years)	45.9 (±8.0)	47.3 (±8.9)	1.87	1/312	0.172
Gender			5.89	3	0.117
Male	76	34			
Female)	148	56			
Ethnicity			2.15	2	0.342
Caucasian	161	58			
African + Asian	34	15			
Mixed	29	17			
Marital status			2.73	2	0.256
Single	27	17			
Stable relationship	154	55			
Divorced/separated/Widowed	43	18			
Years of education	14.0 (±6.4)	12.7 (±5.3)	2.96	1/312	0.086
Tobacco use disorder			5.59	1	0.018
No	142	44			
Yes	82	46			
Mood disorders			0.45	1	0.503
No	141	53			
Yes	83	37			
Hamilton scale	5.7 (±7.9)	6.3 (±7.6)	0.34	1/311	0.562
Use of psychotropics			0.09	1	0.765
Yes	25	9			
No	199	81			
Use of statins			0.15	1	0.696
Yes	17	8			
No	195	77			
Body mass index (kg/m^2)	25.7 (±4.4)	29.6 (±5.0)	45.82	1/310	<0.001
Insulin* (μU/mL)	8.1 (±4.2)	12.7 (±6.9)	53.06	1/312	<0.001
Glucose* (mg/dL)	86.3 (±8.0)	100.6 (±26.2)	53.89	1/312	<0.001
HOMA2B%*	106.1 (±37.9)	113.9 (±45.1)	2.41	1/312	0.122
HOMA2S%*	119.6 (±59.6)	75.6 (±41.2)	41.13	1/312	<0.001
HOMA2IR*	1.030 (±0.521)	1.672 (±0.912)	61.42	1/312	<0.001
Total cholesterol* (mg/dL)	189.6 (±33.8)	206.0 (±53.5)	10.56	1/312	0.001
HDL-c* (mg/dL)	51.2 (±14.8)	39.1 (±9.7)	51.50	1/312	<0.001
Triglycerides* (mg/dL)	104.9 (±53.3)	193.0 (±99.2)	103.06	1/312	<0.001
Atherogenic index of plasma	−0.078 (±0.266)	0.292 (±0.275)	121.66	1/312	<0.001
Uric acid (mg/dL)	4.31 (±1.28)	5.33 (±1.32)	40.52	1/311	<0.001
Malondialdehyde (mmol MDA/mg ptna)	15.6 (±5.4)	17.4 (±6.1)	6.58	1/307	0.011

Continuous variables are shown as mean (±SD) and categorical variables are expressed as n.
All results of analyses of contingency tables (X^2 test) or analyses of variance (F values).
HOMA: Homeostatic model assessment of β-cell function (HOMA2B%), insulin sensitivity (HOMA2S%) and insulin resistance (HOMA2IR).
HDL-c: High density lipoprotein cholesterol.
* These data are processed in Ln transformation.

Table 2
Results of an automatic binary logistic regression analysis with the metabolic syndrome as dependent variable (reference group is no metabolic syndrome) and the listed variables as explanatory variables.

Explanatory variables	B	SE	Wald	df	p	Odds ratio	Lower CI 95%	Upper CI 95%
HOMA2IR	1.49	0.41	13.21	1	<0.001	4.41	1.98	9.83
Atherogenic index of plasma	0.49	0.0.7	47.00	1	<0.001	1.64	1.42	1.88
Body mass index	0.12	0.04	10.32	1	0.001	1.12	1.05	1.20

CI: confidence intervals.
HOMA2IR: Homeostatic model assessment of insulin resistance.

df=2/302, p=0.083) and uric acid (F=0.65, df=2/303, p=0.524) between controls, depressed and bipolar disorder patients after controlling for the effects of TUD, BMI, the MetS, age and gender.

3.3. *Effects of biomarkers on glucose, insulin and lipids*

Table 4 shows the results of a multivariate GLM analysis with glucose, insulin, triglycerides and HDL-c as dependent variables and the same explanatory variables as listed in Table 3. We found that the metabolic variables were significantly associated with age, gender, BMI, mood disorders, TUD, uric acid and MDA. Analyses of parameter estimates showed that age was significantly associated with increased glucose (B=+0.281, SE=0.111), lowered insulin (B=−0.006, SE=0.003) and higher HDL-c (B=+0.189, SE=0.092). Glucose was significantly higher in men than in women (adjusted means ±SEM=93.2 ±1.8 versus 89.0±1.2 mg/dL), while HDL-c was significantly lower in men than in women (42.4±1.5 versus 49.1±1.0 mg/dL). There were significant associations between BMI and glucose (B=+1.129, SE=0.199), insulin (B=+0.041, SE=0.006), triglycerides (B=+0.013, p=0.006) and HDL-c (B=−0.461, SE=0.165). Triglycerides were significantly higher in patients with mood disorders than in controls (145.5±6.8 versus 123.7±5.3 mg/dL), whereas HDL-c was significantly lower in the patients than in controls (43.3±1.3 versus 48.3±1.3 mg/dL). Triglycerides were

Table 3
Outcome of multivariate GLM analysis with HOMA2IR and AIP as dependent variables and age, gender, BMI, diagnosis, uric acid and MDA as explanatory variables.

Dependent variables	Explanatory variables	*F*	df	*p*
HOMA2IR and AIP	Age	2.16	2/298	0.117
	Gender	6.11	2/298	0.003
	BMI	29.36	2/298	< 0.001
	Mood disorders	6.48	2/298	0.002
	TUD	14.18	2/298	< 0.001
	Uric acid	16.70	2/298	< 0.001
	MDA	4.04	2/298	0.019
HOMA2IR	Age	3.01	1/299	0.084
	Gender	3.68	1/299	0.056
	BMI	57.50	1/299	< 0.001
	Mood disorders	0.02	1/299	0.901
	TUD	0.43	1/299	0.511
	Uric acid	7.96	1/299	0.005
	MDA	0.15	1/299	0.695
AIP	Age	2.33	1/299	0.128
	Gender	12.09	1/299	0.001
	BMI	8.50	1/299	0.004
	Mood disorders	12.09	1/299	0.001
	TUD	28.12	1/299	< 0.001
	Uric acid	31.03	1/299	< 0.001
	MDA	7.04	1/299	0.008

BMI: body mass index; TUD: tobacco use disorder; MDA: malondialdehyde.

significantly higher in individuals with TUD than in those without (150.4 ± 6.5 versus 118.8 ± 5.6 mg/dL), whereas HDL-c was significantly lower in those with TUD (43.3 ± 1.2 versus 48.2 ± 1.1 mg/dL). Uric acid was significantly associated with insulin ($B = +0.068$, SE = 0.024), triglycerides ($B = +0.128$, SE = 0.024) and HDL-c ($B = -2.165$, SE = 0.695). MDA was significantly correlated with triglycerides ($B = +0.015$, SE = 0.005).

3.4. *Effects of psychotropic drugs and statins*

Forced entry of use of psychotropic drugs ($F = 0.62$, df = 2/251, $p = 0.537$) and use of statins in the multivariate GLM analysis listed in Table 3 showed that use of statins had a significant effect ($F = 4.17$, df = 2/281, $p = 0.016$) and that the significance levels of the other explanatory variables remained unaffected, i.e. gender ($F = 6.00$, df = 2/281, $p = 0.003$), BMI (F = 26.61, df = 2/281, $p < 0.001$), mood disorders ($F = 5.62$, df = 2/281, $p = 0.004$), TUD ($F = 16.74$, df = 2/281, $p < 0.001$), uric acid ($F = 18.34$, df = 2/281, $p < 0.001$) and MDA ($F = 4.75$, df = 2/281, $p = 0.009$). Tests of between subjects effects showed a mild but significant suppressant effect of statins on AIP ($F = 5.08$, df = 1/282, $p = 0.025$), but not HOMA2IR (F = 1.56, df = 1/282, $p = 0.212$). A multivariate GLM analysis with uric acid and MDA as dependent variables showed that use of psychotropic drugs ($F = 1.04$, df = 2/288, $p = 0.356$) and statins ($F = 0.92$, df = 2/288, $p = 0.399$) as explanatory variables had no significant effects on the biomarkers. Forced entry of use of psychotropic drugs ($F = 0.36$, df = 4/278, $p = 0.839$) and use of statins ($F = 2.47$, df = 4/278, $p = 0.045$) in the multivariate GLM analysis listed in Table 4 shows that the latter was significant and that the significance levels of the other explanatory variables remained unaffected, i.e. BMI ($F = 17.64$, df = 4/278, $p < 0.001$), mood disorders ($F = 3.33$, df = 4/278, $p = 0.011$), TUD ($F = 8.25$, df = 4/278, $p < 0.001$), uric acid ($F = 9.27$, df = 4/278, $p < 0.001$), MDA ($F = 3.50$, df = 4/278, $p = 0.008$). Tests of between subjects effects showed that statins elevated HDL-c ($F = 3.90$, df = 1/281, $p = 0.049$) and that there was a trend towards suppressed triglyceride levels ($F = 3.60$, df = 1/281, $p = 0.059$). We also reran the same GLM analyses in subjects who were free of psychotropic drugs and those who were free of statins. These analyses showed basically the same findings as those listed in Tables 3 and 4.

4. Discussion

As expected, we found that the MetS is strongly predicted by insulin resistance, the AIP and BMI. The MetS thus reflects two pathophysiological processes, i.e. insulin resistance and increased atherogenicity, which are both related to an increased BMI. Therefore, we will discuss our data with respect to these two metabolic components.

The first major finding of this study is that insulin resistance is significantly associated with BMI but not mood disorders (either depression or bipolar depression). As reviewed in the introduction there is a small association between insulin resistance and depressive symptoms (Silva et al., 2012; Kan et al., 2013). However, 15 of the 25 datasets analyzed in the meta-analysis used self-reported depression scales and not a clinical diagnosis, while the number of studies with a diagnostic interview for depression was very limited (Kawada, 2013). Only a few studies included in that meta-analysis adjusted the results for BMI or other intervening factors. The role of increased BMI as intervening factor is important because obesity/overweight and insulin resistance coexist as key components of the MetS. Finally, depression and psychotropic use may increase the risk of obesity/overweight, and this raises the question whether obesity/overweight may mediate a spurious association between insulin resistance and depression.

The negative results showing no relationship between TUD and insulin resistance are not consistent with results from previous studies. There is some evidence that smoking is an aggravating factor for the development of insulin resistance (Facchini et al., 1991; Attvall et al., 1993; Szulinska et al., 2013). Current smoking is also positively associated with the MetS (Cena et al., 2013). Nevertheless, the results of our study indicate that current smoking is more related with the atherogenic component of the MetS than with insulin resistance.

The significant positive correlation between insulin resistance (and the MetS) and uric acid (even after adjusting for the effects of BMI) extent the results of previous studies (Feoli et al., 2014). Increased levels of uric acid may precede the onset of obesity, insulin resistance, diabetes, hypertension and inflammatory responses (Johnson et al., 2013), while lowering the levels of uric acid may improve insulin resistance (Ogino et al., 2010). Uric acid may cause insulin resistance via different mechanisms: (a) Increased uric acid contributes to liver insulin resistance by enhancing oxidative stress in the mitochondria (Lanaspa et al., 2012). (b) Uric acid causes oxidative stress in islets cells and consequently islet cell dysfunction (Roncal-Jimenez et al., 2014). (c) Uric acid induces inflammatory responses in adipose cells and lowers adiponectin production (Baldwin et al., 2011). Increased levels of insulin also cause urate absorption in the proximal tubule and may contribute to hyperurecemia. Therefore, it may be concluded that there are reciprocal relationships between hyperinsulinemia and hyperuricemia in individuals with the MetS. Considering that uric acid is largely responsible of the free-radical scavenging capacity in plasma, it is suggested that increased uric acid in the MetS is an adaptive mechanism preventing oxidative damage caused by free radicals (Fabbrini et al., 2014). Nevertheless, while uric acid is a strong antioxidant in the plasma it has pro-oxidant properties in the cells (Sautin and Johnson 2008; Lanaspa et al., 2012). We could not detect a relationship between MDA and insulin resistance (and insulin and glucose levels), while previous papers reported a significant association between insulin resistance and higher plasma MDA levels (Moreto et al., 2014). Moreto et al. (2014) concluded that the glucolipotoxic state in the MetS is a major risk factor to lipid peroxidation.

The second major finding of this study is that increased AIP is strongly predicted by mood disorders, BMI, TUD, uric acid and MDA. Previously, we have discussed that there is a significant association between mood disorders, either depression or bipolar disorder, and atherogenic indexes (Vargas et al., 2013, 2014). Here

Table 4
Results of a multivariate GLM analysis with glucose, insulin, HDL cholesterol and triglycerides as dependent variables and the listed variables as explanatory variables.

Dependent variables	Explanatory variables	F	df	p
Glucose, insulin, triglycerides and HDL cholesterol	Age	4.14	4/296	0.003
	Gender	5.94	4/296	<0.001
	BMI	18.18	4/296	<0.001
	Mood disorders	3.60	4/296	0.007
	TUD	7.03	4/296	<0.001
	Uric acid	8.46	4/296	<0.001
	MDA	2.76	4/296	0.028
Glucose	Age	6.41	1/299	0.012
	Gender	3.21	1/299	0.074
	BMI	32.11	1/299	<0.001
	Mood disorders	0.13	1/299	0.719
	TUD	2.45	1/299	0.118
	Uric acid	0.05	1/299	0.832
	MDA	0.28	1/299	0.596
Insulin	Age	4.09	1/299	0.044
	Gender	4.46	1/299	0.035
	BMI	52.92	1/299	<0.001
	Mood disorders	0.29	1/299	0.888
	TUD	0.29	1/299	0.592
	Uric acid	8.18	1/299	0.005
	MDA	0.14	1/299	0.712
Triglycerides	Age	0.67	1/299	0.414
	Gender	1.20	1/299	0.274
	BMI	4.97	1/299	0.027
	Mood disorders	6.57	1/299	0.011
	TUD	22.57	1/299	<0.001
	Uric acid	27.20	1/299	<0.001
	MDA	9.59	1/299	0.002
HDL cholesterol	Age	4.27	1/299	0.040
	Gender	11.94	1/299	0.001
	BMI	7.82	1/299	0.006
	Mood disorders	10.22	1/299	0.002
	TUD	10.13	1/299	0.002
	Uric acid	9.72	1/299	0.002
	MDA	0.06	1/299	0.801

BMI: body mass index.
TUD: tobacco use disorder.
MDA: malondialdehyde.

we report that both mood disorders and TUD are accompanied by lowered levels of HDL-c and increased triglyceride levels. Lowered HDL-c has been observed in patients with unipolar major depression, chronic depression, acute depression rather than depression in remission and is more marked in depressed women than in men (review: Vargas et al., 2014).

Our findings that TUD is accompanied by increased AIP (and lower HDL-c, but higher triglyceride levels) are in agreement with previous publications reporting that TUD has a negative impact on the risk of cardiovascular disease and that this is mediated through effects on lipid metabolism, including lowering HDL-c levels (He et al., 2013). These effects may be explained by various mechanisms: carbon monoxide inhibits hepatic microsomal synthesis of HDL-c (Willett et al., 1983); smoking decreases apoA-I levels, which result in impaired HDL synthesis and LCAT activity, which affects maturation of HDL-c; smoking increases the activity of cholesterol ester transfer protein, which facilitates the exchange of cholesteryl ester for triglycerides; smoking may impair the uptake of HDL-c in the liver; and smoking may oxidize HDL-c which may cause loss of the atheroprotective properties (He et al., 2013). It has long been known that smokers have higher triglyceride levels than non-smokers (Willett et al., 1983).

In this study we found a significant positive correlation between MDA levels and atherogenicity. It is known that oxidative stress is related to the development of hyperlipidemia (Ibrahim et al., 1997) and that treatment with antioxidants may decrease MDA levels in association with improving triglyceride levels (Zhao et al., 2014). Oxidative stress may also oxidize HDL-c which has anti-inflammatory, antioxidant and antithrombotic properties and transports excess cholesterol to the liver (the reverse cholesterol transport) thus causing vascular inflammation (Linsel-Nitschke and Tall, 2005; He et al., 2013). In these conditions, assays measuring HDL-c may detect less HDL-c in plasma.

Our finding that the AIP (and triglyceride levels) was significantly and positively associated with increased uric acid levels is in agreement with that of previous studies (Lippi et al., 2010; Baliarsingh et al., 2012). In another report, the changes in triglyceride levels over a 10 year period were significantly related to changes in plasma uric acid and this independently from changes in BMI (Rathmann et al., 1998). As in previous publications (Chu et al., 2000; Lin et al., 2006; Onat et al., 2006a, 2006b) we found significant inverse correlations between serum uric acid and HDL-c. We found no significant differences in serum uric acid levels between subjects with and without depression or bipolar disorder. This contrast studies reviewed in the Introduction showing higher uric acid in bipolar disorder (Albert et al., 2015) and lower levels in depression (Chaudhari et al., 2010; Wen et al., 2012).

We have previously reported that the co-occurrence of mood disorders and TUD is accompanied by higher AIP values as compared to each of these disorders alone (Nunes et al., 2014). This is also reflected in the results of our regression analysis showing cumulative effects of both mood disorders and TUD on AIP. AIP values were significantly higher in men than women, while HDL-c levels were lower in men than in women. It is well established that the average HDL-c values in men are lower than in women and that these differences are mediated by other factors, including smoking, hormonal

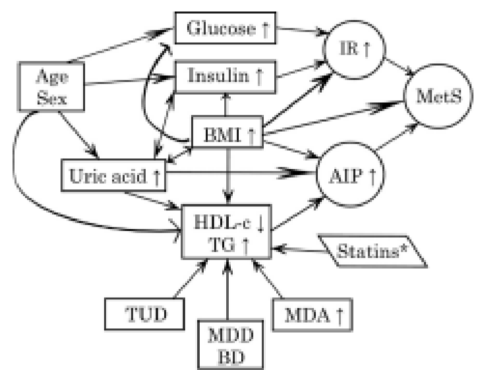

Fig. 1

status, alcohol use, etc (Thelle et al., 1982; Davis et al., 1996). This increased prevalence of lowered HDL-c in males is associated with the increased mortality and morbidity due to CVD in males (Thelle et al., 1982; Davis et al., 1996).

This study has some strengths and limitations that must be considered in the interpretation of the results. This is a cross-sectional study and therefore we cannot draw firm conclusions on causal associations. Strengths are that the results are controlled for possible effects of intervening variables, including alcohol use (exclusion criterion), intake of antioxidants and ω3 polyunsaturated fatty acid (exclusion criteria), BMI, age, gender, and use of psychotropic medication and statins. Thus, we replicated all results reported here in the subgroups of patients who were free of psychotropic medications and statins.

In conclusion: Fig. 1 summarizes our findings. Insulin resistance (and insulin and glucose levels) is correlated with BMI and uric acid levels; and AIP [and triglycerides (TG) and HDL-c] with BMI, depression (MDD) or bipolar disorder (BD), TUD, male gender and MDA. The findings show that depression, bipolar disorder and TUD are closely related to an increased atherogenic potential but not to insulin resistance or the MetS. Comorbid mood disorder and TUD further increase AIP but not insulin resistance. MDA, a marker of lipid peroxidation, was significantly correlated with elevated triglyceride levels and increased AIP. Increased uric acid levels were strongly associated with increased insulin resistance and AIP. Interestingly, MDA and uric acid are not related either to depression or BD after controlling for the MetS, TUD, age, sex, etc. Statins decrease the AIP and increase HDL-c levels. These results show that depression, bipolar disorder, TUD and increasing BMI together with elevated levels of uric acid and MDA independently contribute to insulin resistance and/or atherogenicity. The MetS is not associated with depression or bipolar disorder, but is predicted by insulin resistance, atherogenicity and BMI.

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Conflict of interest

There are none conflicts of interest with any of the authors.

Author contributions

HOV and SOVN conducted the study. CCB and HKM were responsible for blood analyses. MM performed data analysis. All authors contributed equally to data interpretation and manuscript writing.

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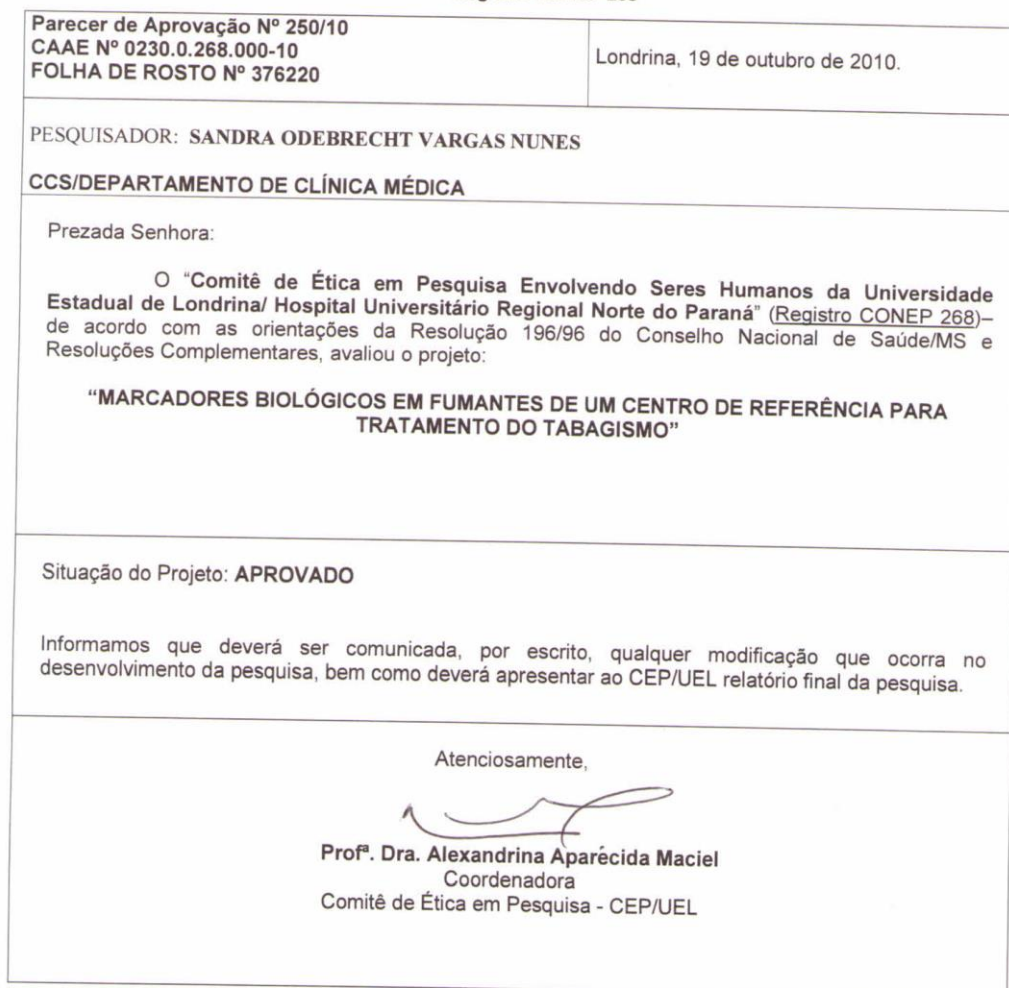
5 CONSIDERAÇÕES FINAIS

Existem diferentes interações dos diferentes Q192R genótipos da PON1 e fatores ambientais nos transtornos do humor. Níveis reduzidos da atividade da PON1 e sua interação com outros fatores de risco podem contribuir para o advento das comorbidades entre depressão e SM. As interações dos genótipos e o uso de tabaco além de predizerem os transtornos de humor, também contribuem para alterações nos níveis de antioxidantes. Interações entre os transtornos de humor, transtorno por uso de tabaco, altos níveis de ácido úrico e malondialdeido contribuem para o aumento do potencial aterogênico.

É importante a detecção de diferentes interações e possíveis comorbidades para uma avaliação mais precisa e individualizada do paciente. Dessa forma, na prática clínica deve-se atentar não apenas para o tratamento do transtorno do humor, mas também para a intervenção em outros fatores que influenciam diretamente no desenvolvimento e progressão desta doença. Essa intervenção pode envolver o auxílio à cessação do tabaco e o uso de antioxidantes.

ANEXOS

Anexo 1 - Parecer de Aprovação do Comitê de Ética



COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná
Registro CONEP 268

Parecer de Aprovação Nº 250/10 **CAAE Nº 0230.0.268.000-10** **FOLHA DE ROSTO Nº 376220**	Londrina, 19 de outubro de 2010.

PESQUISADOR: **SANDRA ODEBRECHT VARGAS NUNES**

CCS/DEPARTAMENTO DE CLÍNICA MÉDICA

Prezada Senhora:

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná**" (Registro CONEP 268)– de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"MARCADORES BIOLÓGICOS EM FUMANTES DE UM CENTRO DE REFERÊNCIA PARA TRATAMENTO DO TABAGISMO"

Situação do Projeto: **APROVADO**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL relatório final da pesquisa.

Atenciosamente,

Profª. Dra. Alexandrina Aparécida Maciel
Coordenadora
Comitê de Ética em Pesquisa - CEP/UEL

Campus Universitário: Rodovia Celso Garcia Cid (PR 445), km 380 - Fone (043) 371-4000 PABX - Fax 328-4440 - Caixa Postal 6.001 - CEP 86051-990 – Internet http://www.uel.br
Hospital Universitário/Centro de Ciências da Saúde: Av. Robert Koch, 60 - Vila Operária – Fone (043) 381-2000 PABX – Fax 337-4041 e 337-7495 - Caixa Postal 791 - CEP 86038-440
LONDRINA - PARANÁ - BRASIL

Form. Código 11.764 – Formato A4 (210x297mm)

Anexo 2 – Questionário

AMBULATÓRIO DE TABAGISMO – AVALIAÇÃO CLÍNICA

Instrumento Número: |___|___|___|. Data da primeira avaliação: _____/_____/_____

Etiqueta de Identificação

Caracterização Sócio-demográfica

Nome:____

2. Data de Nascimento: _____/_____/_____.**3.** Idade (em anos): ....

4. Naturalidade: ______________________________ **5.** Gênero: 1. Masculino 2.

Feminino

6. Situação conjugal: 1. Solteiro 2. União estável 3. Separado/Divorciado 4. Viúvo

7. Cor da pele: 1.Branca 2.Negra 3.Amarela 4.Mulata 5. Parda 6. Indigena

8. Anos de estudo:

9. Nível de Escolaridade: 01. Analfabeto 02. Alfabetizado 03. Fundamental incompleto

04. Fundamental Completo 05. Médio Incompleto 06. Médio Completo 07. Superior Incompleto

08. Superior Completo 09. Pós-graduação latu-sensu 10. Pós-graduação stricto-sensu

10. Reside: 1. Sozinho 2. Parceiro 3. Família 4. Familiares 6. Outros

Endereço:

____

Município: ________________________________ CEP: ________________ Estado: _________

Telefone Contato: _________________ Celular: __________________

Ramal:______________

Situação de Trabalho

13. Local de Trabalho: ____

Endereço: ____

Município: ____________________________ CEP: _______________ Estado: __________

15. Profissão: __________________ 16. Ocupação:_______________

17. Relação com o trabalho: 1. Formal 2. Informal 3. Autônomo 4. Servidor Público

18. Situação trabalhista: 1. Desempregado 2. Auxílio-desemprego 3. Atividade não Remunerada

4. Atividade Remunerada 5. Auxílio-doença 6. Estudante 7. Aposentado 8. Outro ______

19. Possui doença que o afaste do trabalho: 1. sim 2. não

20. Qual é a doença? ____

21. Esta doença torna-o incapaz para o trabalho? 1. sim 2. não

24. No último ano, quantos dias ficou afastado das suas atividades laborais? ______

25. Qual foi o motivo/doença? ____

26. Esta doença o incapacitou para as atividades domésticas? 1. sim 2. não

27. Teve alguma internação geral recente: 1. sim 2. não

28. Por quantas vezes foi internado? ____________________________________

29. Quantos dias duraram cada internação? _________________________________

ABORDAGEM E TRATAMENTO DO TABAGISTA

História Pregressa da Doença

33.(01) Você tem ou teve freqüentemente aftas, lesões (feridas) e/ou sangramento na boca?

1. Sim 2. Não.. ☐

33.1. Está em tratamento? 1. Sim 2. Não.. ☐

34.(02) Você tem diabetes mellitus? 1. Sim 2. Não .... ☐

34.1. Está em tratamento? 1. Sim 2. Não.. ☐

35.(03) Você tem hipertensão arterial? 1. Sim 2. Não .... ☐

35.1. Está em tratamento? 1. Sim 2. Não.. ☐

36. (04) Você tem ou teve algum problema cardíaco? 1. Sim 2. Não .... ☐

36.1. Qual? ____

36.2. Está em tratamento? 1. Sim 2. Não.. ☐

37.(05) Você tem ou teve freqüentemente queimação, azia, dor no estômago, úlcera ou gastrite?

1. Sim 2. Não .... ☐

37.1. Está em tratamento? 1. Sim 2. Não.. ☐

38.(06) Você tem ou teve algum problema pulmonar? 1. Sim 2. Não...................... ☐

38.1. Qual? ____

38.2. Está em tratamento? 1. Sim 2. Não..

39.(07) Você tem alergia respiratória? 1. Sim 2. Não ....

39.1. Está em tratamento? 1. Sim 2. Não..

40.(08) Você usa medicação para dislipidemia ? 1. Sim 2. Não ....

40.1. Está em tratamento? 1. Sim 2. Não..

41.(09) Você tem ou teve alguma lesão ou tumor maligno? 1. Sim 2. Não ....

41.1. Onde (local)? ____

41.2. Está em tratamento? 1. Sim 2. Não..

42. (10) Você tem ou teve crise convulsiva, convulsão febril na infância ou epilepsia?

1. Sim 2. Não ....

42.1. Está em tratamento? 1. Sim 2. Não..

43. (11) Você tem síndrome metabólica ? 1. Sim 2. Não ....

43.1. Está em tratamento? 1. Sim 2. Não..

44. (12) Você costuma ter crises de depressão ou ansiedade? 1. Sim 2. Não ......

44. 1. Está em tratamento? 1. Sim 2. Não..

45. (13) Você faz ou fez algum tratamento psicológico ou psiquiátrico?

1. Sim 2. Não ....

45. 1. Está em tratamento? 1. Sim 2. Não..

45.2. Qual a medicação? ______________________________________

45.3. Você já tentou suicídio? 1. Sim 2. Não..

45.4 Quantas vezes? ....

45.5 Métodos de tentativa de suicídio..

1. Ingestão de medicamento
2. Ingestão de organofosforado
3. Enforcamento
4. Arma branca
5. Arma de fogo
6. Gás
7. Precipitar-se de alturas
8. Precipitar-se de carro em movimento
9. Outros ____

57. Você tem ou teve algum outro problema sério de saúde que não foi citado?

1. Sim 2. Não **57.1.** Qual? ______________________________

57.2. Está em tratamento? 1. Sim 2. Não..

57.3. Qual? ____

58. Algum medicamento em uso atual? 1. Sim 2. Não ....

58.1. Qual? ____

As perguntas 60 e 61 deverão ser respondidas por todos os pacientes do sexo feminino. Se NÃO ir para a questão 62.

60. Está grávida? 1. Sim 2. Não .... ☐

1. Quantos meses? ____________________________

2. Número gestações ____________________________

61. Está amamentando? 1. Sim 2. Não .... ☐

História Tabagistica

62. (01) Com quantos anos você começou a fumar ? .... ☐☐

62. (02) Quantos anos fuma: .... ☐☐

62. (03) Quantos cigarros fuma por dia?.. ☐☐

62. (04) Anos/ Maço. (nº cigarros x anos fumando/20).. ☐☐

65. (04) Quantas vezes você tentou parar de fumar? .... ☐

1. De 1 a 3 vezes 2. Mais de 3 vezes

3. Nunca tentou *(seguir para a questão 69)*

66. (05) Quantas vezes você ficou sem fumar por pelo menos um dia? .... ☐

1. Uma vez 2. Duas vezes

3. Três vezes 4. Mais de três vezes 5. Nenhuma vez

67. (06) Quais foram os motivos que levaram você a voltar a fumar?(Múltipla escolha)

. Bebida..	☐	. Estressor de Perda................	☐
. Briga – Raiva................................	☐	Festa..	☐
. Tensão..	☐	Alegria...	☐
. Influência......................................	☐	Condicionamento.....................	☐
. Medo ganhar peso........................	☐	Ansiedade..................................	☐
. Sem motivo aparente..................	☐	Outro..	☐

☐

68. (07) Alguma vez na vida utilizou algum recurso para deixar de fumar? ....

1. Nenhum 2. Apoio de profissional de saúde

3. Leitura em folhetos, revistas, jornais e outros

4. Medicamento

4.1. Qual? ____

5. Outros____

69. (08) Você participou de algum grupo de apoio para abordagem e tratamento do tabagismo em algum lugar? ☐

1. Sim 2. Não ....

70. Fez uso de tratamento para parar de fumar (pode escolher várias) :

.bupropiona .... ☐ . reposição com adesivo .... ☐

. goma .... ☐ . acupuntura.............................. ☐

. homeopatia .... ☐ . grupo terapêutico.................... ☐

. apoio de profissionais de saúde ☐ . outros medicamentos .... ☐

. Qual? ____

71. A última vez que ficou abstinente foi por quanto tempo (em meses) ________ ☐☐☐

72. (09) Por que você quer deixar de fumar agora? (Pode assinalar várias alternativas)

. Por que está afetando minha saúde .... ☐

. Outras pessoas estão me pressionando.. ☐

. Pelo bem-estar de minha família... ☐

. Estou preocupado com minha saúde no futuro..................... ☐

. Porque meus filhos pedem... ☐

. Porque não gosto de ser dependente... ☐

. Fumar é anti-social.. ☐

. Porque gasto muito dinheiro com cigarro .... ☐

. Fumar é um mal exemplo para as crianças.................................... ☐

. Por conta das restrições de fumar em ambientes fechados.......... ☐

. Outros.. ☐

73. (10) Você convive com fumantes na sua casa? 1. Sim 2. Não .... ☐

73.1. Qual o grau de parentesco? ____

74. (11) Você se preocupa em ganhar peso ao deixar de fumar?

1. Sim 2. Não.. ☐

Escala de Tolerância de Fagerström – *Gravidade à Dependência de Nicotina*

75. (01) Quanto tempo depois de acordar fuma o primeiro cigarro? .... ☐

0. Após 60 minutos

1. Entre 31 a 60 minutos
2. Entre 06 a 30 minutos
3. Nos primeiros 5 minutos

76. (02) Você acha difícil não fumar em lugares onde é proibido, como em igrejas, bibliotecas, local de trabalho, shoppings, etc? 1. Sim 2. Não .... ☐

77. (03) Qual o cigarro do dia traz mais satisfação? .... ☐

1.O primeiro da manhã   0. Outros

78. (04) Quantos cigarros você fuma por dia?.. ☐

0. Menos de 10
1. De 11 a 20
2. De 21a 30
3. Mais de 31

79. (05) Você fuma mais pela manhã? 1. Sim   2. Não .... ☐

80. (06) Você fuma mesmo doente quando precisa ficar na cama a maior parte do tempo?

1. Sim   2. Não .... ☐

80.1. *Pontuação*.. ☐☐

História Familiar de Tabagismo em Primeiro Grau

81. Seu pai fuma ou já fumou?   1. Sim   2. Não.. ☐

82. Sua mãe fuma ou já fumou?   1. Sim   2. Não .... ☐

83. Número de irmãos? ________________ **84.** Quantos irmãos fumam? ☐☐

85. Número de filhos? ___________________ **86.** Quantos filhos fumam? ☐☐

87. História familial: 1. Positiva   2. Negativa   3. Desconhece ☐

88. História familiar de transtorno mental: 1.Sim   2. Não ☐

88.1. Qual familiar? ____

88.2. Qual transtorno mental? ____

TABELA

118. Sessões Terapêuticas - (Marcar na Tabela)

(1). Situação Paciente

1. Fumante   2. Não fumante   3. Não compareceu
4. Lapso recaida   5. Lapso abstinência   6. Abandono

(2). Tratamento

01. Grupo   02. Grupo+adesivo   03. Grupo+goma
04. Grupo+adesivo+goma   05. Grupo+bupropriona   06. Grupo+bupropriona+adesivo
07. Grupo+bupropriona+adesivo+goma   08. Grupo+bupropriona+goma
09. Grupo+ISRS   10. Grupo+ISRS+adesivo   11. Grupo+ISRS+goma
12. Grupo+ISRS+goma+adesivo   13. Outro _______________

(3). Em uso de NAC ou placebo?   1. Sim   2. Não

(4). Monóxido de Carbono exalado (CO exal) - PPM e %

	(1) Situação do Paciente	(2) Tratamento	(3) NAC / placebo	CO exalado	
				%	ppm
Fase 0 (Semana 1)					
Semana 2					
Semana 3					
Fase 1 (Semana 4)					
Semana 6					
Fase 2 (Semana 8)					
Fase 3 (Semana 12)					

FASE 0

AVALIAÇÃO – SEMANA 1

1ª Semana

120. 1. Data de retirada do medicamento na farmácia ☐☐/☐☐/☐☐

120.2. Data da próxima retirada de medicamento na farmácia ☐☐/☐☐/☐☐

122. Exame Físico

122.1. Altura do paciente: ☐☐☐ cm **122.2.** Peso: ☐☐☐Kg☐☐☐g

IMC – Índice de Massa Corpórea (peso/ altura²):☐☐,☐

122.4. PA: ☐☐☐ x ☐☐☐ **122.5.** FC:☐☐☐

122.6 Circunferência Abdominal:☐☐☐cm **122.7** Circunferência Quadril: ☐☐☐

126. Incapacidade de Sheehan (0 – 10) – Fase 0

01. Trabalho______________ ☐☐

02. Vida social___________ ☐☐

03. Vida familiar____________

Dias perdidos ou improdutivos por mês:

Marcar 1 (sim) ou 2 (não)

Os sintomas têm causado faltas no trabalho, escola ou incapacidade em trabalhar em casa?

Mesmo que tenha ido ao trabalho ou escola, os sintomas têm diminuido sua produtividade?

127.1 Hamilton - Fase 0..

EXAMES (SEMANA 1) - Fase 0

129.21 Hemograma Anemia – **Fase 0:** 1- Normal 2- Alterado

129.22 Hemograma Infecção – **Fase 0:** 1- Normal 2- Alterado

129.23 VHS - **Fase 0:**

129.24 Hepatite B – **Fase 0:** 1- Reagente 2- Não Reagente

129.25 Hepatite C – **Fase 0:** 1- Reagente 2- Não Reagente

129.26 HIV – **Fase 0:** 1- Reagente 2- Não Reagente

129.27 TGO – **Fase 0:**

129.28 TGP – **Fase 0:**

129.29 Creatinina – **Fase 0:**

124.1 Glicemia:

124.2 Colesterol Total: **124.3-** Colesterol HDL:

124.4- Colesterol LDL: **124.5-** Triglicerídeos:

FASE 1

AVALIAÇÃO 4ª SEMANA - FASE 1

4ª Semana

150. 1. Data de retirada do medicamento na farmácia / /

150.2. Data da próxima retirada de medicamento na farmácia / /

150.3. Iniciou tratamento medicamentoso? 1-sim 2-não

150.4 Situação do Paciente **(TABELA)** **150.5.** Tratamento **(TABELA)**

152. Exame Fisico – Fase 1

152.1. Altura do paciente: ☐☐☐ cm **152.2.** Peso: ☐☐☐Kg ☐☐☐g

152.3.IMC – Índice de Massa Corpórea (peso/ altura²): ☐☐ ☐

152.4. PA: ☐☐☐ x ☐☐☐ **152.5.** FC: ☐☐☐

152.6 Circunferência Abdominal: ☐☐☐cm **152.7** Circunferência Quadril: ☐☐☐

155.Efeitos Colaterais – Fase 1

1. Não 2. Sim, leve. 3. Sim, moderado. 4. Sim, grave.

157.1. Náuseas e/ ou vômitos___ ☐ **157.2.**Diarréia_______________ ☐

157.3. Alergia de pele_________ ☐ **157.4.** Alergia respiratória_____ ☐

157.5. Outro(s)?_____________ ☐ Quais?_____________________

156. Escala de Adesão Terapêutica – Fase 1 1. Sim 2. Não

.1. Alguma vez se esqueceu de tomar a sua medicação?____________________

.2. Por vezes é descuidado a tomar a sua medicação?______________________

.3. Quando se sente melhor, deixa, por vezes, de tomar a sua medicação?______

.4. Por vezes, se sente pior quando toma a medicação, deixa da tomar?________

.5. Só tomo a medicação quando me sinto doente?________________________

.6. Não é natural para minha mente e meu corpo ser controlado pela medicação?

.7. Os meus pensamentos são mais claros com a medicação?________________

.8. Por usar a medicação posso prevenir de ficar doente?___________________

.9. Sinto-me estranho com a medicação?_________________________________

.10. A medicação faz com que eu me sinta cansado e lento?_________________

159. Escala de abstinência de nicotina de Minnesota (MNWS) : 0, 1, 2, 3 e 4 – Fase 1

1. Raiva, irritabilidade e frustração ___________ ☐
2. Ansiedade e nervosismo_________________ ☐
3. Humor deprimido e tristeza_______________ ☐
4. Desejo e fissura para fumar_______________ ☐

5. Dificuldade de concentração_______________

6. Aumento do apetite, fome e ganho de peso___ ☐

7. Insônia, problemas de sono e acordar a noite_ ☐

8. Incapacidade de relaxar__________________ ☐

9. Impaciência___________________________ ☐

10. Obstipação___________________________ ☐

11. Tontura_______________________________ ☐

12. Tosse________________________________ ☐

13. Pesadelo, sonhos______________________ ☐

14. Náusea______________________________ ☐

15. Nó na garganta_______________________ ☐

AVALIAÇÃO 8ª SEMANA - FASE 2

170. 1. Data de retirada do medicamento na farmácia ☐☐ / ☐☐ / ☐☐ 8ªSemana

170.2. Data da próxima retirada de medicamento na farmácia ☐☐ / ☐☐ / ☐☐

170.4 Situação do Paciente (TABELA) **170.5.Tratamento** **170.6. Monóxido**

172. Exame Fisico – Fase 2

172.1. Altura do paciente: ☐☐☐ cm **172.2.** Peso: ☐☐☐ Kg ☐☐☐ g

172.3.IMC – Índice de Massa Corpórea (peso/ altura²): ☐☐ ☐

172.4. PA: ☐☐☐ x ☐☐☐ **172.5.** FC: ☐☐☐

172.6 Circunferência Abdominal: ☐☐☐ cm **172.7** Circunferência Quadril: ☐☐☐ cm

177.Efeitos Colaterais – Fase 2

1. Não 2. Sim, leve. 3. Sim, moderado. 4. Sim, grave.

177.1. Náuseas e/ ou vômitos___ ☐ **177.2.**Diarréia______________ ☐

177.3. Alergia de pele_________ ☐ **177.4.** Alergia respiratória______ ☐

177.5. Outro(s)?______________ Quais?_____________________

178. Escala de Adesão Terapêutica – Fase 2 1. Sim 2. Não

178.1.Alguma vez se esqueceu de tomar a sua medicação?___________________

178.2. Por vezes é descuidado a tomar a sua medicação?____________________

178.3. Quando se sente melhor, deixa, por vezes, de tomar a sua medicação?____

178.4. Por vezes, se sente pior quando toma a medicação, deixa da tomar?______

178.5. Só tomo a medicação quando me sinto doente?_______________________

178.6. Não é natural para minha mente e meu corpo ser controlado pela medicação?

178.7. Os meus pensamentos são mais claros com a medicação?_______________

178.8. Por usar a medicação posso prevenir de ficar doente?___________________

178.9. Sinto-me estranho com a medicação?________________________________

178.10. A medicação faz com que eu me sinta cansado e lento?_________________

179 . Escala de abstinência de nicotina de Minnesota (MNWS) : 0, 1, 2, 3 e 4 (FASE 2)

1. Raiva, irritabilidade e frustração ___________ ☐
2. Ansiedade e nervosismo________________ ☐
3. Humor deprimido e tristeza______________ ☐
4. Desejo e fissura para fumar_______________ ☐
5. Dificuldade de concentração_______________ ☐
6. Aumento do apetite, fome e ganho de peso___ ☐
7. Insônia, problemas de sono e acordar a noite_ ☐
8. Incapacidade de relaxar__________________ ☐
9. Impaciência___________________________ ☐
10. Obstipação___________________________ ☐
11. Tontura_______________________________ ☐
12. Tosse________________________________ ☐
13. Pesadelo, sonhos______________________ ☐
14. Náusea______________________________ ☐

15. Nó na garganta______________________ ☐

FASE 3

AVALIAÇÃO 12ª SEMANA

180. 1. Data de retirada do medicamento na farmácia ☐☐ / ☐☐ / ☐☐ 3ª Mês

180.2. Data da próxima retirada de medicamento na farmácia ☐☐ / ☐☐ / ☐☐

180.4 Situação do Paciente (TABELA) **180.5. Tratamento** **180.6. Monóxido**

182. Exame Fisico – Fase 3

182.1. Altura do paciente: ☐☐☐ cm **182.2.** Peso: ☐☐☐Kg ☐☐☐g

182.3.IMC – Índice de Massa Corpórea (peso/ altura²): ☐☐ ☐

182.4. PA: ☐☐☐ x ☐☐☐ **182.5.** FC: ☐☐☐

182.6 Circunferência Abdominal: ☐☐☐cm **182.7** Circunferência Quadril: ☐☐☐

EXAMES – FASE 3

185.21 Hemograma Anemia – **Fase 3**: 1- Normal 2- Alterado ☐

185.22 Hemograma Infecção – **Fase 3**: 1- Normal 2- Alterado ☐

185.23 VHS - **Fase 3**: ☐☐☐

185.27 TGO – **Fase 3**: ☐☐☐

185.28 TGP – **Fase 3**: ☐☐☐

185.29 Creatinina – **Fase 3**: ☐☐☐

186. Escala de abstinência de nicotina de Minnesota (MNWS) : 0, 1, 2, 3 e 4 – Fase 3

1. Raiva, irritabilidade e frustração ___________ ☐
2. Ansiedade e nervosismo________________ ☐
3. Humor deprimido e tristeza______________ ☐

4. Desejo e fissura para fumar______________

5. Dificuldade de concentração______________ ☐

6. Aumento do apetite, fome e ganho de peso___ ☐

7. Insônia, problemas de sono e acordar a noite_ ☐

8. Incapacidade de relaxar________________ ☐

9. Impaciência_________________________ ☐

10. Obstipação________________________ ☐

11. Tontura_____________________________ ☐

12. Tosse______________________________ ☐

13. Pesadelo, sonhos____________________ ☐

14. Náusea____________________________ ☐

15. Nó na garganta______________________ ☐

187. Efeitos Colaterais – Fase 3:

1. Não 2. Sim, leve. 3. Sim, moderado. 4. Sim, grave.

187.1. Náuseas e/ ou vômitos___ ☐ **187.2.**Diarréia______________ ☐

187.3. Alergia de pele_________ ☐ **187.4.** Alergia respiratória_____ ☐

187.5. Outro(s)?_____________ ☐ Quais?____________________

188. Escala de Adesão Terapêutica - Fase 3: 1. Sim 2. Não

188.1. Alguma vez se esqueceu de tomar a sua medicação?_________________

188.2. Por vezes é descuidado a tomar a sua medicação?__________________

188.3. Quando se sente melhor, deixa, por vezes, de tomar a sua medicação?____

188.4. Por vezes, se sente pior quando toma a medicação, deixa da tomar?______

188.5. Só tomo a medicação quando me sinto doente?_____________________

188.6. Não é natural para minha mente e meu corpo ser controlado pela medicação?

188.7. Os meus pensamentos são mais claros com a medicação?____________

188.8. Por usar a medicação posso prevenir de ficar doente?_________________

188.9. Sinto-me estranho com a medicação?_____________________________

188.10. A medicação faz com que eu me sinta cansado e lento?________________

189.1. Pontuação – Hamilton(Fase 3)..

189.2. Escala de Tolerância de Fagerström (Fase 3)..

189.3.Escala de Incapacidade de Sheehan (0 – 10) – Fase 3

01. Trabalho______________

02. Vida social____________

03. Vida familiar___________

189.4. Dias perdidos ou improdutivos por mês:

Marcar S (sim) ou N (não)

189.5. Os sintomas têm causado faltas no trabalho, escola ou têm causado incapacidade em trabalhar em casa?

189.6 Mesmo que tenha ido ao trabalho e escola ou trabalho em casa , os sintomas tem diminuido sua produtividade?

300. SCID - Transtorno de HUMOR..

0- Sem alteração de Humor
1- Transtorno Bipolar, tipo Maniaco
2- Transtorno Bipolar, tipo Hipomaniaco
3- Transtorno Bipolar, tipo Depressivo
4- Transtorno Bipolar, tipo Misto
5- Transtorno Depressivo Maior, unipolar
6- Transtorno Depressivo Maior, em Remissão
7- Transtorno Distimico
8- Transtorno de Humor, devido a uma Condição Médica Geral
9- Transtorno de Humor, Induzido por Substância
10- Transtorno Bipolar em Remissão

301. SCID - Transtorno de ANSIEDADE...

0- Sem alteração de Ansiedade
1- Transtorno de Ansiedade Generalizada
2- Transtorno do Pânico
3- Agorafobia
4- Fobia Social
5- Transtorno de Estresse Pós Traumático
6- Transtorno Obsessivo Compulsivo
7- Outro

Anexo 3 – Determinação do Malondialdeído

MDA (MALONALDEIDO) - TBARS – Colorimétrico

(JENTZSCH, M. A.; BACHMANN, H.; FURST, P. Improved analysis of malondialdehide in human body fluids. Free Radic. Bio. Med., Tarrytown, v. 20, p. 251-256, 1996.)

REAGENTES

- Todos os reagentes podem ficar a temperatura ambiente, apenas são guardados em isopor.

1 - Ácido ortofosfórico 0,2 mol/L (1,685/ml)

- Em balão volumétrico pipetar 1,163mL de ácido fosfórico e completar com água destilada
- Transferir para frasco de vidro
- Pode ser estocado por tempo indeterminado, mas sempre verificar se há a presença de fungos

2 - NaOH 0,1 mol/L

- Pesar 4,0 g de NaOH, colocar em balão volumétrico de 1000mL e completar com água destilada
- Preparar a quantidade que for usar
- Pode ser armazenado por tempo indeterminado

3 - TBA 0,11 mol/L

- Pesar 800 mg TBA e dissolver em 50 mL de NaOH (0,1 mol/L)

4 - NaCl Saturado

- Adicionar NaCl em água até não dissolver mais (formar corpo de fundo)
- Pode ser armazenado por tempo indeterminado

PADRÃO

Solução de 0,03 µmol MDA

- PIPETAR 5µl de MDA + 50 ml de H2O
- Diluir esta solução 1/20 (1mL do padrão + 19mL de água

PROCEDIMENTO

- ✓ - Realizado em tubos de vidro de 8mL
- ✓ - 1 tubo – Branco
- ✓ - 6 tubos para a curva padrão – P1 a P6
- ✓ - E os tubos das amostras

Reagentes	Branco	01	02	03	04	05	06	Amostra
Agua	0,8 ml							
Padrao		0,8 ml	0,8 ml	0,8 ml	0,8 ml	0,8 ml	0,8 ml	
Plasma heparina								0,8 ml
BHT	0,1 ml	0,1 ml	0,1 ml	0,1 ml	0,1 ml	0,1 ml	0,1 ml	0,1 ml
Ac. Ortofosfo.	0,8 ml	0,8 ml	0,8 ml	0,8 ml	0,8 ml	0,8 ml	0,8 ml	0,8 ml
Concentr.	0,0	0,03	0,015	0,0075	0,00375	0,001875	0,000937	X

- ➢ Agitar por 10 segundos (mixer)
- ➢ Adicionar 1,0 ml de TBA – agitar (mixer)
- ➢ Incubação 45 minutos (banho maria a 90º c)
- ➢ No banho maria, tampar os tubos com bolinhas de vidro (evita a evaporação da reação)
- ➢ Resfriar os tubos – em um recipiente com água fria ou colocar os tubos na geladeira
- ➢ Adicionar 0,2 ml de NaCl saturado (presença de corpo de fundo)
- ➢ Adicionar 2,0 ml de n-butanol (na capela)
- ➢ Agitar por 40 segundos
- ➢ Centrifugar 3.000 rpm por 10 minutos
- ➢ Leitura – ler em 572 nm e ler em 535 nm (ler contra o branco)

CURVA DE CALIBRAÇÃO DO MDA

1 MOL – 164,2g – 1.000 ml
1 mmol – 0,1642 g – 1.000 ml
10 mmol – 1,642 g – 1.000 ml

Concentração do padrão 0,99 g – 1 ml

Anexo 4 – Determinação do Potencial Antioxidante Total Plasmático

TRAP SÉRICO

(Repetto, M. Oxidative stress in blood of HIV infected pacients.
Clin Chim Acta, 107-117, 1996)

REAGENTES

1. **ABAP** (azobis, PM = 271,2) 200mM
   Pesar 54,24mg de ABAP e dissolver em 1mL H_2O destilada em tubo de ensaio de 8mL
   Agitar no vórtex e preparar no dia de uso.
   Proteger o tubo com papel alumínio
   Manter no gelo

2. **Luminol** (PM = 199,1) – **Solução mãe**
   Pesar 3,98mg de Luminol e dissolver em 10mL H_2O destilada em tudo de ensaio de 8mL
   Agitar em vórtex
   Forma corpo de fundo
   Proteger o tubo com papel alumínio
   Conservar em geladeira

3. **Luminol – Solução de trabalho**
   Preparar no dia do uso
   A partir da Solução Mãe, agitar o tubo e pipetar 100μL LUMINOL – SOL. MÃE e diluir com 900μL de H_2O destilada
   Proteger o tubo com alumínio
   Agitar em vórtex

4. **TROLOX** (PM = 250,29) - **Solução mãe** (20μM)
   Pesar 5mg e dissolver em 10mL Tampão Glicina pH=8,6 em tubo de ensaio de 8mL
   Proteger com papel alumínio
   Agitar em vórtex
   Conservar em geladeira

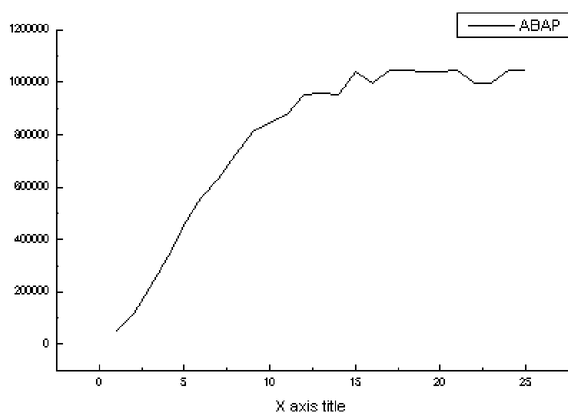
5. **TROLOX - Solução de trabalho** – PREPARO NO DIA DE USO
   Agitar e pipetar 4μL TROLOX SOL. MÃE e diluir com 796μL de Tampão Glicina pH = 8,6
   Manter no gelo

6. **Tampão glicina**
   Pesar 3,75g de glicina dissolver com $\pm$400mL H_2O destilada em béquer de vidro
   Acertar pH em 8,6 com KOH 1M
   Transferir o tampão para balão volumétrico de 500mL e completar o volume
   Transferir o tampão para frasco de vidro identificado e conservar em geladeira

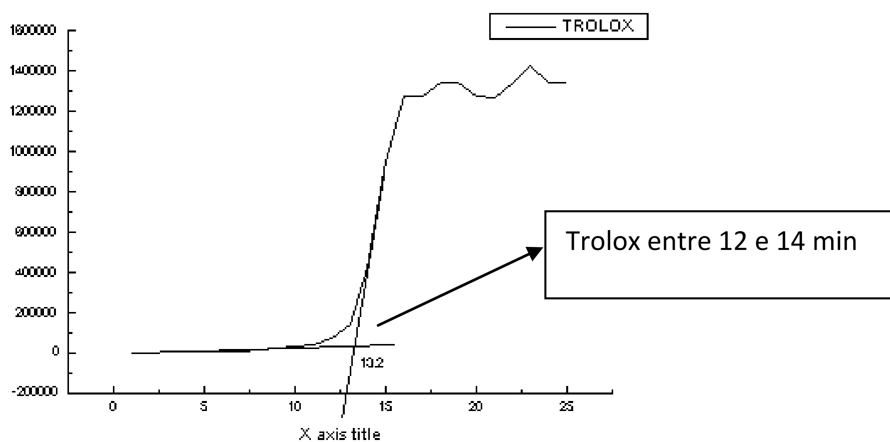
PROCEDIMENTO

✓ Antes de iniciar as reações com soro, fazer as curvas ABAP e com TROLOX

Curva ABAP
Tampão Glicina ______ 1,8mL
Luminol ___________ 0,1mL
ABAP _____________ 0,1mL



Curva Trolox
Tampão Glicina ______ 1,8mL
Luminol ___________ 0,1mL
Trolox _____________0,1 mL
ABAP _____________ 0,1mL
Tempo da curva Trolox entre 12 e 14 min



Reação com Soro
Tampão Glicina ______ 1,8mL
Luminol ___________ 0,1mL
Soro diluido 1 :2 c H_2O__ 5 uL
ABAP _____________ 0,1mL

PROGRAMAÇÃO DO APARELHO

- ✓ Main Menu – sel review and edit user program
- ✓ Review/Edit – sel luminescencia
- ✓ Review/Edit – sel counting time:25min
    - ➢ Edit Other Parameters
- ✓ Edit Other Parameters – sel data calculation
- ✓ Data calculation – sel number of data points: 25
    - ➢ sel count time/data point: 0,10
    - ➢ sel count sample set: 1
    - ➢ sel factor: 0,01
- ✓ Voltar ao menu principal: sel count single rack
- ✓ Count single rack: sel select user program
- ✓ User selection: sel luminescencia
- ✓ Count single rack: sel count with program user: 1
- ✓ Count single rack: sel [START]

Selecionar previamente os passos acima e deixar a tela start até que a rack contendo o frasco esteja na posição

CÁLCULO

$$\text{TRAP} = 802 \times \frac{\text{Tempo da amostra}}{\text{Tempo de trolox}} = \text{cpm}$$

Anexo 5 – Determinação do *Status* da Paraoxonoxase 1

Determinação do PON1*status*
Ensaio triplo enzimático - Espectrofotometria

Richter, R.J.; Jarvink, G. P.; Furlong, C. E. Determination of Paraoxonase 1 status Without the Use of Use of Toxic Organophosphate Substrates. Circulation: cardiovascular genetics (2008) 1:147-152

- ✓ **Preparo da Solução de Estoque de Tampão TRIS-HCl 1M**
  ($C_4H_{11}NO_3 \cdot HCl$ = 157,64)

Pesar 39,41g e diluir em 250mL de H_2O (utilizar balão volumétrico)
CUIDADO: reação exotérmica!

*Ajustar o pH para 8,0 (antes de acertar o menisco) e armazenar em geladeira
**Corrigir pH todos os dias antes do uso – para o uso, o pH poderá variar entre 8,0 e 8,5

- ✓ **Preparo da Solução de Estoque de $CaCl_2$ 1M**
  ($CaCl_2 \cdot 2H_2O$ = 147,02)

Pesar 7,35g e diluir em 50mL de H_2O (utilizar balão volumétrico)
CUIDADO: reação exotérmica!

*Filtrar a solução em papel de filtro após o preparo e armazenar em geladeira.

- ❖ **Preparo do Tampão A – Preparar no dia do uso**
  20mM TRIS-HCl (pH=8,0) e 1mM $CaCl_2$

** Tampão para a *diluição das amostras* e preparo do *Substrato de CMPA* (cloro-metil-fenil-acetato)
Para 100mL de H_2O: 2mL Solução Estoque de TRIS-HCl pH8,0
100μL Solução Estoque de $CaCl_2$

- ❖ **Preparo do Tampão B – Preparar no dia do uso**
  20mM TRIS-HCl (pH=8,0), 1mM $CaCl_2$ e 2M NaCl

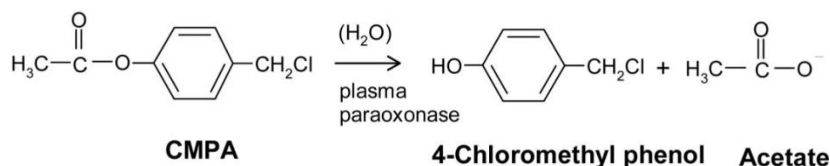
** Tampão para o preparo do Substrato de PA-*High Salt* (fenil-acetato em alta concentração de sal)
Para 50mL de H_2O: 1mL Solução Estoque de TRIS-HCl pH8,0
50μL Solução Estoque de $CaCl_2$
5,84g de NaCl

- ❖ **Preparo do Tampão C – Preparar no dia do uso**
  9,0mM TRIS-HCl (pH=8,0) e 0,9mM $CaCl_2$

** Tampão para o preparo do Substrato de PA-*No Salt* (fenil-acetato sem a adição de sal)
Para 50mL de H_2O: 450μL Solução Estoque de TRIS-HCl pH8,0
45μL Solução Estoque de $CaCl_2$

Preparo das amostras:
Antes de diluir as amostras, os tubos/criotubos de armazenamento deverão ser centrifugados a 10000 RPM, por 5min e refrigerados a 10ºC.

- **Reação 1 – Atividade CMPAse**



*** Leitura avalia a formação do produto de hidrólise do CMPA (o 4-clorometil-fenol) durante 4 minutos**

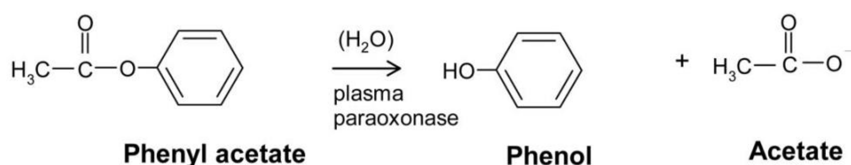
Diluir a amostra 1:40 – 10µL da amostra e 390µL do Tampão A
*Utilizar tubos de vidro
As amostras, após serem diluídas, deverão ser processadas em até 30min.

Preparo do Substrato de CMPA: $C_8H_7O_2CH_2Cl$ - PM: 184,62; Conc. final: 3mM
10mL do Tampão A + 4,6µL do Reagente CMPA
*Esta solução de substrato deve ser preparada em Tubo tipo *Falcon*, ao abrigo de luz, NO MOMENTO DO USO e **não** deverá ser utilizada após **1 hora** de seu preparo.
**Agitar vigorosamente por 30 segundos!

PROCEDIMENTO:
Adicionar 20µL da amostra diluída 1:40 nos poços em que ocorrerão a reação. Ao fim, adicionar 200µL da solução do Substrato de CMPA e proceder a leitura.

Parâmetros de leitura – Utilizar modelo pré-definido no leitor de microplacas *EnSpire*®: Protocolo "CMPAse"
Leitura cinética a 25ºC – 16 leituras, em 280nm, com intervalo de 15 segundos entre as leituras; o tempo total da reação é de 4min.
Após as leituras cinéticas, serão realizadas mais duas leituras (em 900nm e 977nm) para a correção do *path length* (caminho ótico);
*ver cálculos de correção ao final.

- **Reação 2 – Atividade AREase em alta concentração de sal**



*** Leitura avalia a formação do produto de hidrólise do fenil-acetato (fenol) durante 4 minutos**

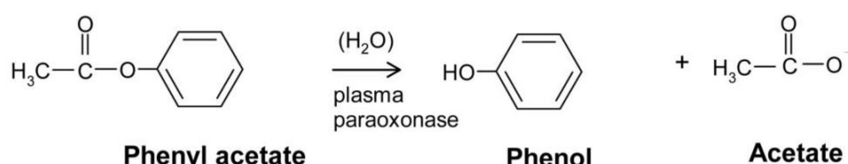
Diluir a amostra 1:40 – 10µL da amostra e 390µL do Tampão A
*Utilizar tubos de vidro
As amostras, após serem diluídas, deverão ser processadas em até 30min.

Preparo do Substrato de PA-*High Salt*: $C_8H_7O_2$ - PM: 136,15; Conc. final: 5,52mM
10mL do Tampão B + 10µL do Reagente PA (fenil-acetato)
*Esta solução de substrato deve ser preparada em Tubo tipo *Falcon*, ao abrigo de luz, NO MOMENTO DO USO e **não** deverá ser utilizada após **2 hora** de seu preparo.
**Agitar vigorosamente por 30 segundos!

PROCEDIMENTO:
Adicionar 20µL da amostra diluída 1:40 nos poços em que ocorrerão a reação. Ao fim, adicionar 200µL da solução do Substrato de PA-*High Salt* e proceder a leitura.
*Adicionar o substrato com cuidado, para não fazer bolhas!

Parâmetros de leitura – Utilizar modelo pré-definido no leitor de microplacas *EnSpire*®: Protocolo "AREase"
Leitura cinética a 25ºC – 16 leituras, em 270nm, com intervalo de 15 segundos entre as leituras; o tempo total da reação é de 4min.
Após as leituras cinéticas, serão realizadas mais duas leituras (em 900nm e 977nm) para a correção do *path length* (caminho ótico);
*ver cálculos de correção ao final.

- **Reação 3 – Atividade AREase sem adição de sal**



*** Leitura avalia a formação do produto de hidrólise do fenil-acetato (fenol) durante 4 minutos**

Diluir a amostra 1:80 – 5µL da amostra e 395µL do Tampão A
*Utilizar tubos de vidro
As amostras, após serem diluídas, deverão ser processadas em até 30min.

Preparo do Substrato de PA-*No Salt*: $C_8H_7O_2$ - PM: 136,15; Conc. final: 3,26mM
10mL do Tampão B + 5µL do Reagente PA (fenil-acetato)
*Esta solução de substrato deve ser preparada em Tubo tipo *Falcon*, ao abrigo de luz, NO MOMENTO DO USO e **não** deverá ser utilizada após **2 hora** de seu preparo.

**Agitar vigorosamente por 30 segundos!

PROCEDIMENTO:
Adicionar 20µL da amostra diluída 1:80 nos poços em que ocorrerão a reação. Ao fim, adicionar 200µL da solução do Substrato de PA-*No Salt* e proceder a leitura.

Parâmetros de leitura – Utilizar modelo pré-definido no leitor de microplacas *EnSpire*®: Protocolo "AREase"
Leitura cinética a 25ºC – 16 leituras, em 270nm, com intervalo de 15 segundos entre as leituras; o tempo total da reação é de 4min.
Após as leituras cinéticas, serão realizadas mais duas leituras (em 900nm e 977nm) para a correção do *path length* (caminho ótico);
*ver cálculos de correção ao final.

✓ **ANÁLISE DOS RESULTADOS**

Os resultados das 16 leituras em todas as reações deverão ser corrigidos da mesma forma;
* A primeira etapa é a correção por *base line correction*; esta correção deverá ser feita para realizar a comparação da atividade da cinética enzimática;
* As amostras cujas replicatas variarem mais de 10% ou que apresentarem um valor de r^2 inferior a 0,99 deverão ser reprocessadas;
* Somente a primeira porção linear da curva deverá ser utilizada na análise final;
* A análise deverá ser feita em mDO, e o valor das médias das leituras deverá ser utilizado na fórmula de cálculo de atividade (expresso em U/mL).

FÓRMULA

$$Atv = \frac{mDO \times Vol.total\ da\ reação\ (mL) \times Fator\ de\ diluição}{\varepsilon \times Volume\ de\ amostra\ (\mu L)}$$

Onde:
ε → Coeficiente de extinção molar
Para o produto de hidrólise do CMPA (4-clorometil-fenol) = 1,30mMol/Lcm^{-1}
Para o produto de hidrólise do fenil-acetato (fenol) = 1,31mMol/Lcm^{-1}

Correção do caminho ótico (*Path length correction)*
Greiner bio-one. Aplication Note: UV/VIS Spectroscopy in Microplates UV-Star®, µClear®, MICROLON® and CELLSTAR® < http://www.greinerbioone.com/en/row/articles/literatures/application_notes/>

a) Correção pela geometria da placa – para placas com poços cilíndricos de fundo chato:
   U tilizando as especificações do fabricante quanto ao diâmetro dos poços, fazer o seguinte cálculo:

$$h = \frac{4 \times V}{\pi \times d^2}$$

Onde:
h = fator de correção para a placa
V = volume final que será utilizado
d = média dos diâmetros especificados

b) Correção por volume de água:
Utilizando as leituras em 900nm e 977nm, é possível realizar a correção do *path length* para cada poço de reação, utilizando-se a seguinte fórmula:

$$h = \frac{(A_{977} - A_{900})_{amostra}}{(A_{977} - A_{900})_{1cm\ água}}$$

** Como referência para água, utilizar o valor padrão de 0,180.