UFRGS
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO: CIÊNCIAS EM

GASTROENTEROLOGIA E HEPATOLOGIA

Tese de Doutorado

Pirose e Resposta Encefálica ao Estímulo Ácido Esofágico:

Estudo da sua Modulação por Alimento e Nortriptilina na

Doença do Refluxo Gastroesofágico Não Erosiva

Aluno: Cassiano Mateus Forcelini

Orientador: Prof. Fernando Fornari

Abril - 2014

CIP - Catalogação na Publicação

"Su le finestre mostra a tutti il mio cuore che hai acceso chiudi dentro me la luce che hai incontrato per strada."

(Das janelas o meu coração mostra a todos que acendeste, bem dentro de mim, a luz que encontraste pela estrada)

Trecho da canção "Com Te Partirò", de Lucio Quarantotto, popularizada por Andrea Bocelli

SUMÁRIO

LISTA DE ABREVIATURAS

(em ordem alfabética)

- DRGE: doença do refluxo gastroesofágico

- DRNE: doença do refluxo gastroesofágico não erosiva

- ELISA: ensaio de imunoabsorção enzimática

- IBPs: inibidores da bomba de prótons

- JEG: junção esofagogástrica

- PPGCGH: Programa de Pós-Graduação: Ciências em Gastroenterologia e Hepatologia

- RM: ressonância magnética

- RMf: ressonância magnética funcional

- SNC: sistema nervoso central

- TCLE: termo de consentimento livre e esclarecido

- TRPV1: receptor vaniloide de capsaicina do tipo 1

- UFRGS: Universidade Federal do Rio Grande do Sul

RESUMO

Introdução: Pacientes com doença do refluxo gastroesofágico não erosiva (DRNE) geralmente apresentam a pirose como principal sintoma, comumente após as refeições. O papel de potenciais fatores desencadeadores e agravantes não está esclarecido. Antidepressivos poderiam ser úteis para aliviar a pirose modificando a percepção periférica e central do refluxo. **Objetivos:** Avaliar o efeito do alimento, de fatores psicológicos, do cortisol sérico e da nortriptilina sobre a percepção da pirose e a resposta encefálica, frente à infusão ácida esofágica em pacientes com DRNE, estudados com ressonância magnética funcional (RMf) do encéfalo. **Metodologia:** Em ensaio clínico duplo cego e cruzado, vinte pacientes com DRNE e que não estavam em uso de inibidores de bomba de prótons (36,1 ± 9,3 anos, 75% mulheres) foram randomizados para receber 21 dias de nortriptilina e placebo, de forma alternada, com um periodo de 21 dias de *washout* entre tratamentos. Mudanças nas respostas encefálicas mensuradas por RMf e na percepção de pirose ácido-induzidas foram avaliadas ao fim de cada tratamento. Aspectos psicológicos foram avaliados na amostra. Dezesseis destes pacientes foram também estudados quanto à percepção da pirose e à resposta encefálica frente à infusão ácida esofágica após a administração ou não de alimento, e dez dos pacientes tiveram o cortisol sérico medido em diferentes passos deste protocolo. **Resultados:** A nortriptilina reduziu significativamente a resposta encefálica induzida por ácido no cortex pré-frontal, caudado, ínsula, cíngulo e hipocampo em comparação com placebo. Contudo, não houve diferença significativa entre nortriptilina e placebo nos desfechos clínicos. Ambos os tratamentos diminuíram a pirose em relação ao estado pré-tratamento e a sinalização de pirose durante a estimulação ácida esofágica. No experimento que testou o efeito do alimento, a percepção da pirose aumentou no segundo período de estimulação ácida,

independentemente da administração ou não de alimento. Níveis séricos de cortisol e escores de ansiedade e depressão não tiveram relação com a percepção de pirose.

Discussão e conclusões: A nortriptilina diminuiu a resposta encefálica à infusão ácida esofágica mais intensamente que placebo, mas sem vantagem clínica. Tanto placebo quanto nortriptilina diminuíram a percepção de pirose. A estimulação ácida esofágica repetida é mais importante como fator sensibilizador do que o alimento. A possibilidade de influência de fatores psicológicos na modução da pirose ácido-induzida não pode ser excluída dado o tamanho amostral pequeno do estudo.

Palavras-chave: infusão ácida esofágica; RM funcional; pirose; doença do refluxo não erosiva; nortriptilina; alimento; cortisol.

INTRODUÇÃO

A doença do refluxo gastroesofágico (DRGE) é condição prevalente em todo o mundo, especialmente na sua forma não-erosiva (DRNE). Sua fisiopatologia e o papel de fatores agravantes ou desencadeantes ainda não estão completamente esclarecidos. O surgimento de tecnologias inovadoras para a avaliação do eixo esôfago-sistema nervoso central (SNC), como a ressonância magnética funcional (RMf) de encéfalo, ensejou a possibilidade de um estudo empolgante e desafiador da DRGE. Ao mesmo tempo em que se aprende cada vez mais acerca desta nova ferramenta de pesquisa - a RMf - abre-se o caminho para novas perguntas fisiopatológicas e terapêuticas acerca da DRGE. O papel do alimento, de fatores psicológicos e da liberação de cortisol na percepção dos sintomas, bem como a potencial utilidade de antidepressivos nesta condição que apresenta uma parcela de pacientes refratários ao tratamento padrão foram os motivadores deste estudo, um grande trabalho em equipe.

Como é intrínseco ao método científico, foram as perguntas acima que motivaram a busca pelas respostas, algumas delas obtidas e aqui apresentadas.

REVISÃO BIBLIOGRÁFICA

Doença do refluxo gastroesofágico (DRGE)

A DRGE é condição comum, afetando até 20% da população adulta mundial (1-6), com evidência de aumento de prevalência nos últimos anos (6,7), e implicando comprometimento da qualidade de vida dos pacientes e custos significativos (8). Segundo consenso global de 2006, a DRGE é conceituada como a "condição que ocorre quando o refluxo de conteúdo gástrico para o esôfago causa sintomas incomodativos e/ou complicações" (9). O sintoma cardinal da DRGE em adulto é a pirose (sensação de queimação retroesternal), porém outras queixas como regurgitação, disfagia e tosse crônica podem ocorrer (9). Na população pediátrica a definição diagnóstica da doença é menos clara abaixo dos 8 anos de idade (10), mas o sintoma de regurgitação é o mais comum (11,12), principalmente no primeiro ano de vida (13).

A fisiopatologia da DRGE em adultos é complexa. Envolve a falência da barreira antirrefluxo, situada na junção esofagogástrica (JEG), mas também o comprometimento de mecanismos de defesa do esôfago (14). Episódios de refluxo costumam ocorrer após as refeições, seja pela maior frequência de relaxamentos transitórios do esfíncter esofágico inferior, seja pelo aumento da pressão gástrica relacionada à presença de alimento (15). A formação de um bolsão ácido sobre o alimento na região da cárdia do estômago tem sido recentemente apontada como facilitadora para a ocorrência do refluxo (16-19), especialmente em portadores de hérnia de hiato (17). O hábito de deitar logo após uma refeição também se associa com a ocorrência de sintomas da DRGE (20).

A percepção da pirose pode variar em momentos diferentes no mesmo indivíduo. Por exemplo: estímulo no esôfago proximal pode ser mais sintomático em

comparação ao esôfago distal (21,22). Há evidências, também, da ocorrência do fenômeno de sensibilização ácida, como demonstrado por Emerenziani e colegas (23), no qual episódios espontâneos de refluxo ácido facilitam a ocorrência de sintomas em episódios de refluxo posteriores, mesmo que pouco ácidos (pH entre 4,0 e 7,0). Isso pode ser causado pela dilatação de espaços intercelulares em resposta à exposição esofágica ao refluxo ácido, e mesmo aos episódios pouco ácidos, facilitando a chegada de moléculas de hidrogênio às terminações nervosas da mucosa (24). De fato, o contato prolongado da mucosa com o conteúdo do refluxo, provavelmente causado por defeito no mecanismo de barreira da JEG e nas defesas esofágicas parece ser fator sensibilizador de sintomas (25).

Existe a demonstração de que refluxo de suco gástrico pouco ácido ou não ácido (pH > 7,0), e mesmo de sais biliares pode produzir sintomas de DRGE (26,27). Dentre os mecanismos apontados como responsáveis por isso estão: 1. contrações sustentadas da musculatura longitudinal esofágica (28); 2. inflamação microscópica, com dilatação dos espaços intercelulares, infiltração linfocitária e liberação de citocinas pró-inflamatórias em resposta à agressão química (29-32); 3. hipersensibilidade visceral (33,34).

Já a variação inter-indivíduo na DRGE é considerável. Com base nos achados endoscópicos, a DRGE tem sido categorizada em dois grupos (9,28,35,36): 1. DRGE *erosiva*, com presença de esofagite de refluxo macroscopicamente identificada como soluções de continuidade na mucosa; 2. DRGE *não erosiva* (DRNE), quando a mucosa esofágica tem aspecto normal. Esta é mais comum como forma de apresentação sintomática do que a doença erosiva (16,37,38). De fato, até um terço dos pacientes com doença erosiva mostra-se assintomático (37). O uso de inibidores da bomba de prótons (IBPs) costuma ser eficaz no manejo de pacientes com doença erosiva (39), mas uma

parcela de pacientes (até 40% dos casos) mostra-se refratária aos IBPs, especialmente os portadores de DRNE (40,41,42). Tal heterogeneidade de resposta provavelmente se deve à sua complexidade fisiopatológica.

A DRGE não erosiva, por sua vez, tem sido subdividida em dois tipos conforme a demonstração de exposição da mucosa esofágica ao refluxo ácido: 1. Associada ao aumento de exposição ácida comprovada por pHmetria; 2. Sem evidência de aumento de exposição ácida, mas com associação positiva entre refluxo e sintoma à pHmetria, e resposta a medicamentos com efeito ácido-supressor. Além disso, tem-se usado o termo *pirose funcional* para diagnosticar o paciente com sintomas sugestivos de DRGE, porém sem erosões esofágicas à endoscopia, sem aumento da exposição ácida à pHmetria (pH <4), ausência de associação entre refluxo e sintoma, e sem resposta à terapia anti-ácida (33,43,44). Contudo, a diferenciação entre pirose funcional e DRNE suscita debates, reconhecendo-se que pode haver sobreposição entre as condições (28,45-47).

Hipersensibilidade Visceral

A hipersensibilidade visceral constitui a disfunção da neuropercepção periférica e/ou central de estímulos nocivos e inócuos sobre o trato gastrintestinal. Postula-se sua participação na geração de sintomas na DRGE, especialmente na DRNE, e na pirose funcional. Estudos recentes têm apontado o papel do eixo esôfago-sistema nervoso central (SNC) na modulação dos sintomas de DRGE (48,49). Tal sensibilização pode se dar tanto em nível periférico, isto é, nas terminações nervosas amielínicas sensitivas espinhais e autonômicas presentes no epitélio esofágico, como em nível central, ou seja, na medula espinhal e encéfalo (33,34). Como exemplo de elementos periférios que

podem contribuir para a hipersensibilidade visceral está a expressão de receptores vanilóides de capsaicina do tipo 1 (TRVP1) (50,51).

Dentre fatores com potencial de modular a percepção de pirose estão os aspectos psicológicos. Recentemente, foi demonstrada maior prevalência de ansiedade em pacientes com DRNE que em controles (52). Além disso, encontrou-se relação inversa entre qualidade de vida em pacientes com DRNE e a presença de ansiedade ou depressão (53). A associação positiva entre estresse e sintomas de DRGE já foi apontada no passado (54). Os mecanismos pelos quais fatores psicológicos poderiam modular a percepção de pirose não estão esclarecidos, mas poderiam atuar tanto em nível periférico quanto central. Farré e colaboradores demonstraram que o estresse induz alterações estruturais no epitélio esofágico a ponto de facilitar a penetração de substâncias contidas no refluxo até as terminações nervosas, potencializando a percepção de pirose (55,56).

Um mediador em potencial para este processo pode ser o cortisol, pois é liberado na corrente sanguínea pelo córtex adrenal em resposta a estímulos estressores. Tal liberação poderia levar tanto às alterações estruturais do epitélio esofágico como à maior sensibilização periférica e central a estímulos. Porém, os resultados dos poucos estudos com modelos de estresse agudo não demonstraram relação clara entre níveis de cortisol e sintomas de DRGE (57,58).

Por outro lado, em indivíduos sadios, a perfusão do esôfago distal com soluções ácidas leva à dilatação dos espaços intercelulares do epitélio esofágico tanto na área perfundida quanto mais proximal, sem provocar sintomas (24). Isso sugere que outros elementos, além da dilatação dos espaços intercelulares, podem compor a sensibilização visceral na DRGE.

Representação encefálica da pirose

Na esteira da investigação do eixo esôfago-SNC tem-se estudado, nos últimos anos, a representação encefálica da pirose (59-65). O uso inicial da magnetoeletroencefalografia e da tomografia por emissão de pósitrons evoluiu, posteriormente, para o emprego da ressonância magnética funcional (RMf) de encéfalo, a qual combina atraentes elementos para a pesquisa fisiológica em relação às tecnologias anteriores (63): localização precisa de áreas encefálicas ativadas; menor latência entre estímulo e resposta evocada; maior disponibilidade de aparelhagem.

Kern e colegas mostraram que a infusão de solução acidificada na luz esofágica, simulando refluxo, é capaz de desencadear pirose em pacientes com DRGE e controles, bem como a ativação de áreas encefálicas avaliadas pela RMf (61). Tal ativação foi mais intensa e precoce nos paciente em relação aos controles. Mais recentemente, compararam-se subgrupos de pacientes com DRGE em termos de ativação encefálica durante perfusão ácida no esôfago distal, tendo-se demonstrado que a ansiedade de antecipação ao estímulo já é capaz de desencadear pirose, especialmente em pacientes com DRNE (64). Dentre as áreas encefálicas mais consistentemente ativadas nos estudos acima estão a ínsula, o giro do cíngulo e o córtex pré-frontal, regiões envolvidas na interpretação e no dimensionamento emocional da sensibilidade dolorosa (33,34,63).

Embora o refluxo e seus sintomas sejam mais comuns após refeições (15,20), até hoje não se estudou o efeito da presença de alimento no estômago sobre a ativação encefálica na DRGE. Curiosamente, já foram avaliados os resultados, em termos de resposta encefálica à RMf, do consumo de líquidos de diferentes sabores por pessoas sadias, com maior ativação para líquidos com sabores em relação à água e à saliva (66).

Uso de antidepressivos para dor visceral

O rol de terapêuticas disponíveis para a DRGE é relativamente restrito. O tratamento clínico fundamenta-se no uso de fármacos que bloqueiam a secreção gástrica de ácido e pepsina, com eficácia e segurança significativas. Contudo, a refratariedade ao tratamento ácido-supressor abrange considerável parcela dos pacientes com DRNE (40,41). Além disso, a possibilidade de dependência do uso de IBPs é preocupação contemporânea (67). Tais fatores ensejam a pesquisa de novas modalidades terapêuticas para a DRGE, com alvos terapêuticos diferentes da inibição da secreção ácida.

O uso de antidepressivos tem sido uma ferramenta de uso crescente no controle de dores viscerais na prática clínica. Atuam em sítios encefálicos, medulares e periféricos, tanto em modelos animais quanto clínicos (68,69). O efeito analgésico dos antidepressivos independe de sua ação sobre o humor, apresentando período de latência menor e processando-se com doses inferiores às antidepressivas (70).

Dentre as classes de antidepressivos, os tricíclicos são os que apresentam maior quantidade de estudos, mostrando-se eficazes e seguros em diversas condições de dor crônica. Seus representantes têm efeito analgésico semelhante, mas a nortriptilina apresenta um perfil de segurança melhor em relação aos outros tricíclicos de uso comum, como a amitriptilina e a imipramina (70). A nortriptilina apresenta várias ações farmacológicas, como inibição da recaptação da noradrenalina e, em menor grau, da serotonina, além de bloqueio de canais de sódio, efeito parassimpaticolítico (anticolinérgico) e o antagonismo de receptores do tipo aspartato-glutamato. Em doses baixas apresenta efeito analgésico, observado mesmo em pacientes que não se apresentem com quadro de depressão (71,72). Seus efeitos colaterais mais comuns

incluem sonolência, xerostomia, constipação, retenção urinária, palpitações e aumento do apetite (73).

Em recente metanálise, baixas doses de antidepressivos tricíclicos mostraram-se clinicamente benéficos no controle de sintomas da síndrome do intestino irritável (74). Em pacientes com esta condição, o uso de amitriptilina reduziu a ativação de áreas corticais avaliadas por RMf durante distensão retal com balão (75). Também já foi sugerido o seu uso no manejo da dispepsia funcional (76). Em pessoas saudáveis, a clomipramina diminuiu a ativação em áreas encefálicas semelhantes às induzidas pela infusão ácida esofágica (77). A clomipramina faz parte do grupo dos antidepressivos tricíclicos, mas sua inibição da recaptação de serotonina é maior que a promovida por nortriptilina e amitriptilina (73).

O uso de antidepressivos foi avaliado para a modulação da sensibilidade esofágica em controles sadios, em pacientes com dor torácica presumivelmente esofágica e em portadores de DRGE. Três estudos empregaram antidepressivos para dor esofágica induzida experimentalmente em voluntários sadios, sem história de depressão ou ansiedade, comparando com placebo. Dois deles utilizaram tricíclicos (78,79), enquanto um avaliou um agente serotonérgico (80). Tratamento com tricíclico pouco ou em nada alterou o limiar de dor por distensão esofágica (78,79). Por outro lado, uma dose única de citalopram intravenoso aumentou o limiar de percepção da distensão e da estimulação ácida esofágica (80).

Na síndrome da dor torácica funcional, condição comumente atribuída à hipersensibilidade esofágica, oito ensaios clínicos randomizados e controlados contra placebo foram realizados (81-88), sendo que os resultados foram favoráveis em cinco deles (82-84,87,88): imipramina (tricíclico), sertralina (bloqueador da recaptação da

serotonina) e venlafaxina (bloqueador da recaptação da noradrenalina, serotonina e dopamina) tiveram efeitos benéficos sobre a intensidade e frequência da dor torácica. O mesmo não foi obtido com paroxetina e trazodona (81,85,86).

Dentro do limitado conhecimento acerca dos mecanismos de hipersensibilidade visceral na DRGE, aventa-se um papel potencial para os antidepressivos nessa condição (42,43). Nesse sentido, três estudos com metodologias e resultados diversos foram recentemente publicados (89-91), sendo um na forma de resumo (89). Hershcovici e colegas relataram que nortriptilina e placebo não diferiram no alívio da pirose de pacientes com DRGE, sem avaliar separadamente os pacientes quanto à presença ou não de esofagite (89). Por outro lado, dois estudos com agentes serotonérgicos (citalopram e fluoxetina) mostraram resultados superiores ao placebo (90,91). Porém, em um deles não foi empregado questionário validado para aferição dos sintomas (90), enquanto que em outro foram incluídos somente pacientes com DREN refratários a inibidores de bomba de protons (91). Ou seja, persiste o questionamento acerca da utilidade ou não de antidepressivos para o tratamento da DRGE, especialmente da DRNE.

JUSTIFICATIVA

Há necessidade de um melhor entendimento da fisiopatologia da DRGE não erosiva, especialmente no tocante à interação esôfago-SNC. Para tanto, pode contribuir o estudo dos fenômenos biológicos envolvidos na percepção da pirose e em sua resposta encefálica.

Além disso, frente à falha do tratamento clínico convencional em parcela significativa dos pacientes com DRGE, justifica-se a busca de novas terapêuticas farmacológicas que atuem sobre mecanismos de hipersensibilidade visceral.

OBJETIVOS

Geral

Avaliar a representação encefálica do estímulo ácido esofágico e da pirose ácido-induzida mediante RMf, em pacientes com DRGE não erosiva, bem como os potenciais efeitos moduladores da presença de alimento no estômago, de aspectos psicológicos, dos níveis séricos de cortisol e do tratamento com nortriptilina sobre a pirose e sua resposta encefálica em pacientes adultos com DRGE não erosiva.

Específicos

Capítulo 1 (Estudo Observacional – Artigo 1)

1. Comparar os achados de RMf e a percepção de pirose durante a infusão ácida no esôfago distal em pacientes com DRNE *antes* e *depois* da ingestão de uma refeição, e estes resultados com os obtidos *antes* e *depois* de um intervalo sem refeição.

2. Avaliar o papel de aspectos psicológicos (escores de depressão e ansiedade) na percepção de pirose frente à infusão ácida esofágica.

3. Quantificar os níveis séricos de cortisol antes e após a infusão ácida esofágica, durante a RMf, bem como depois de intervalos com e sem refeição, e os correlacionar com a percepção de pirose frente à infusão ácida esofágica.

Capítulo 2 (Ensaio Clínico - Artigo 2):

1. Comparar a resposta encefálica à perfusão ácida no esôfago distal, avaliada pela RMf, ao término de dois tratamentos diferentes: nortriptilina e placebo.

2. Comparar a ocorrência de pirose durante a perfusão ácida no esôfago distal, em vigência de cada tratamento.

3. Avaliar o efeito da nortriptilina sobre os sintomas durante o período de tratamento, em comparação com placebo.

4. Quantificar os dias com pirose e o número de comprimidos de antiácidos consumidos durante os tratamentos.

5. Comparar a frequência de efeitos adversos da nortriptilina com a do placebo.

6. Avaliar o papel de aspectos psicológicos (escores de depressão e ansiedade) na percepção da pirose frente à infusão ácida esofágica, em vigência de tratamento com nortriptilina e placebo.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Corder AP, Jones RH, Sadler GH, Daniels P, Johnson CD. Heartburn, oesophagitis and Barrett's oesophagus in self-medicating patients in general practice. *Br J Clin Pract* 1996; 50:245-248.

2. Nader F, da Costa JS, Nader GA, Motta GL. [Prevalence of heartburn in Pelotas, RS, Brasil: population-based study]. *Arq Gastroenterol* 2003; 40:31-34.

3. de Oliveira SS, dos Santos IS, da Silva JF, Machado EC. [Gastroesophageal reflux disease: prevalende and associated factors]. *Arq Gastroenterol* 2005; 42:116-121.

4. Dent J, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastroesophageal reflux disease: a systematic review. *Gut* 2005; 54:710-717.

5. Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Graffner H, Vieth M, Stolte M, Engstrand L, Talley NJ, Agréus N. High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: a Kalixanda study report. *Scand J Gastroenterol* 2005; 40:275-285.

6. Jung HK. Epidemiology of gastroesophageal reflux disease in Asia: a systematic review. *J Neurogastroenterol Motil* 2011; 17:14-27.

7. El-Seraq HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2013. doi:10.1136/gutjnl-2012-304269.

8. Shaheen NJ, Hansen RA, Morgan DR, Gangarosa LM, Ringel Y, Thiny MT, Russo MW, Sandler RS. The Burden of Gastrointestinal and Liver Diseases, 2006. *Am J Gastroenterol* 2006; 100:1010-1016.

9. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; 101:1900-1920.

10. Sherman PM, Hassall E, Fagundes-Neto U, Gold BD, Kato S, Koletzko S, Orenstein S, Rudolph C, Vakil N, Vandenplas Y. A global, evidence-based consensus on the definition of gastroesophageal reflux disease in the pediatric population. *Am J Gastroenterol* 2009; 104:1278-1295.

11. Campanozzi A, Boccia G, Pensabene L, Panetta F, Marseglia A, Strisciuglio P, Barbera C, Pettoello-Mantovani M, Staiano A. Prevalence and natural history of gastroesophageal reflux: pediatric prospective survey. *Pediatrics* 2009; 123:779-783.

12. Martigne L, Delaage PH, Thomas-Delecourt F, Bonnelye G, Barthélémy P, Gottrand F. Prevalence and management of gastroesophageal reflux disease in chindren

and adolescents: a nationwide cross-sectional observational study. *Eur J Pedriat* 2012; 171:1767-1773.

13. Osatakul S, Sriplung H, Puetpaiboon A, Junjana CO, Chamnongpakdi S. Prevalence and natural course of gastroesophageal reflux symptons: a 1 year cohort study in Thai infants. *J Pediatr Gastroenterol Nutr* 2002; 34:63-67.

14. Penagini R, Carmagnola S, Cantu P. Review article: gastro-oesophageal reflux disease – pathophysiological issues of clinical relevance. *Aliment Pharmacol Ther* 2002; 16 Suppl 4:65-71.

15. Fass R. The pathophysiological mechanisms of GERD in the obese patients. *Dig Dis Sci* 2008; 53:2300-2306.

16. Clarke AT, Wirz AA, Manning JJ, Ballantyne SA, Alcorn DJ, McColl KE. Severe reflux disease is associated with an enlarged unbuffered proximal gastric acid pocket. *Gut* 2008; 57:292-297.

17. Beaumont H, Bennink RJ, de Jong J, Boeckxstaens GE. The position of the acid pocket as a major risk factor for acidic reflux in healthy subjects and patients with GORD. *Gut* 2010; 59:441-451.

18. Sauter M, Curcic J, Menne D, Goetze O, Fried M, Schwizer W, Steingoetter A. Measuring the interaction of meal and gastric secretion: a combined quantitative magnetic resonance imaging and pharmacokinetic modeling approach. *Neurogastroenterol Motil* 2012; 24:632-638.

19. Rohof WO, Bennink RJ, Smout AJ, Thomas E, Boeckxstaens GE. An alginate-antacid formulation localizes to the acid pocket to reduce acid reflux in patients with gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2013; 11:1585-1591.

20. Mansour-Ghanaei F, Joukar F, Atshani SM, Chagharvand S, Souti F. The epidemiology of gastroesophageal reflux disease: a survey on the prevalence and the associated factors in a random sample of the general population in the Northern part of Iran. *Int J Mol Epidemiol Genet* 2013; 4:175-82.

21. Bredenoord AJ, Weusten BL, Curvers WL, Timmer R, Smout AJ. Determinants of perception of heartburn and regurgitation. *Gut* 2006; 55:313-318.

22. Rohof WO, Bennink RJ, de Jonge H, Boeckxstaens GE. Increased proximal reflux in a hypersensitive esophagus might explain symptoms resistant to proton pump inhibitors in patients with gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2013. doi: 10.1016/j.cgh.2013.10.026.

23. Emerenziani S, Ribolsi M, Guarino MP, Balestrieri P, Altomare A, Rescio MP, Cicala M. Acid reflux episodes sensitize the esophagus to perception of weakly acidic and mixed reflux in non-erosive reflux disease patients. *Neurogastroenterol Motil* 2014; 26:108-114.

24. Farre R, Fornari F, Blondeau K, Vieth M, De Vos R, Bisschops R, Mertens V, Pauwels A, Tack J, Sifrim D. Acid and weakly acidic solutions impair mucosal integrity of distal exposed and proximal non-exposed human oesophagus. *Gut* 2010; 59:164-169.

25. Altomare A, Guarino MP, Cocca S, Emerenziani S, Cicala M. Gastroesophageal reflux disease: Update on inflammation and symptom perception. *World J Gastroenterol* 2013; 19:6523-6528.

26. Sifrim D. Acid, weakly acidic and non-acid gastroesophageal reflux: differences, prevalence and clinical relevance. *Eur J Gastroenterol Hepatol* 2004; 16:823-830.

27. Savarino E, Zentilin P, Tutuian R, Pohl D, Casa DD, Frazzoni M, Cestari C, Savarino V. The role of nonacid reflux in NERD: lessons learned from impedance-pH monitoring in 150 patients off therapy. *Am J Gastroenterol* 2008; 103:2685-2693.

28. Winter JW, Heading RC. The nonerosive reflux disease – gastroesophageal reflux disease controversy. *Curr Opin Gastroenterol* 2008; 24:509-515.

29. Zentilin P, Savarino V, Mastraccci L, Spaggiari P, Dulbecco P, Ceppa P, Savarino E, Parodi A, Mansi C, Fiocca R. Reassessment of the diagnostic value of histology in patients with GERD, using multiple biopsy sites and an appropriate control group. *Am J Gastroenterol* 2005; 100:2299-2306.

30. Rieder F, Cheng L, Harnett KM, Chak A, Cooper GS, Isenberg G, Ray M, Katz JA, Catanzaro A, O'Shea R, Post AB, Wong R, Sivak MV, McCormick T, Philips M, West GA, Willis JE, Biancani P, Fiocchi C. Gastroesophagel reflux disease-associated esophagitis induces endogenous cytokine production leading to motor abnormalities. *Gastroenterology* 2007; 132:154-165.

31. Souza RF, Huo X, Mittal V, Schuler CM, Carmack SW, Zhang HY, Zhang X, Yu C, Hormi-Carver K, Genta RM, Spechler SJ. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. *Gastroenterology* 2009; 137:1776-1784.

32. Tadiparthi RA, Bansal A, Wani S, Mathur S, Hall SB, Rastogi A, Higbee A, Gaddam S, Sharma P. Dilated intercellular spaces and lymphocytes on biopsy relate to symptoms in erosive GERD but not NERD. *Aliment Pharmacol Ther* 2011; 33:1202-1208.

33. Chua YC, Aziz Q. Perception of gastro-oesophageal reflux. *Best Practice & Research Clinical Gastroenterology* 2010; 24:883-891.

34. Knowles CH, Aziz Q. Basic and clinical aspects of gastrointestinal pain. *Pain* 2009; 141:191-209.

35. Modlin IM, Hunt RH, Malfertheiner P, Moayyedi P, Quigley EM, Tytgat GN, Tack J, Heading RC, Holtman G, Moss SF; Vevey NERD Consensus Group. *Digestion* 2009; 80:74-88.

36. Fock KM, Poh CH. Gastroesophageal reflux disease. *J Gastroenterol* 2010; 45:808-825.

37. Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Graffner H, Vieth M, Stolte M, Engstrand L, Talley NJ, Agréus L. High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: a Kalixanda study report. *Scand J Gastroenterol* 2005; 40:275-285.

38. Minatsuki C, Yamamichi N, Shimamoto T, Kakimoto H, Takahashi Y, Fujishiro M, Sakaguchi Y, Nakayama C, Konno-Shimizu M, Matsuda R, Mochizuki S, Asada-Hirayama I, Tsuji I, Kodashima S, Ono S, Niimi K, Mitsushima T, Koike K. Background factors of reflux esophagitis and non-erosive reflux disease: a cross-sectional study of 10,837 subjects in Japan. *PLoS One* 2013. doi:10.1371/journal.pone.0069891.

39. Kahrilas PJ, Shaheen NJ, Vaezi MF. American Gastroenterological Association Institute technical review on the management of gastroesophageal reflux disease. *Gastroenterology* 2008; 135:1392-1413.

40. Dean BB, Gano AD Jr, Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in nonerosive reflux disease. *Clin Gastroenterol Hepatol* 2004; 2: 656-664.

41. Fass R, Sifrim D. Management of heartburn not responding to proton pump inhibitors. *Gut* 2009; 58:295-309.

42. Herschcovici T, Fass R. Nonerosive reflux disease (NERD) – An update. *J Neurogastroenterol Motil* 2010; 16:8-21.

43. Galmiche JP, Clouse RE, Bálint A, Cook IJ, Kahrilas PJ, Paterson WG, Smout AJPM. Functional esophageal disorders. *Gastroenterology* 2006; 130:1459-1465.

44. Fass R. Functional heartburn: what it is and how to treat it. *Gastrointest Endoscopy Clin N Am* 2009; 19:23-33.

45. Noh YW, Yung HK, Kim SE, Jung SA. Overlap of erosive and non-erosive reflux disease with functional gastrointestinal disorders according to Rome III criteria. *J Neurogastroenterol Motil* 2010; 16:148-156.

46. Ke MI. How to differentiate non-erosive reflux disease from functional heartburn. *J Dig Dis* 2012; 13:605-608.

47. Savarino E, Zentilin P, Savarino V. NERD: an umbrella term including heterogeneous subpopulations. *Nat Rev Gastroenterol Hepatol* 2013; 10:371-380.

48. Frobert O, Rendt-Nielsen L, Bak P, Funch-Jensen P, Bagger JP. Oesophageal sensation assessed by electrical stimuli and brain evoked potentials – a new model for viscelar nociception. *Gut* 1995; 37:603-609.

49. Nagahara A, Miwa H, Minoo T, Hojo M, Kawabe M, Osada T, Kurosawa A, Asaoka D, Terai T, Ohkusa T, Sato N. Increased esophageal sensitivity to acid and saline in patients with nonerosive gastroesophageal reflux disease. *J Clin Gastroenterol* 2006; 40: 891-895.

50. Matthews PJ, Aziz Q, Facer P, Davis JB, Thompson DG, Anand P. Increased capsaicin receptor TRVP1 nerve fibres in the inflamed human oesophagus. *Eur J Gastroenterol Hepatol 2004*; 16:897-902.

51. Guarino MP, Cheng L, Ma J, Harnett K, Biancani P, Altomare A, Panzera F, Behar J, Cicala M. Increased TRPV1 gene expression in esophageal mucosa of patients with non-erosive and erosive reflux disease. *Neurogastroenterol Motil* 2010; 22: 746-751.

52. Lee KJ, Kwon HC, Cheong JY, Cho SW. Demographic, clinical, and psychological characteristics of the heartburn groups classified using the Rome III criteria and factors associated with the responsiveness to proton pump inhibitors in the gastroesophageal reflux disease group. *Digestion* 2009; 79:131-136.

53. Oh JH, Kim TS, Choi MG, Lee H, Jeon EJ, Choi SW, Lee C, Chung IS. Relationship between Psychological Factors and Quality of Life in Subtypes of Gastroesophageal Reflux Disease. *Gut Liver* 2009; 3:259-265.

54. Bradley LA, Richter JE, Pulliam TJ, Haile JM, Scarinci IC, Schan CA, Dalton CB, Salley AN. The relationship between stress and symptoms of gastroesophageal reflux: the influence of psychological factors. *Am J Gastroenterol* 1993; 88:11-19.

55. Farre R, De Vos R, Geboes K, Verbecke K, Vanden BP, Depoortere I, Blondeau K, Tack J, Sifrim D. Critical role of stress in increased oesophageal mucosa permeability and dilated intercellular spaces. *Gut* 2007; 56:1191-1197.

56. Soderholm SJ. Stress-related changes in oesophageal permeability: filling the gaps of GORD? *Gut* 2007; 56:1177-1180.

57. Wright CE, Ebrecht M, Mitchell R, Anggiansah A, Weinman J. The effect of psychological stress on symptom severity and perception in patients with gastro-oesophageal reflux. *J Psychosom Res* 2005; 59:415-424.

58. Fass R, Naliboff BD, Fass SS, Peleg N, Wendel C, Malagon IB, Mayer EA. The effect of auditory stress on perception of intraesophageal acid in patients with gastroesophageal reflux disease. *Gastroenterology* 2008; 134:696-705.

59. Aziz Q, Andersson JLR, Valind S, Sundin A, Hamdy S, Jones AKP, Foster ER, Langstrom B, Thompson DG. Identification of human brain loci processing esophageal sensation using positron emission tomography. *Gastroenterology* 1997; 113:50-59.

60. Kern M, Birn RM, Jaradeh S, Jesmanowicz A, Cox RW, Hyde JS, Shaker R. Identification and characterization of cerebral cortical reponse to esophageal mucosal acid exposure and distension. *Gastroenterology* 1998; 115:1353-1362.

61. Kern M, Hofmann C, Hyde J, Shaker R. Characterization of the cerebral cortical representation of heartburn in GERD patients. *Am J Physiol Gastrointest Liver Physiol* 2004; 286:G174-181.

62. Lawal A, Kern M, Sanjeevi A, Antonik S, Mepani R, Rittmann T, Hussaini S, Hofmann C, Tatro L, Jesmanowicz A, Verber M, Shaker R. Neurocognitive processing of the esophageal central sensitization in the insula and cingulated gyrus. *Am J Physiol Gastrointest Liver Physiol* 2008; 294:G787-794.

63. Mayer EA, Aziz Q, Coen S, Kern M, Labus JS, Lane R, Kuo B, Naliboff B, Tracey I. Brain imaging approaches to the study of functional GI disorders: a Rome working Team report. *Neurogastroenterol Motil* 2009; 21:579-596.

64. Wang K, Duan LP, Zeng XZ, Liu JY, Weng XC. Differences in cerebral response to esophageal acid stimuli and psychological anticipation in GERD sybtypes – An fMRI study. *BMC Gastroenterol* 2011. doi:10.1186/1471-230X-11-28.

65. Forcelini CM, Tomiozzo Jr JC, Farré R, Van Oudenhove L, Callegari-Jacques SM, Ribeiro M, Madalosso BH, Fornari F. Effect of nortriptyline on brain responses to painful esophageal acid infusion in patients with non-erosive reflux disease. *Neurogastroenterol Motil* 2014; 26:187-195.

66. Babaei A, Kern M, Antonik S, Mepani R, Ward BD, Li SJ, Hyde J, Shaker R. Enhancing effects of flavored nutritive stimuli on cortical swallowing network activity. *Am J Physiol Gastrointest Liver Physiol* 2010; 299:G422-G429.

67. Reimer C, Sondergaard B, Hilsted L, Bytzer P. Proton-pump inibitor therapy induces acid-related symptoms in healthy volunteers after withdrawal of therapy. *Gastroenterology* 2009; 137:80-87.

68. Sawynok J, Esser MJ, Reid AR. Antidepressants as analgesics: an overview of central and peripheral mechanisms of action. *J Psychiatry Neurosci* 2001; 26:21-29.

69. Sawynok J. Topical and peripherally acting analgesics. *Pharmacol Rev* 2003; 55:1-20.

70. Caumo W. Fármacos utilizados em dor crônica. In: Fuchs FD, Wannmacher L (eds). Farmacologia Clínica: Fundamentos da Terapêutica Racional. 4ª ed. Rio de Janeiro, Guanabara Koogan, 2010.

71. Gilron I, Bailey JM, Tu D, Holden RR, Jackson AC, Houlden RL. Nortriptyline and gabapentin, alone and in combination for neuropathic pain: a double-blind, randomized controlled crossover trial. *Lancet* 2009; 374:1252-1261.

72. Dwoekin RH, O'Connoer AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, Portenoy RK, Rice AS, Stacev BR, Treede RD, Turk DC, Wallace MA. Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain* 2007; 132:237-251.

73. Forcelini CM. Farmacologia do sistema nervoso central. In: Fuchs FD, Wannmacher L (eds). Farmacologia Clínica: Fundamentos da Terapêutica Racional. 4ª ed. Rio de Janeiro, Guanabara Koogan, 2010.

74. Rahimi R, Nikfar S, Rezaie A, Abdollahi M. Efficacy of tricyclic antidepressants in irritable bowel sydrome: a meta-analysis. *World J Gastroenterol* 2009; 15:1548-1553.

75. Morgan V, Pickens D, Gautam S, Kessler R, Mertz H. Amitriptyline reduces rectal pain related activation of the anterior cingulated cortex in patients with irritable bowel syndrome. *Gut* 2005; 54:601-607.

76. Mertz H, Fass R, Kodner A, Yan-Go F, Fullerton S, Mayer EA. Effect of amitriptyline on symptoms, sleep, and visceral perception in patients with functional dyspepsia. Am J Gastroenterol 1998; 93:160-165.

77. De Almeida JRC, Phillips ML, Cerqueira CT, Zilberman M, Lobo D, Henna E, Tavares H, Amaro E, Gorenstein C, Gentil V, Busatto GF. Neural activity changes to emotional stimuli in healthy individuals under chronic use of clomipramine. *J Psychopharmacol* 2010; 24:1165-1174.

78. Gorelick AB, Koshy SS, Hooper FG, Bennett TC, Chey WD, Hasler WL. Differential effects of amitriptyline on perception of somatic and visceral stimulation in healthy humans. *Am J Physiol* 1998; 275:G460-G466.

79. Peghini PL, Katz PO, Castell DO. Imipramine decreases oesophageal pain perception in human male volunteers. *Gut* 1998; 42:807-813.

80. Broekaert D, Fischler B, Sifrim D, Janssens J, Tack J. Influence of citalopram, a selective serotonin reuptake inhibitor, on oesophageal hypersensitivity: a double-blind, placebo controlled study. *Aliment Pharmacol Ther* 2006; 23:365-370.

81. Clouse RE, Lustman PJ, Eckert TC, Ferney DM, Griffith LS. Low-dose trazodone for symptomatic patients with esophageal contraction abnormalities. A double-blind, placebo-controlled trial. *Gastroenterology* 1987; 92:1027-1036.

82. Cannon RO 3rd, Quyyumi AA, Mincemoyer R, Stine AM, Gracely RH, Smith WB, Geraci MF, Black BC, Uhde TW, Waclawiw MA, Maher K, Benjamin SB. Imipramine in patients with chest pain despite normal coronary angiograms. *N Engl J Med* 1994; 330:1411-1417.

83. Cox ID, Hann CM, Kaski JC. Low dose imipramine improves chest pain but not quality of life in patients with angina and normal coronary angiograms. *Eur Heart J* 1998; 19:250-254.

84. Varia I, Logue E, O'Connor C, Newby K, Wagner HR, Davenport C, Rathey K, Krishnan KR. Randomized trial of sertraline in patients with unexplained chest pain of noncardiac origin. *Am Heart J* 2000; 140:367-372.

85. Doraiswamy PM, Varia I, Hellegers C, Wagner HR, Clary GL, Beyer JL, Newby LK, O'Connor JF, Beebe KL, O'Connor C, Krishnan KR. A randomized controlled trial of paroxetine for noncardiac chest pain. *Psychopharmacol Bull* 2006; 39:15-24.

86. Spinhoven P, Van der Does AJ, Van Dijk E, Van Rood YR. Heart-focused anxiety as a mediating variable in the treatment of noncardiac chest pain by cognitive-behavioral therapy and paroxetine. *J Psychosom Res* 2010; 69:227-235.

87. Lee H, Kim JH, Min BH, Lee JH, Son HJ, Kim JJ, Rhee JC, Suh YJ, Kim S, Rhee PL. Efficacy of venlafaxine for symptomatic relief in young adult patients with functional chest pain: a randomized, double-blind, placebo-controlled, crossover trial. *Am J Gastroenterol* 2010; 105:1504-1512.

88. Keefe FJ, Shelby RA, Somers TJ, Varia I, Blazing M, Waters SJ, McKee D, Silva S, She L, Blumenthal JA, O'Connor J, Knowles V, Johnson P, Bradley L.Effects of coping skills training and sertraline in patients with non-cardiac chest pain: a randomized controlled study. *Pain* 2011; 152:730-741.

89. Hershcovici T, Jha LK, Gadam R, Cui H, Gerson L, Thomson S, Fass R. Comparison of therapeutic strategies for patients with refractory gastroesophageal reflux disease (GERD) – A randomized, double blind, placebo-controlled trial. *Gastroenterology* 2011; 140:S-579.

90. Viazis N, Keyoglou A, Kanellopoulos AK, Karamanolis G, Vlachogiannakos J, Triantafyllou K, Ladas SD, Karamanolis DG. Selective Serotonin Reuptake Inhibitors for the Treatment of Hypersensitive Esophagus: A Randomized, Double-Blind, Placebo-Controlled Study. *Am J Gastroenterol* 2012; 107:1662-1667.

91. Ostovaneh MR, Saeidi B, Hajifathalian K, Farrokhi-Khajeh-Pasha Y, Fotouhi A, Mirbagheri SS, Emami H, Barzin G, Mirbagheri SA. Comparing omeprazole with fluoxetine for treatment of patients with heartburn and normal endoscopy who failed once daily proton pump inhibitors: Double-blind placebo-controlled trial. *Neurogastroenterol Motil* 2014; doi: 10.1111/nmo.12313.

ARTIGO 1 (para submissão à revista *Am J Physiol Gastrointest Liver Physiol* – FI 3,65 – A2)

Heartburn perception and brain response before and after a meal in patients with non-erosive reflux disease

Cassiano Mateus Forcelini[1,2], José Carlos Tomiozzo Jr.[1,2], Sidia M. Callegari-Jacques[3],Ricard Farré[4], Lukas Van Oudenhove[4], Marcelo Ribeiro[2,5], Fernando Fornari[1,2]

[1]Faculdade de Medicina, Universidade de Passo Fundo (UPF), Brazil;

[2]Programa de Pós-Graduação: Ciências em Gastroenterologia e Hepatologia, Faculdade de Medicina, and [3]Departamento de Estatística, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil;

[4]Translational Research Center for Gastrointestinal Disorders (TARGID), Department of Clinical and Experimental Medicine, Catholic University of Leuven, Belgium;

[5]Clínica Kozma, Passo Fundo.

Authors' contributions:

Fernando Fornari, *Cassiano M. Forcelini and *José C. Tomiozzo Jr. designed the study and performed the research. Fernando Fornari, Cassiano M. Forcelini, José C. Tomiozzo Jr., Sidia M. Callegari-Jacques, Ricard Farré, Lukas Van Oudenhove and Marcelo Ribeiro analysed the data and wrote the paper. *CMF and JCTJ contributed equally to the study.

Running head: Effect of a meal on NERD

Corresponding author: Cassiano Mateus Forcelini, MD; Faculdade de Medicina, Universidade de Passo Fundo; Rua Teixeira Soares, 817, 99010-080, Passo Fundo-RS, Brazil; Tel/Fax: +55.54.33168553 cmforcelini@gmail.com.

ABSTRACT

Gastroesophageal reflux patients perceive heartburn particularly after a meal. We assessed heartburn and brain response to esophageal acid infusion before and after a meal in 16 non-erosive reflux disease (NERD) patients (aging 38.1 ± 8.7 y.o., 75% women) who underwent two sessions of functional magnetic resonance imaging (fMRI) in different days. The sessions were composed of two 20-minute runs of esophageal infusion, alternating between water and acid every 5 minutes. Between the runs, a liquid meal was given orally in the first session, but no meal in the second session. Blood samples for cortisol measurement were obtained at baseline and after each run. Psychological aspects were also evaluated. Heartburn perception was significantly higher in the second run of infusion in both sessions (median score pre and post meal: 5 [IQR 0 to 9.7] vs. 12 [IQR 5.2 to 13]; p = 0.006) (pre and post no meal: 5 [IQR 2 to 9,2] vs. 8.5 [IQR 5.7 to 11.7]; p = 0.03). The enhancement of heartburn neither differed between the sessions, nor was related to serum cortisol levels and anxiety/depression scores. As compared to the first run, a second run did not lead to any change in cortical activation. A significant difference in brain response was demonstrated between the second runs of each session (with and without a meal) in left supramarginal gyrus (-0.041% vs. -0.144%; $p < 0.01$). We concluded that NERD patients showed increased heartburn perception after a second stimulus with acid, regardless of a meal. These findings suggest a state of esophageal sensitization.

Keywords: esophageal acid infusion; functional MRI; heartburn; meal; NERD; cortisol.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a worldwide prevalent condition with evidence of increasing incidence (13, 28). Heartburn represents GERD main symptom and causes noticeable impact on the patients' quality of life (29, 43, 46). Non-erosive reflux disease (NERD) is more common than erosive reflux disease (ERD) as presentation of symptomatic GERD (9, 38, 42). In fact, even one third of subjects with erosive esophagitis are asymptomatic (42). On the other hand, a considerable proportion of GERD patients, especially NERD, may not respond adequately to proton pump inhibitors (PPIs) (11). This raises the possibility that other factors beyond the acid reflux may intervene in heartburn perception, like nonacid reflux (16, 45), proximal esophageal reflux (40), receptors on afferent nerves from esophagus (7, 24), brain modulation (17), and psychological aspects (6, 7, 15).

GERD symptoms commonly occur after meals, particularly in obese patients, probably not only due to gastric distention, but also to an increased frequency of lower esophageal sphincter relaxation (18) and the formation of an acid pocket at the gastric cardia (3, 8, 44). Besides, lying down immediately after meals facilitates the occurrence of heartburn (37). However, the mechanisms underlying such increase in heartburn are not completely understood. It is not known if chemical and physical esophageal stimuli produced by the meal can enhance the perception of symptoms in NERD and functional heartburn patients, like the esophageal sensitization produced by the gastric acid (14).

Recent studies on the perception of heartburn have focused on the interactions between the esophagus and the central nervous system (CNS) with the aid of functional magnetic resonance imaging (fMRI) (21, 30, 49). This new technology has produced interesting

data about the brain response to esophageal acid infusion in GERD patients, including the determination of specific brain areas involved in this process.

The present study intended to apply fMRI in the evaluation of brain response parallel to the heartburn perception during esophageal acid infusion in NERD patients before and after the oral administration of a meal, controlling for psychological aspects and serum cortisol.

MATERIALS AND METHODS

Participants

This cross-sectional study recruited NERD patients from January to July 2010 by local advertisement. Sixteen subjects (38.1 ± 8.7 y.o.; 75% women) were enrolled after clinical and endoscopic evaluation. The sample size was calculated based on data from the study of Babaei and colleagues who evaluated cerebral response related to the perception of swallowing flavored liquids (2). An adequate statistical power (0.8) was considered to identify a 0.5 % signal change, with a two-tailed p-value of 0.05.

This protocol was approved by the Ethical Committee of Universidade de Passo Fundo (UPF) (150/2009; CAAE nº 0102.0.398.000-09) and every patient signed informed consent before the enrollment.

Inclusion and exclusion criteria

The patients fulfilled the following criteria to be included in the study protocol: troublesome heartburn at least twice a week in the last 30 days; no use of proton pump inhibitors (PPIs) or H2 blockers for at least one month before enrollment; normal endoscopic examination performed during the screening period; right-handed subjects.

Subjects were excluded in case of any of the following criteria: history of gastroesophageal surgery or brain neurosurgery; untreated chronic diseases; current use of centrally acting medications; body mass index > 35 kg/m^2; pregnancy; claustrophobia.

Clinical evaluation

After the enrollment, demographic, anthropometric and clinical data were obtained from every patient. Questionnaires were applied to assess GERD symptoms (GERD-SQ) (22, 48), anxiety (Hamilton Anxiety Scale) (25), depression (Beck Depression Inventory) (4, 5), and stress (Lipp Stress Inventory) (36).

Responsiveness to PPIs was assessed on all subjects after the end of the study procedures and consisted of a challenge with pantoprazole 40 mg/day for two months. Patients were considered responsive if they had a decrease of at least 50% on GERD-SQ score at the end of the period on pantoprazole, in comparison to baseline score.

Upper gastrointestinal endoscopy

All endoscopies were performed at Clínica Endopasso (Passo Fundo – RS, Brazil) after an 8-hour fasting period under intravenous sedation with midazolam 0.05mg/kg and with a video endoscope (Olympus GIF-130, Tokyo, Japan). The finding of mucosal breaks in the distal esophagus was considered as reflux esophagitis. The screening for *Helicobacter pylori* infection consisted on rapid urease test in mucosal specimens collected from the gastric antrum.

Functional Magnetic Ressonance Imaging (fMRI) protocol

In order to determine the pH turning point (PTP) for each patient, a pH mapping of the gastroesophageal junction was carried as previously described (35). This procedure guided the transnasal insertion of a catheter positioned 7 cm above the PTP just before each fMRI session, allowing infusion of solutions in the distal esophagus.

Two fMRI sessions, the first with a meal (fMRI-m) and the second without a meal (fMRI-wm) were performed on different days, both in the afternoon. Patients were examined after 6 hours fasting.

In the beginning of each session (Figure 1), a 10-minute anatomical MRI image was obtained. Then, a 20-minute fMRI run was carried out, during which the esophagus was perfused with 1 ml/min solutions with the aid of an infusion pump (Infusomat compact, Braum S. A., São Gonçalo, RJ, Brazil), in the following order: 1) distilled water at pH 6.5 for 5 min; 2) acid solution (HCl, pH 1.5) for 5 min; 3) distilled water for 5 min; 4) acid solution in the last 5 min. This procedure was adapted from a study by Kern and colleagues (30) and was employed previously by our group (21).

The first run of the each fMRI session described above was followed by an interval of 10 minutes when a liquid meal was orally administered to the subjects (fMRI-m), or by a period without any meal (fMRI-wm). After that, the 20-minute fMRI run was acquired again for evaluating the effect of a meal (or its absence) in the study outcomes.

Patients remained on supine position during the fMRI procedures, including the intervals between the first and the second runs of each fMRI session and were allowed to swallow saliva when necessary. They were instructed to signalize with the left hand when perceiving heartburn during esophageal infusion in the following way: index finger for mild heartburn and index plus medium fingers for severe heartburn. The sum

of the total finger signaling during the 20 minutes of esophageal infusion in each run of the fMRI session resulted in the heartburn signaling score.

Thus, at the end of the study, four runs of evaluation were available, each one rendering a heartburn signaling score and the brain response on fMRI (see below): 1) before an interval with a meal; 2) after an interval with a meal; 3) before an interval without a meal; 4) after an interval without a meal.

The chocolate flavored liquid meal administered during the 10-minute interval of the first fMRI session was a 200 ml cool bottle of Nutridrink (Nutricia, Zetermeer, The Netherlands), which was composed of 12g of protein, 36g of carbohydrates, and 12g of fat, without gluten or lactose, resulting in 300 Kcal.

fMRI - Data acquisition

The fMRI protocol was performed at Clínica Kozma (Passo Fundo – RS, Brazil). Gradient echo planar magnetic resonance (MR) images were obtained using a 1.5 Tesla Magnetom Avanto (Siemens AG, Munich, Germany) with a standard 8-channel head coil. The MR scanner and head coil acquired a time series of echo planar images (EPI) covering the entire brain. High-resolution tridimensional anatomic images were obtained from 176 sagittal spoiled gradient recalled sequence slices, considering a voxel size of 1.0 x 1.0 x 1.0 mm. An EPI mosaic composed of 36 axial images of 64 x 64 pixels over a 220 mm field of view (voxel size of 3.0 x 3.0 x 3.75 mm) was build, with a repetition time (TR) of 4270 ms and echo time (TE) of 50 ms.

fMRI - Image registration and data analysis

MR data preprocessing and statistical analyses were accomplished with the aid of Analysis of Functional NeuroImaging (AFNI) software package (10). Changes in head position were realigned to the first volume with tridimensional volume registration by cubic interpolation. The images were smoothed with a 6 mm full width at half maximum (FWHM) Gaussian filter. Signal intensity data over the time series were scaled to represent the percentage of change in comparison to the mean signal over the time series. A multiple regression technique that computes the voxel-wise hemodynamic response function from the MR signal time series was used to detect brain regions which exhibit significant blood oxygenation level dependent (BOLD) changes. Volumes with movement artifacts that could interfere with the measurement of brain response were automatically excluded by AFNI censorship. In order to complete the regression model of acid exposure, regressors of hand signaling were included for analysis. After the individual analysis, the compilation of the areas with consistent signal change in the patient group was accomplished with the aid of AFNI software 3dANOVA2. Multiple testing correction using Monte Carlo simulation was performed by AFNI allowing the formation of clusters of voxels with statistical significance in terms of signal change on group level. Data were stereotaxically transformed into the Talairach-Tournoux coordinate system.

Assessment of serum cortisol

Blood samples were obtained from 10 of the 16 patients in both fMRI sessions (fMRI-m and fMRI-wm) in three different moments: 1) before the first run of acid infusion; 2) at the end of the first run of acid infusion; 3) at the end of the second run of acid infusion, which was undertaken after the interval with or without a meal (fMRI-m and fMRI-wm sessions).

Each blood sample (5 ml) was obtained from a catheter inserted in right cubital vein before the fMRI procedures and maintained during the entire session. After a 20-minute period of rest, blood samples were centrifuged at 3,250 rpm during 5 minutes (Centurion Centrifuge, Labor Line, Osasco, SP, Brazil). The supernatant serum was separated and immediately frozen and stored (- 21° C).

At the end of the study, all serum samples were defrosted on the same day and the cortisol levels were determined by competitive enzyme-linked immunosorbent assay (ELISA) in the Laboratório de Pesquisa, Faculdade de Medicina, Universidade de Passo Fundo (Passo Fundo – RS, Brazil). Specific microtiter plates (dbc-Diagnostic Biochem Canada Inc., Lonfon, Ontario, Canada) were employed for assessing serum cortisol on duplicate with the aid of ELISA washer and reader instruments (Biotek Instruments Inc, Winooski, VT, EUA). Final results were expressed in µg/dl.

Statistical analysis

Quantitative variables are presented as median and interquartile range (IQR), or, when otherwise stated, mean ± standard deviation (SD). Categorical data is described as percentage. The Wilcoxon test, with Bonferroni correction when appropriate, was used for comparisons of quantitative data. Linear regression analysis was performed to evaluate the relationship between individual quantitative characteristics and heartburn rating. The analyses were accomplished with commercially available *PASW Statistics for Windows* version 18.0 (SPSS Inc, Chicago, IL, USA) and *GraphPad Prism* version 5.00 (GraphPad Software Inc, San Diego, CA, USA). Statistical significance was assumed when a two-tailed *p*-value ≤ 0.05.

RESULTS

Patients

Demographic characteristics of the study sample are presented in Table 1. All patients were Caucasian (Eurodescendants), reflecting the local ethnic composition. Most of them were women (75%) and responsive to PPIs (82%). The presence of stress, anxiety and depression among the patients was considerable (35%, 29% and 35%, respectively), as well as the scores [median (IQR)] on Hamilton Anxiety Scale [10.5 (4.5-21.0)] and Beck Depression Inventory [5.0 (3.0-15.7)].

Heartburn signaling scores and serum cortisol levels

Heartburn signaling scores were significantly higher in the second run of esophageal infusion both in fMRI-m (median score pre and post: 5 [IQR 0 to 9.7] vs. 12 [IQR 5.2 to 13]; p = 0.006) and fMRI-wm (5 [IQR 2 to 9,2] vs. 8.5 [IQR 5.7 to 11.7]; p = 0.033) sessions (Table 2). The increase of heartburn signaling scores did not differ statistically between fMRI-m and fMRI-wm sessions.

The serum cortisol levels (Table 3) did not change significantly in fMRI-m session, but exhibited a decrease at the end of the second run (after an interval without a meal) in comparison to baseline in fMRI-wm session.

The potential effect of age, BMI, anxiety and depression scores, and serum cortisol levels on the enhancement of heartburn rating either with a meal or without a meal was investigated by simple linear regression. No significant regression was found, although trends were observed for the influence of anxiety score on the enhancement of heartburn perception with a meal (p = 0.095) and without a meal (p = 0.086), as well for the

influence of depression score on the increase of heartburn signaling with a meal (p = 0.067).

Brain responses at fMRI

Specific areas exhibited a collective significant brain response in the first run of esophageal infusion (Table 4): left parahippocampal gyrus (p < 0.01) in fMRI-m session; right precuneus (p < 0.001) and left lingual gyrus (p < 0.01) in fMRI-wm session. No significant change was found in the second run in comparison with the first run of each session, either after an interval with a meal (fMRI-m), or without a meal (fMRI-wm). Comparisons between the second runs of each session (fMRI-m and fMRI-wm) revealed a significant difference in left supramarginal gyrus (p < 0.01) (Table 5; Figure 2).

On average 14% of image volumes of fMRI time series were excluded by AFNI censorship because of movement artifacts that could interfere with the measurement of brain response. There was no significant difference in the percentage of excluded volumes between the runs of both fMRI-wm and fMRI-m sessions.

DISCUSSION

The present study intended to evaluate heartburn perception and the brain response to esophageal acid infusion with the aid of fMRI in NERD patients before and after a liquid meal. For this purpose we used a previously developed fMRI protocol that simulates the repetitive presence of acid in the distal esophagus (21, 30, 49).

An increased heartburn signaling score after a second challenge of acid perfusion was demonstrated, regardless of the oral administration of a chocolate-flavored liquid meal. Not only the absolute figures of heartburn signaling, but also the amount of enhancement of heartburn scores were similar between the sessions with a meal and without a meal. These findings showed that the repeated acid stimulation of distal esophagus, a simulation of frequent acid reflux episodes, is a peripheral determinant of esophageal sensitization, even in the absence of erosive esophagitis. Esophageal sensitization represents an increase in the awareness of the reflux episodes caused by previous stimuli. Our findings are in accordance with those from a recent study of Emerenziani and colleagues who evaluated NERD patients with impedance-pH monitoring (14). They found that spontaneous acid reflux enhances subsequent reflux perception, regardless of acidity or liquid/mixed composition of subsequent episodes. This may be due to the generation of dilated intercellular spaces in response to esophageal exposure to acid or weekly acid solutions, which impairs esophageal mucosal integrity (16). In fact, the prolonged contact of the esophageal mucosa with the refluxed content, probably caused by a defective anti-reflux barrier and luminal clearance mechanisms is a factor that contributes to the generation of symptoms and esophagitis in GERD (1).

There is compelling evidence that psychopathologies like depression and anxiety are common in GERD patients and may enhance the burden in their quality of life (27, 39). However, studies on the symptomatic response of GERD patients face to an acute stressor stimulation have rendered contradictory results: while Fass and colleagues found that acute auditory stress can exacerbate heartburn symptoms, with no change in plasma cortisol, adrenocorticotropic hormone (ACTH) and norepinephrine (19), the group of Wright obtained an increase in saliva cortisol and in anxiety measurement face to acute stress, despite no change in heartburn and objetive reflux monitoring (50). Due to this issue, the possibility of psychological factors and of cortisol (a known systemic marker of acute stress) intervening in the perception of heartburn was evaluated. In our sample, serum cortisol levels actually decreased in comparison to baseline during the fMRI session without a meal and did not change with a meal. We recognize that data was not available from all patients (10 out of 16 subjects), but this points to a lack of relation between cortisol secretion and heartburn perception, in accordance with previous studies (19, 50).

We performed a simple linear regression analysis that did not show a significant influence of anxiety and depression scores, as well as cortisol levels on the amount of increase of heartburn signaling. However, some trends have emerged for anxiety and depression scores, suggesting that such influence could be found in studies with larger populations. The small sample of patients in the present study precluded the use of a multiple regression analysis for a better evaluation of the issue, a limitation that must be emphasized.

The enhancement in heartburn perception and the differences in brain response cannot probably be attributed to novelty effects produced by the study protocol because of two reasons: 1) it would be expected an inverse result in case of a novelty effect, since it is

supposed that the patients would get accustomed to the esophageal acid stimulation during the time, especially in the second run; 2) similarly, the second fMRI session (fMRI-wm) would render a lower increase in heartburn signaling than that observed in the first session (fMRI-m). We believe that the order of the sessions established by the protocol (first the "meal" session, and some days later the "without a meal" session) did not influence the results, because the theoretical background supported the notion that a higher heartburn signaling would be expected with the presence of a meal (18, 37). No difference was demonstrated between the sessions indeed. Nevertheless, we cannot completely exclude an "order effect" and recognize that a random counterbalanced order should be more adequate to the study protocol.

Despite an increased heartburn signaling score after a second challenge of acid perfusion (second runs of both fMRI-m and fMRI-wm sessions) was evident, there was no change in collective brain response from the first to the second run of each fMRI session. It probably means that the brain areas which exhibited activity during the first acid stimulation did the same face to the second acid challenge, independently from the administration or not of a meal. The chocolate-flavored liquid employed in the study protocol is a commercially available product that has content and volume that resembles a small ordinary meal. Sauter and colleagues administered a similar product to fourteen healthy volunteers and demonstrated the formation of the secretion layer at the meal-air interface in the proximal stomach (44), the "acid pocket" that has been recently linked to the generation of acidic reflux (3, 41). Nevertheless, it is possible that a bigger volume could better simulate a copious meal and thus lead to more prominent effects in brain response.

On the other hand, the comparison between the second runs of each fMRI sessions (fMRI-m and fMRI-wm) revealed a different pattern of brain response in left

supramarginal gyrus. That is, the oral administration of a meal followed by esophageal acid stimulation resulted in different brain responses on fMRI in comparison to the maintenance of fasting condition. Since there was not a difference in heartburn signaling between the sessions with a meal and without a meal, we do not believe that these fMRI findings represent an enhancement in esophageal reflux, but actually meal-related effects on cortical activation, including its sensitive effect on esophagus and stomach. Left supramarginal gyrus activation has been linked to emotional responsiveness to sensitive stimulation (20, 33, 47), as well as to verbal processing of meanings (12, 32, 34). It is not probable that the aforementioned response has been related to the taste perception since the right, and not the left supramarginal gyrus, was described as a region with increased cerebral blood flow in reaction to taste tests (23). Besides, the study of Babaei and colleagues has already shown the list of brain areas with consistent response to swallowing flavored liquid meals (2), where the left supramarginal gyrus was not included.

In the present study, left parahippocampal gyrus showed a collective increase in activation during the first run of fMRI-m session (before a meal), instead of negative values in right precuneus and left lingual gyrus before an interval without a meal (first run of fMRI-wm session). Although positive figures are linked to a real increase in vascular supply and enhanced neuronal activity, the meaning of negative values is still a matter of debate. It is possible that brain areas with negative figures are a result of reallocation of cortical blood resources that overcomes a local demand for increased cerebral blood flow induced by enhanced neuronal activity (26). Caution is recommended for the interpretation of negative values expressed by fMRI, since these may not reflect an actual hypoactivation.

Left parahippocampal gyrus, right precuneus and left lingual gyrus are brain areas not included in the roll of regions that responded to the acid esophageal infusion in previous studies (21, 30, 31, 49), namely: insula, cingulate, caudate, hippocampus, as well as prefrontal, parieto-occipital and sensory/motor cortex. These discrepancies may have two origins: a change in our methodological procedures of fMRI analysis in relation to previous studies; artifacts not controlled by the censorship of AFNI software 3dANOVA2. Our methodological novelties included: 1) The adoption of a 6 mm (FWHM) Gaussian filter in order to make a more selective definition of activated voxels; 2) Signal intensity data over the time series scaled to represent the percentage of change in comparison to the mean signal. This resource was previously employed by Babaei and colleagues and promotes a measurement of brain response to a specific stimulation (2), instead of the sum of diverse influences on cortical activation as basic oscillation of signal and movement artifacts. This procedure delivers an intensity of signal response lower than the figures of previous publications with the same esophageal acid stimulation (21, 30, 49), but with a probable more reliable meaning. 3) The use of regressors of hand signaling in the analysis for the purpose of excluding hand movement artifact; 4) The employment of AFNI software 3dANOVA2 after individual analysis for assessing the patient group as a whole, thus determining the collective brain areas with significant response. We recognize that these methodological innovations may have produced conservative results in terms of number of brain regions with significant response, an explanation for the description of fewer areas than the previous studies. Nevertheless, we cannot exclude that the finding of activation in diverse brain regions in two different sessions of the same protocol (first runs of fMRI-m and of fMRI-wm, both before the interval with or without a meal, respectively) may be an artifact. Besides, on average 14% of the volumes of fMRI time series were

excluded by AFNI censorship because of movement artifacts. Although there was no significant difference in the percentage of excluded volumes between the runs of both fMRI-wm and fMRI-m sessions, it is possible that such exclusions may have influenced qualitatively the results of brain activity.

There are other limitations that must be taken into account for the interpretation of the results of this study, some of them already discussed above: the sample size may have hidden a possible influence of psychological factors in the enhancement of heartburn; the small volume of the liquid meal administered to the subjects; the possibility of an order effect caused by a predetermined first session with a meal; the lack of impedance-pH monitoring precludes an assessment of non-acid reflux; the paucity of men and PPIs non responders avoids the obtainment of data about subpopulations that may be clinically different from the average NERD patients.

In summary, the findings of the present study suggest that a state of esophageal sensitization to repeated acid stimuli is more relevant to elicitation of heartburn in NERD patients than meal-related effects.

Acknowledgements

REFERENCES

1. **Altomare A, Guarino MP, Cocca S, Emerenziani S, Cicala M.** Gastroesophageal reflux disease: Update on inflammation and symptom perception. *World J Gastroenterol* 19: 6523-6528, 2013.
2. **Babaei A, Kern M, Antonik S, Mepani R, Ward BD, Li S-J, Hyde J, Shaker, R.** Enhancing effects of flavored nutritive stimuli on cortical swallowing network activity. *Am J Physiol Gastrointest Liver Physiol* 299: G422-G429, 2010.
3. **Beaumont H, Bennink RJ, de Jong J, Boeckxstaens GE.** The position of the acid pocket as a major risk factor for acidic reflux in healthy subjects and patients with GORD. *Gut* 59: 441-451, 2010.
4. **Beck AT, Steer RA, Garbin MG.** Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin Psychol Rev* 8: 77-100, 1988.
5. **Beck AT, Ward CH, Mendelson M, Mock J, Erbaug J.** An inventory for measuring depression. *Arch Gen Psychiatry* 4: 561-571, 1961.
6. **Bradley LA, Richter JE, Pulliam TJ, Haile JM, Scarinci IC, Schan CA, Dalton CB, Salley AN.** The relationship between stress and symptoms of gastroesophageal reflux: the influence of psychological factors. *Am J Gastroenterol* 88: 11-19, 1993.
7. **Chua YC, Aziz Q.** Perception of gastro-oesophageal reflux. *Best Pract Res Clin Gastroenterol* 24: 883-891, 2010.
8. **Clarke AT, Wirz AA, Manning JJ, Ballantyne SA, Alcorn DJ, McColl KE.** Severe reflux disease is associated with an enlarged unbuffered proximal gastric acid pocket. *Gut* 57: 292-297, 2008.
9. **Corder AP, Jones RH, Sadler GH, Daniels P, Johnson CD.** Heartburn, oesophagitis and Barrett's oesophagus in self-medicating patients in general practice. *Br J Clin Pract* 50: 245-248, 1996.
10. **Cox RW, Hyde JS.** Software tools for analysis and visualization of fMRI data. *NMR in Biomed* 10: 171-178, 1997.
11. **Dean BB, Gano AD Jr, Knight K, Ofman JJ, Fass R.** Effectiveness of proton pump inhibitors in nonerosive reflux disease. *Clin Gastroenterol Hepatol* 2: 656-664, 2004.
12. **Deschamps I, Baum SR, Gracco VL.** On the role of the supramarginal gyrus in phonological processing and verbal working memory: Evidence from rTMS studies. *Neuropsychologia* 53: 39-46, 2014.
13. **El-Seraq HB, Sweet S, Winchester CC, Dent J.** Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* (Jul 13, 2013). doi:10.1136/gutjnl-2012-304269.
14. **Emerenziani S, Ribolsi M, Guarino MP, Balestrieri P, Altomare A, Rescio MP, Cicala M.** Acid reflux episodes sensitize the esophagus to perception of weakly acidic and mixed reflux in non-erosive reflux disease patients. *Neurogastroenterol Motil* 26: 108-114, 2014.
15. **Farré R, De Vos R, Geboes K, Verbecke K, Vanden Berghe P, Depoortere I, Blondeau, Tack J, Sifrim D.** Critical role of stress in increased oesophageal mucosa permeability and dilated intercellular spaces. *Gut* 56: 1191-1197, 2007.
16. **Farré R, Fornari F, Blondeau K, Vieth M, De Vos R, Bisschops R, Mertens V, Pauwels A, Tack J, Sifrim D.** Acid and weakly acidic solutions impair

mucosal integrity of distal exposed and proximal non-exposed human oesophagus. *Gut* 59: 164-169, 2010.

17. **Fass R, Tougas G.** Functional heartburn: the stimulus, the pain, and the brain. *Gut* 51: 885-892, 2002.

18. **Fass R.** The pathophysiological mechanisms of GERD in the obese patient. *Dig Dis Sci* 53: 2300-2306, 2008.

19. **Fass R, Naliboff BD, Fass SS, Peleg N, Wendel C, Malagon IB, Mayer EA.** The effect of auditory stress on perception of intraesophageal acid in patients with gastroesophageal reflux disease. *Gastroenterology* 134: 696-705, 2008.

20. **Ferraro S, Grazzi L, Mandelli ML, Aquino D, Di Fiore D, Usai S, Bruzzone MG, Di Salle F, Bussone G, Chiapparini L.** Pain processing in medication overuse headache: a functional magnetic resonance imaging (fMRI) study. *Pain Med* 13: 255-262, 2012.

21. **Forcelini CM, Tomiozzo Jr JC, Farré R, Van Oudenhove L, Callegari-Jacques SM, Ribeiro M, Madalosso BH, Fornari F.** Effect of nortriptyline on brain responses to painful esophageal acid infusion in patients with non-erosive reflux disease. *Neurogastroenterol Motil* 26: 187-195, 2014.

22. **Fornari F, Gruber AC, Lopes AB, Cecchetti D, Barros SGS.** Questionário de sintomas na doença do refluxo gastroesofágico. *Arq Gastroenterol* 41: 263-267, 2004.

23. **Gautier JF, Chen K, Uecker A, Bandy D, Frost J, Salbe AD, Pratley RE, Lawson M, Ravussin E, Reiman EM, Tataranni PA.** Regions of the human brain affected during a liquid-meal taste perception in the fasting state: a positron emission tomography study. *Am J Clin Nutr* 70: 806-810, 1999.

24. **Guarino MP, Cheng L, Ma J, Harnett K, Biancani P, Altomare A, Panzera F, Behar J, Cicala M.** Increased TRPV1 gene expression in esophageal mucosa of patients with non-erosive and erosive reflux disease. *Neurogastroenterol Motil* 22: 746-751, 2010.

25. **Hamilton M.** The assessment of anxiety states by rating. *Br J Med Psychol* 32: 50-55, 1959.

26. **Harel N, Lee SP, Nagaoka T, Kim DS, Kim SG.** Origin of negative blood oxygenation level-dependent fMRI signals. *J Cereb Blood Flow Metab* 22: 908-917, 2002.

27. **Hartono J L, Mahadeva S, Goh KL.** Anxiety and depression in various functional gastrointestinal disorders: do differences exist? *J Dig Dis* 13: 252-257, 2012.

28. **Jung HK.** Epidemiology of gastroesophageal reflux disease in Asia: a systematic review. *J Neurogastroenterol Motil* 17: 14-27, 2011.

29. **Kahrilas PJ, Jonsson A, Denison H, Wernersson B, Hughes N, Howden CW.** Impact of regurgitation on health-related quality of life in gastro-oesophageal reflux disease before and after short-term potent acid suppression therapy. *Gut* (Jul 5, 2013). doi:10.1136/gutjnl-2013-304883.

30. **Kern M, Hofmann C, Hyde J, Shaker R.** Characterization of the cerebral cortical representation of heartburn in GERD patients. *Am J Physiol Gastrointest Liver Physiol* 286: G174-G181, 2004.

31. **Kern MK, Birn RM, Jaradeh S, Jesmanowicz A, Cox RW, Hyde JS, Shaker R.** Identification and characterization of cerebral cortical response to esophageal mucosal acid exposure and distention. *Gastroenterology* 115: 1353-1362, 1998.

32. **Köchel A, Schöngassner F, Schienle A.** Cortical activation during auditory elicitation of fear and disgust: a near-infrared spectroscopy (NIRS) study. *Neurosci Lett* 549: 197-200, 2013.

33. **Kong J, Gollub RL, Rosman IS, Webb JM, Vangel MG, Kirsch I, Kaptchuk TJ.** Brain activity associated with expectancy-enhanced placebo analgesia as measured by functional magnetic resonance imaging. *J Neurosci* 26: 381-388, 2006.

34. **Kraemer DJM, Hamilton RH, Messing SB, Desantis JH, Thompson-Schill SL.** Cognitive style, cortical stimulation, and the conversion hypothesis. *Front Hum Neurosci* (Jan 29, 2014). doi: 10.3389/fnhum.2014.00015.

35. **Liell TP, Tomiozzo Jr JC, Denti F, de Lima LA, Fornari F.** Determination of pH turning point with pH mapping of the gastroesophageal junction: an alternative technique to orientate esophageal pH monitoring. *Dis Esophagus* 24: 305-311, 2011.

36. **Lipp M, Guevara A.** Validação empírica do inventário de sintomas de stress. *Estudos de Psicologia* 11: 43-49, 1994.

37. **Mansour-Ghanaei F, Joukar F, Atshani SM, Chagharvand S, Souti F.** The epidemiology of gastroesophageal reflux disease: a survey on the prevalence and the associated factors in a random sample of the general population in the Northern part of Iran. *Int J Mol Epidemiol Genet* 4: 175-82, 2013.

38. **Minatsuki C, Yamamichi N, Shimamoto T, Kakimoto H, Takahashi Y, Fujishiro M, Sakaguchi Y, Nakayama C, Konno-Shimizu M, Matsuda R, Mochizuki S, Asada-Hirayama I, Tsuji I, Kodashima S, Ono S, Niimi K, Mitsushima T, Koike K.** Background factors of reflux esophagitis and non-erosive reflux disease: a cross-sectional study of 10,837 subjects in Japan. *PLoS One* (Jul 26, 2013). doi:10.1371/journal.pone.0069891.

39. **Oh JH, Kim TS, Choi MG, Lee H, Jeon EJ, Choi SW, Lee C, Chung IS.** Relationship between Psychological Factors and Quality of Life in Subtypes of Gastroesophageal Reflux Disease. *Gut Liver* 3: 259-65, 2009.

40. **Rohof WO, Bennink RJ, de Jonge H, Boeckxstaens GE.** Increased proximal reflux in a hypersensitive esophagus might explain symptoms resistant to proton pump inhibitors in patients with gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* (Nov 1, 2013). doi: 10.1016/j.cgh.2013.10.026.

41. **Rohof WO, Bennink RJ, Smout AJ, Thomas E, Boeckxstaens GE.** An alginate-antacid formulation localizes to the acid pocket to reduce acid reflux in patients with gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 11: 1585-1591, 2013.

42. **Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Graffner H, Vieth M, Stolte M, Engstrand L, Talley NJ, Agréus L.** High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: a Kalixanda study report. *Scand J Gastroenterol* 40: 275-285, 2005.

43. **Ronkainen J, Aro P, Storskrubb T, Lind T, Bolling-Sternevald E, Junghard O, Talley NJ, Agréus L.** Gastro-oesophageal reflux symptoms and health-related quality of life in the adult general population--the Kalixanda study. *Aliment Pharmacol Ther* 23: 1725-1733, 2006.

44. **Sauter M, Curcic J, Menne D, Goetze O, Fried M, Schwizer W, Steingoetter A.** Measuring the interaction of meal and gastric secretion: a combined quantitative magnetic resonance imaging and pharmacokinetic modeling approach. *Neurogastroenterol Motil* 24: 632-638, 2012.

45. **Savarino E, Zentilin P, Tutuian R, Pohl D, Casa DD, Frazzoni M, Cestari R, Savarino V.** The role of nonacid reflux in NERD: lessons learned from impedance-pH monitoring in 150 patients off therapy. *Am J Gastroenterol* 103: 2685-2693, 2008.

46. **Shaheen NJ, Hansen RA, Morgan DR, Gangarosa LM, Ringel Y, Thiny MT, Russo MW, Sandler RS.** The Burden of Gastrointestinal and Liver Diseases, 2006. *Am J Gastroenterol* 101: 2128-2138, 2006.

47. **Van der Heiden L, Scherpiet S, Konicar L, Birbaumer N, Veit R.** Inter-individual differences in successful perspective taking during pain perception mediates emotional responsiveness in self and others: an fMRI study. *Neuroimage* 65: 387-394, 2013.

48. **Velanovich V, Vallance SR, Gusz JR, Tapia FV, Harkabus MA.** Quality of life scale for gastroesophageal reflux disease. *J Am Coll Surg* 183: 217-224, 1996.

49. **Wang K, Duan LP, Zeng XZ, Liu JY, Xu-Chu W.** Differences in cerebral response to esophageal acid stimuli and psychological anticipation in GERD subtypes--an fMRI study. *BMC Gastroenterol* (Mar 26, 2011). doi:10.1186/1471-230X-11-28.

50. **Wright CE, Ebrecht M, Mitchell R, Anggiansah A, Weinman J.** The effect of psychological stress on symptom severity and perception in patients with gastro-oesophageal reflux. *J Psychosomatic Res* 59: 415-424, 2005.

FIGURE CAPTIONS

Figure 1. After 10 minutes of an anatomical MRI mapping, a series of fMRI signals were obtained during a 20-minute run of esophageal infusion of alternate solutions (five minutes each one: water; acid solution; water; acid solution), followed by a 10-minute interval with a chocolate flavored liquid meal orally administered (fMRI-m session). Then, the 20-minute fMRI run was reproduced. A few days later, the entire protocol was repeated, but with an interval without a meal (fMRI-wm session).

Figure 2. Illustration of the areas that exhibited a consistent difference in brain response between the second runs of each functional magnetic resonance imaging (fMRI) session (with a meal *vs.* without a meal). The clusters are colored yellow and orange and their central mass is located in left supramarginal gyrus. Images refer to brain areas from all patients.

TABLES

Table 1. Demographic characteristics of the study sample ($n = 16$) [median (interquartile range)].

Characteristics	Descriptive Statistics
Women (%)	75
Age (years) §	38.1 ± 8.7
Caucasian (%)	100
Weight (kg)	62.0 (53.0-80.7)
Height (cm)	164.5 (157.5-170.0)
BMI (kg/m^2)	23.4 (20.4-28.7)
GERD-SQ	13.5 (9.2-16.0)
PPI responsiveness (%)	82
Helicobacter pylori + (%)	29
Anxiety score	10.5 (4.5-21.0)
Presence of anxiety (%)	29
Depression score	5.0 (3.0-15.7)
Presence of depression (%)	35
Presence of stress (%)	35

BMI: Body mass index;

GERD-SQ: Gastroesophageal reflux disease symptoms questionnaire.

PPI: Protom pump inhibitors

§ mean ± standard deviation.

Table 2. Perception of heartburn between the periods before and after a meal, as well as before and after an interval without a meal (*n* = 16) [median (interquartile range)].

Before a meal	After a meal
5 (0 to 9.7)*	12 (5.2 to 13)

Before an interval without a meal	After an interval without a meal
5 (2 to 9.2)†	8.5 (5.7 to 11.7)

fMRI: functional magnetic resonance imaging.

* *p* = 0.006 vs. after a meal

† *p* = 0.033 vs. after an interval without a meal

Comparisons performed with Wilcoxon test.

Table 3. Serum cortisol levels (µg/dl) measured in three moments: baseline; at the end of first run of esophageal acid perfusion; at the end of second run of esophageal acid perfusion after a meal, as well as after an interval without a meal ($n = 10$) [median (interquartile range)].

Baseline	Before a meal	After a meal
9.8 (5.2 to 13.8)	5.7 (4.5 to 20.3)	9.9 (4.8 to 15.5)

Baseline	Before an interval without a meal	After an interval without a meal
10.7 (6.7 to 13.0)	4.9 (1.3 to 17.3)	5.8 (3.8 to 7.7)*

* $p = 0.041$ vs. baseline

Comparisons performed with Wilcoxon test with Bonferroni correction.

Table 4. Description of the brain areas with collective significantly change in brain response measured by fMRI during the first run of each fMRI sessions with esophageal acid stimulation, before an interval with or without a meal ($n = 16$).

Brain areas (before an interval *with* a meal)	Cluster volume (µl)	Mean of signal change	Coordinates of cluster central mass (x, y, z)
Left parahippocampal gyrus*	428	0.181 %	+6.2, +34.4, -0.6

Brain areas (before an interval *without* a meal)	Cluster volume (µl)	Mean of signal Change	Coordinates of cluster central mass (x, y, z)
Right precuneus**	2036	-0.180%	-12.0, +57.6, +21.4
Left lingual gyrus†	468	-0.157%	+26.5, +70.4, -1.5

fMRI: functional magnetic resonance imaging.

* $p < 0.01$

** $p < 0.001$

† $p < 0.01$

Comparisons performed by AFNI 3dANOVA2.

Table 5. Description of the brain areas which exhibited a collective significant difference in brain response between the second runs of each session of esophageal acid perfusion [after an interval with a meal (fMRI-m) and without a meal (fMRI-wm)] ($n = 16$).

Brain areas (after an interval)	Cluster volume (µl)	Coordinates of cluster central mass (x, y, z)
Left supramarginal Gyrus*	260	+41.8, +50.8, +37.0

fMRI-m: functional magnetic resonance imaging session with a meal

fMRI-wm: functional magnetic resonance imaging session with an interval without a meal

* -0.041% vs. -0.144% ($p < 0.01$)

Comparison performed by AFNI 3dANOVA2.

FIGURAS DO ARTIGO 1

Figure 1
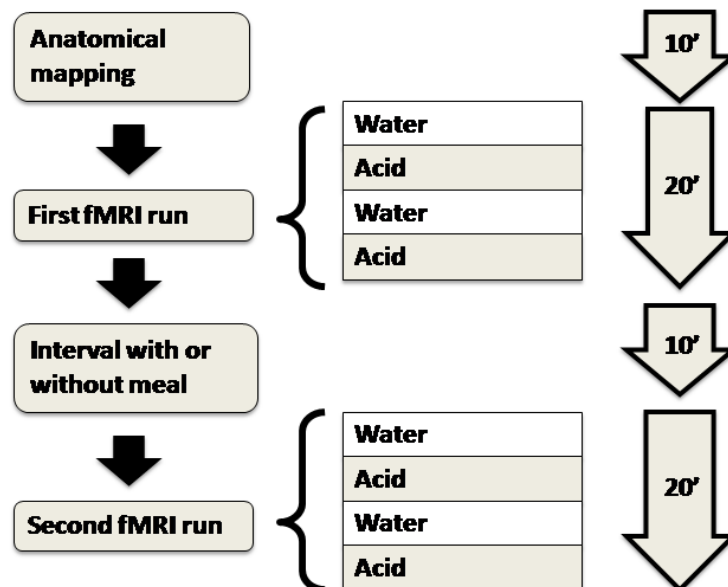
Figure 2
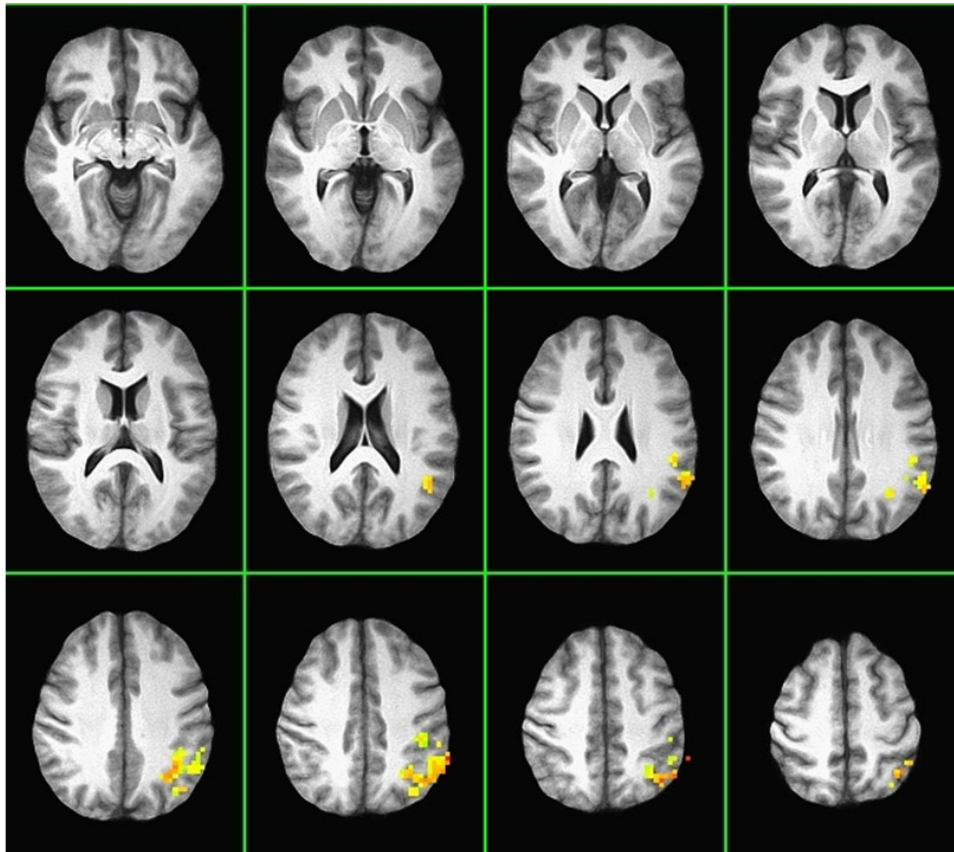
ARTIGO 2

(Publicado na revista *Neurogastroenterol Motil* [FI 2,93 – A2] em 2014, n°65 da lista de referências bibliográficas da tese)

Running title: **Effect of nortriptyline on NERD**

Effect of nortriptyline on brain responses to painful esophageal acid infusion in patients with non-erosive reflux disease

*Cassiano Mateus Forcelini[1,2], *José Carlos Tomiozzo Jr.[1,2], Ricard Farré[3], Lukas Van Oudenhove[3], Sidia M Callegari-Jacques[4], Marcelo Ribeiro[2,5], Ben Hur Madalosso[5], Fernando Fornari[1,2]

*CMF and JCTJ contributed equally to the study.

[1]Faculdade de Medicina, Universidade de Passo Fundo (UPF), Passo Fundo, Brazil; [2]Programa de Pós-Graduação: Ciências em Gastroenterologia e Hepatologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; [3]Translational Research Center for Gastrointestinal Disorders (TARGID), Department of Clinical and Experimental Medicine, University of Leuven, Belgium; [4]Departamento de Estatística, UFRGS; [5]Clínica Kozma, Passo Fundo.

Corresponding author: Cassiano Mateus Forcelini, MD; Faculdade de Medicina, Universidade de Passo Fundo; Rua Teixeira Soares, 817, 99010-080, Passo Fundo-RS, Brazil; Tel/Fax: +55.54.33168553 cmforcelini@gmail.com.

ABSTRACT

Background: Non-erosive reflux disease (NERD) patients generally present with heartburn as the main symptom. Antidepressants might help to relieve heartburn by acting on the esophagus-brain axis. We aimed to assess the effect of nortriptyline on behavioural and brain responses to painful esophageal acid infusion in NERD patients evaluated with functional magnetic resonance imaging (fMRI). **Methods:** In a randomized double-blind crossover design, 20 NERD patients off proton pump inhibitors (36.1 ± 9.3 years, 75% women) were assigned to 21 days of nortriptyline and placebo, in counterbalanced order, with a 21 days washout period in between both treatment periods. Changes in acid-induced brain response on fMRI and heartburn perception were assessed and at the end of each treatment. **Key Results:** Nortriptyline significantly reduced the acid-induced brain response in prefrontal cortex [median (IQR): -1.9 (-4.5 to -0.1) vs. -0.3 (-2.5 to 2.3); $p = 0.050$], caudate [-3.0 (-5.1 to -0.01) vs. 0.48 (-1.9 to 3.1); $p = 0.029$], insula [-2.4 (-4.8 to -0.6) vs. -0.2 (-1.5 to 1.5); $p = 0.029$], cingulate [-4.2 (-8.8 to -0.1) vs. -0.6 (-1.8 to 3.0); $p = 0.017$] and hippocampus [-2.7 (-6.0 to 0.5) vs. -0.04 (-2.3 to 1.9); $p = 0.006$] in comparison to placebo. However, there was no significant difference between nortriptyline and placebo in clinical outcomes and side effects. **Conclusions & Inferences:** Nortriptyline decreased the brain response to esophageal acid infusion more markedly than placebo, but without clinical significance.

Keywords: esophageal acid infusion; functional MRI; heartburn; NERD; nortriptyline.

INTRODUCTION

Gastroesophageal reflux disease (GERD) affects a substantial part of the worldwide population and causes considerable social and economic burden (1-3). The main symptoms of GERD are heartburn and regurgitation, although other complaints such as dysphagia, chronic cough and non-cardiac chest pain may occur (4). GERD is generally classified in two main types according to endoscopic findings: erosive reflux disease (ERD) and non-erosive reflux disease (NERD) (4,5).

Proton pump inhibitors (PPIs) are the cornerstone of GERD treatment, but a substantial proportion of NERD patients are not responsive to these pharmacological agents (6). Recently, the term *functional heartburn* has been applied to a subgroup of patients with NERD who experience persistent symptoms despite the absence of objective parameters as increased acid exposure and reflux-symptom association, as well as lack of responsiveness to PPIs (7,8). Moreover, the demonstration that weakly acidic reflux may cause symptoms has contributed to the debate on the pathophysiology of GERD (9).

The complaint of heartburn in NERD patients despite absence of erosive esophagitis points to the role of other factors beyond acid reflux in the generation of GERD symptoms (7). Disruption of esophageal mucosal integrity in healthy subjects due to weakly acidic solutions (10) and in rats exposed to stress (11) suggests that impairment of mucosal defense may be caused by elements other than the gastric acid. It is possible that the perception of physical and chemical esophageal stimuli in NERD and functional heartburn can be peripherally and centrally modulated, like in neuropathic pain (12,13,14).

Studies on the perception of esophageal stimulation with balloon distention, acid infusion or even electrical stimuli have been performed as models for visceral pain (15-20). Nowadays, the method that has mostly been applied for studying the interactions between the esophagus and the central nervous system (CNS) is functional brain imaging by

magnetic resonance (fMRI) (18,21,22). In fact, fMRI has brought new data about the pathophysiology of GERD by evaluating the brain responses during esophageal acid infusion of in patients and healthy subjects (21,22). In these studies, brain areas consistently activated were insula, cingulate, and prefrontal cortex, regions involved in homeostatic-afferent processing as well as emotional and cognitive modulation of visceral pain (12,23,24).

The existence of esophagus-CNS interactions results in different potential targets for therapeutic interventions, with drugs potentially acting in both peripheral and central pathways. Antidepressants such as nortriptyline are potential candidates to this role, since they have recognized analgesic properties and are widely used for treating neuropathic pain (25). Antidepressants have already been applied in terms of modulating gastrointestinal sensitivity (26,27). However, no study has investigated the effect of these drugs, neither on the perception of heartburn during esophageal acid infusion, nor on its brain representation, nor on heartburn symptoms. We hypothesized that nortriptyline would significantly change brain responses to esophageal acid infusion and reduce heartburn perception in NERD patients by acting on the esophagus-brain axis. Therefore, we conducted a mechanistic study to test this hypothesis.

METHODS

Subjects

Twenty-five adult patients were recruited from January to July 2010 to participate in the study after clinical and endoscopic evaluation. Inclusion criteria were: 1 - troublesome heartburn at least twice a week in the last month; 2 - naïve to acid suppressive therapy or interruption of PPIs or H2 blockers for at least 30 days; 3 - normal esophageal mucosa at endoscopy performed off acid suppressive therapy. Patients were excluded if they have any of the following criteria: 1 - use of centrally acting medication in the last 30 days; 2 - gastroesophageal surgery; 3 - body mass index > 35 kg/m^2; 4 - untreated chronic diseases; 5 - pregnancy; 6 - claustrophobia.

From the initial 25 patients, 20 subjects completed the study (36.1 ± 9.3 years, 75% women): one patient was excluded before starting the first treatment because of an accidental finding on the basal anatomical MRI; two were found to be claustrophobic during the first fMRI session; one was diagnosed to have functional dyspepsia instead of NERD after symptoms reevaluation; one dropped out from the study before taking the first treatment.

Based on data from a study which evaluated amitriptyline for reducing cerebral activation related to the perception of painful rectal distension in patients with irritable bowel syndrome (28), a sample size of 20 patients was calculated to give adequate statistical power (0.8) to identify a 30% signal change, with a two-tailed p-value of 0.05.

Clinical evaluation

At the evaluation for patient enrollment, heartburn was scored using a validated GERD symptoms questionnaire (GERD-SQ) with the following question (29,30): "How bad is the heartburn?". The possible scores were: 0 - no symptoms; 1 - noticeable symptoms, but non-

troublesome; 2 - troublesome symptoms, but not every day; 3 - troublesome symptoms every day; 4 - symptoms affect daily activities; 5 - incapacitating symptoms. Heartburn evaluation at baseline and following each treatment was carried out with the above mentioned question and 5 more questions: "Heartburn when lying down?"; "Heartburn when standing up?"; "Heartburn after meals?"; "Does heartburn change your diet?"; "Does heartburn wake you from sleep?". A total score for heartburn with the sum of 6 questions ranged between 0 (best) and 30 (worst).

Responsiveness to PPIs was assessed after the end of the trial. Every patient with a decrease of at least 50% in GERD-SQ during a period of continuous use of pantoprazole 40 mg/day for two months was considered a responsive patient.

As part of the clinical evaluation, the presence of anxiety, depression and stress was assessed before starting the trial and at the end of each treatment with the aid of the Hamilton Anxiety Scale (31), Beck Depression Inventory (32,33), and Lipp Stress Inventory (34). Epworth Sleepiness Scale was also completed by the patients for measuring sleepiness as a potential adverse effect of treatments (35). The cut-off point for defining the presence of mild depression was a score of at least 10 on the Beck Depression Inventory (33), while the corresponding threshold for diagnosing mild anxiety was 21 on the Hamilton Anxiety Scale (31).

Upper gastrointestinal endoscopy

Endoscopy was performed after an 8-hour fasting period under intravenous sedation with midazolam 0.05mg/kg, using a video endoscope (Olympus GIF-130, Tokyo, Japan). Reflux esophagitis was identified by the presence of mucosal breaks in the distal esophagus. A rapid urease test was employed to screen for *Helicobacter pylori* infection in mucosal

specimens collected from the gastric antrum. All endoscopies were carried out by the same investigator.

Drug administration

The trial had a randomized placebo-controlled, double-blind crossover design. After recruitment, subjects were randomly enrolled (by raffle of numbers) in one of the two treatment groups: 'nortriptyline first' *vs*. 'placebo first' (Figure 1 A). Patients and medical staff remained blind until the end of the entire study. The person who performed the randomization and assigned the treatments was not involved in the patients' evaluations. Nortriptyline and placebo were packed in identical pills. Patients undergoing treatment with nortriptyline first received 10 mg/day during 7 days, followed by 25 mg/day during 14 days. After that, a washout period of 21 days was built in, followed by 21 days of placebo. The patients enrolled in the 'placebo first' arm received the inverse treatment schema. All subjects received the recommendation of taking the pill at bedtime.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Universidade de Passo Fundo (UPF) (150/2009; CAAE nº 0102.0.398.000-09). Every participant signed informed consent before entry in the trial. The study has registration in ClinicalTrials.gov (NCT01065649).

Symptom rating

Clinical evaluations occurred in three different moments just before fMRI sessions (Figure 1 A): in the beginning of the trial (baseline); at the end of the first treatment (week 3); and at the end of the second treatment (week 9). The following data were obtained: scores on GERD-SQ; presence of stress, anxiety, and depression according to Lipp Stress Inventory, Hamilton Anxiety Scale, Beck Depression Inventory, respectively; scores on anxiety scale

and on depression inventory; quantity of antacid pills consumed during each treatment; number of days with heartburn; prevalence of adverse events, including sleepiness (score on Epworth sleepiness scale).

fMRI protocol

fMRI sessions were performed at three moments (Figure 1 A): baseline, week 3, and week 9. In each session, patients were examined after 6 hours fasting, in the afternoon. A pH mapping of the gastroesophageal junction (36) was carried before the first fMRI in order to determine a pH turning point (PTP). A catheter was then inserted transnasally and positioned 7 cm above the PTP, allowing infusion of solutions in the distal esophagus.

The fMRI protocol was adapted from a study by Kern and colleagues (21). After obtaining a 10-minutes anatomical MRI image, an fMRI time series was recorded during 20 minutes (Figure 1 B). During fMRI acquisition the esophagus was perfused with 1 ml/min solutions in the following order: distillated water at pH 6.5 for 5 min, acid solution (HCl 0.1 N) at pH 1.5 for 5 min, distillated water for 5 min, and acid solution in the last 5 min. This was performed with the aid of an infusion pump (Infusomat compact, Braum S. A., São Gonçalo, RJ, Brazil).

Patients were instructed to lie still during image acquisition and to signalize with the left hand when perceiving heartburn during esophageal infusion as follows: index finger for mild heartburn and index plus medium fingers for severe heartburn. The symptom score during esophageal perfusion was calculated as the sum of the total finger signaling during 20 min of esophageal perfusion, regardless of water or acid.

Functional magnetic resonance imaging (fMRI)

Data acquisition

Gradient echo planar magnetic resonance (MR) images were acquired at Clínica Kozma using a 1.5 Tesla Magnetom Avanto (Siemens AG, Munich, Germany), equipped with a standard 8 channel head coil. The MR scanner and head coil were used to acquire a time series of echo planar images (EPI) covering the whole brain. High resolution tridimensional anatomic images were obtained from 176 sagittal spoiled gradient recalled sequence slices with a voxel size of 1.0 x 1.0 x 1.0 mm, to rule out anatomical abnormalities and for co-registration with the functional images. EPI were obtained in a mosaic composed of 36 axial slices of 64 x 64 pixels over a 220 mm field of view (voxel size of 3.0 x 3.0 x 3.75 mm), with a repetition time (TR) of 4270 ms and echo time (TE) of 50 ms. All MR data were stereotaxically transformed into the Talairach-Tournoux coordinate system for comparison and display purposes.

Image registration and data analysis

MR data preprocessing and statistical analyses were performed using the Analysis of Functional NeuroImaging (AFNI) software package (37). Small changes in head position were realigned to the first volume with tridimensional volume registration using quintic interpolation. The images were smoothed with a 4 mm full width at half maximum (FWHM) Gaussian filter. All MR signal intensity data were scaled to mean signal intensity over the time series to represent percentage of change in comparison to water perfusion performed in the first five minutes of fMRI procedure (basal period). The first three slices were excluded in order to avoid magnetic artifacts generally present in the beginning of each fMRI session. A multiple regression technique that computes the voxel-wise hemodynamic response function from the MR signal time series, based on a predefined

global spline waveform (Figure 2), was used to detect brain regions which exhibit significant blood oxygenation level dependent (BOLD) changes. The onset of the predefined global spline waveform model was constrained to occur within 5 minutes and 15 minutes, with peaks at 10 and 20 minutes, after the beginning of the fMRI procedure. The comparisons were undertaken considering the first five minutes of esophageal water infusion as the basal period for defining contrasts of functional measurements. AFNI accomplished multiple test correction using Monte Carlo simulation in order to form the clusters of voxels with statistical significance in terms of signal change.

Study outcomes

The brain response detected by fMRI to painful esophageal acid infusion was predefined as the primary outcome to be compared between the treatments, for which the sample size was calculated. The heartburn rating during acid perfusion, the amount of change of heartburn score on GERD-SQ, the number of days with heartburn and the quantity of antacid pills consumed were considered secondary outcomes to be compared between the treatments. Patients were asked about the adherence at the end of each treatment. A minimum of 80% of self-reported adherence during the treatments was considered adequate.

Statistical analysis

Quantitative variables are presented as median and 25%-75% interquartile range (IQR), or, when otherwise stated, mean ± standard deviation (SD). Categorical data is described as number of cases and percentage. The Wilcoxon test was used to compare quantitative variables. The McNemar test was employed to compare matched categorical data. Linear regression analysis was performed to evaluate the relationship between quantitative variables. The analyses were accomplished with commercially available *Statistical*

Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc, Chicago, IL, USA) and *GraphPad Prism* version 5.00 (GraphPad Software Inc, San Diego, CA, USA). Statistical significance was assumed when a two-tailed *p*-value ≤ 0.05.

RESULTS

Patients

Demographic and clinical characteristics of the study population are shown in Table 1. Women comprised 75% of the included patients. Most subjects (95%) were Caucasian (Brazilians of European descent), reflecting the local ethnic composition. A considerable proportion of subjects (35%) had *Helicobacter pylori* infection. The presence of stress, anxiety and depression among the patients was noticeable (40%, 35%, and 45%, respectively), as well as the scores [median (1st–3rd IQR)] on Hamilton Anxiety Scale [10.5 (4.5-22.5)] and Beck Depression Inventory [6.5 (3.0-15.0)]. Regarding PPI challenge after the end of the trial, 75% (15/20) of patients were responsive to PPIs, whereas 20% (4/20) were non-responsive. Such data was not available for one patient (1/20) who moved to another city.

Brain responses at fMRI

There was no statistical difference in the volume of activated voxels in whole brain between the nortriptyline and placebo ($p = 0.080$). Nortriptyline reduced cerebral activation in five areas (Table 2), namely: prefrontal cortex, insula, caudate, cingulate and hippocampus. A more prominent effect was observed on anterior cingulate, but as there was not a significant difference between the two divisions (anterior and posterior), cingulate was reported as a whole area. Furthermore, we evaluated a potential influence of

anxiety and depression on brain response, and we did not find any correlation between the variables.

Heartburn perception, psychological symptoms and side effects

There was no difference between nortriptyline and placebo, either in clinical outcomes (Table 3), or in the incidence of side effects (Table 4). All patients reported an adequate adherence to each treatment. The decreases of heartburn rating at the end of each treatment phase were similar [2.5 (0.8-8.7) *vs.* 2.5 (0-11.2), respectively; $p = 0.520$]. Results obtained in the evaluation of heartburn by GERD-SQ were similar to those of rating during esophageal acid infusion in fMRI sessions. The magnitude of decrease was not significantly different between nortriptyline and placebo treatments [4.5 (1.5-7.7) *vs.* 5.0 (3.2-7.0), respectively; $p = 0.670$]. The percentage of subjects who had stress, depression and anxiety did not differ between the treatments ($p = 0.620$; $p = 0.500$; $p = 0.500$, respectively). The potential effect of anxiety on the heartburn scores (GERD-SQ and acid-induced heartburn rating) was analyzed by linear regression separately for nortriptyline and placebo treatments. The regression coefficient of GERD-SQ score on anxiety levels was statistically significant when nortriptyline was considered ($p < 0.001$), but no significant regression was found when placebo was analyzed ($p = 0.150$). Heartburn rating during painful esophageal acid infusion did not depend on anxiety levels, either on nortriptyline ($p = 0.400$) or placebo ($p = 0.780$). The anxiety-adjusted decrease of heartburn assessed by GERD-SQ remained similar between the treatments.

DISCUSSION

The study design was planned to test the hypothesis that nortriptyline could reduce brain responses to painful esophageal acid stimulation and consequently relieve heartburn in NERD patients off PPI. This hypothesis was based on the use of chemical or physical esophageal stimulation as a model of visceral pain (17-20), and the assumption that the perception of heartburn could be modulated like neuropathic pain. Both nortriptyline and placebo promoted a decrease in whole brain response to painful esophageal acid infusion measured by fMRI. However, the effect of nortriptyline in predetermined areas of interest according to the literature (18,21,22,28,38) was strikingly more pronounced than placebo. Nortriptyline reduced cerebral response in five out of nine areas of interest in comparison to placebo. We recognize the possibility of a generalized non-specific effect of nortriptyline on brain activity, instead of a specific sensory effect on the esophagus-CNS axis. It cannot be excluded that the effect of nortriptyline observed in this study is non-sensory specific, which would ideally have been tested by studying the effect of nortriptyline on a non-sensory task. However, we specifically analyzed the brain responses to acid infusion during nortriptyline and placebo, and therefore believe a specific sensory effect is the most likely explanation.

The finding of lower activity in specific brain areas measured by fMRI during chronic antidepressant treatment has been recently reported: in healthy subjects, clomipramine reduced brain response to emotional stimuli in amygdala, insula, and anterior cingulate in comparison to non-medicated state (38). Insula, cingulate, prefrontal cortex, and hippocampus have been linked to the emotional perception of stimuli (39). In our study, insula, prefrontal cortex, caudate, cingulate and hippocampus exhibited lower activation with nortriptyline than with placebo. Morgan and cols. found that rectal painful distention in irritable bowel syndrome patients provoked activation of anterior cingulate, insula, and

prefrontal cortex, and that amitriptyline reduced pain related cerebral activation in cingulate and posterior parietal cortex in comparison to placebo, but only during concomitant psychological stress induction (28).

It would be reasonable to suppose that such a marked difference in brain response could favor nortriptyline over placebo in terms of heartburn relief. However, the clinical effect of nortriptyline was not different from that of placebo. Such discrepancy between clinical response and the extent of fMRI findings related to nortriptyline enables the possibility that the CNS modulation promoted by the drug could be counterbalanced by its peripheral anticholinergic effects, such as slowing of peristalsis and dry mouth. Perhaps an antidepressant agent with less anti-cholinergic action could be more helpful for heartburn relief in NERD patients, as well as in functional gastrointestinal disorders such as functional heartburn. In this setting, the antidepressant venlafaxine has already been proposed for treating non cardiac chest pain, a functional esophageal disorder (40).

The neural mechanisms whereby placebo can lead to analgesia remain unclear, but in the last decade a couple of studies have evaluated the placebo effect with the aid of fMRI (18,41,42). Interestingly, Lu and colleagues found improvement in psychophysical inventories as well as reduction of pain extent and brain response to esophageal balloon distention upon placebo treatment (18). The decrease in brain response was encountered in the visceral pain matrix (thalamus, somatossensory cortices, insula, prefrontal cortex, and cingulate) (18), a circuit known to be involved in the processing and modulation of pain (24). This suggests that placebo effect plays an important role in any treatment attempt for functional gastrointestinal disorders (18). Most of the aforementioned areas also exhibited lower activation in our study upon nortriptyline treatment. An adequate explanation for such a discrepancy between the brain responses to placebo in our NERD patients and those from the pain literature is lacking, although the different nature of the stimulus used

(repeated short-lived pain stimuli versus longer and slower esophageal acid infusion) could play a role. Nevertheless, nortriptyline did not differ from placebo concerning the clinical outcomes: heartburn rating during esophageal acid infusion in the fMRI and scores on GERD-SQ. Recently, other studies investigated the potential usefulness of new agents for the relief of heartburn in NERD patients (43,44), including patients non responsive to PPI, but also with disappointing results.

The putative mechanisms underlying the modulation of brain response are not understood, but we hypothesized that psychological comorbidities could be at least partially responsible. The prevalence of anxiety and depression in NERD patients has been pointed out (45,46), with percentages similar to those obtained in our study. Actually, a study demonstrated a higher prevalence of anxiety in NERD patients than in controls (46), while another group found a higher percentage in subjects with functional heartburn than in those with NERD (47). An inverse relation between both anxiety and depression and the quality of life in NERD patients has been stated (48). In our study, psychological symptoms ratings were in general comparable between the treatments. In order to evaluate the possibility of relationship between the heartburn indices and the anxiety score, a linear regression analysis was performed; such a relationship was found for GERD-SQ scores during nortriptyline treatment only. No significant statistical effect of anxiety on GERD-SQ score was observed in the placebo treatment, nor on heartburn rating during esophageal acid infusion either with nortriptyline or with placebo. Aiming to separate the effect of nortriptyline on anxiety and on heartburn, we adjusted the GERD-SQ scores for the anxiety score. The decrease of heartburn assessed by the adjusted GERD-SQ remained similar between treatments, suggesting that heartburn diminishes independently from the decrease in anxiety score.

Our study has some limitations. Due to the difficulty of finding pure non responsive patients to PPI and the invasiveness of study procedures, we recruited NERD patients based on clinical and endoscopic criteria, without categorization of NERD according to reflux testing (5). Nevertheless, we believe that our patients suffer mainly from esophageal hypersensitivity, since the majority of them had heartburn during esophageal acid perfusion in the absence of mucosal breaks. We could not exclude that some of our patients have functional heartburn. For answering this, we accomplished a pantoprazole challenge after the end of the trial, which rendered that most of our patients were PPI-responsive. We did not perform a stratified analysis based on PPI responsiveness because the sample size of non responsive patients' group was too small.

We calculated the sample size based on the findings from a previous fMRI study (28), as the brain response measured by fMRI was our primary outcome. Although the size of our sample was adequate to the fMRI evaluation, the number of subjects could have been insufficient to detect a mild clinical effect on heartburn of one treatment. The employment of such technology for investigating putative mechanisms of brain modulation of heartburn brings logistic difficulties (cost and complexity of fMRI evaluation) for recruiting a number of subjects bigger than the sample needed for the fMRI study. Despite the small number of patients, the differences in brain response to painful esophageal acid infusion were markedly clear between the treatments. The evaluation of symptoms at the end of the washout period could also be potential valuable information that is lacking.

Another concern about the fMRI protocol is the fact that patients remained on supine instead of semi-sitting position, which can lead to proximal esophageal stimulation with consequent more extensive brain response. This could be better assessed in future studies with two or more sensors in the esophagus or even impedance-pHmetry, which will bring technical challenges in an already complex procedure.

Another limitation may be the short period of treatment and the low dose of antidepressant. Even though the dose of nortriptyline was relatively low, the effect of tricyclic antidepressants on neuropathic pain generally begins with a shorter latency and with lower doses than the required for treating depression (25,49). Nevertheless, we cannot exclude the possibility of an improvement of heartburn over placebo with higher doses of nortriptyline for a longer period. Besides, the anticholinergic effects of nortriptyline could have facilitated NERD symptoms and blunted a potential advantage over placebo, since there is evidence of increase of heartburn with anticholinergic drugs (50). An impaired esophageal peristalsis and consequently a reduced esophageal clearance (51) and lower sphincter pressure (52) provoked by anticholinergic drugs can be behind this effect.

In conclusion, nortriptyline had a stronger attenuation in brain response to painful esophageal acid infusion, but without clinical benefit over placebo. Perhaps an agent with different pharmacological profile or higher doses of nortriptyline for longer periods could be helpful in this setting. We propose that the issue on the usefulness of antidepressants for heartburn should be further investigated in more extensive trials on NERD, especially with patients non responsive to PPI.

REFERENCES

1. Dent J, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut 2005; 54: 710-717.

2. Jung HK. Epidemiology of gastroesophageal reflux disease in Asia: a systematic review. J Neurogastroenterol Motil 2011; 17: 14-27.

3. Shaheen NJ, Hansen RA, Morgan DR et al. The Burden of Gastrointestinal and Liver Diseases, 2006. Am J Gastroenterol 2006; 100: 1010-1016.

4. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am J Gastroenterol 2006; 101: 1900-1920.

5. Modlin IM, Hunt RH, Malfertheiner P et al. Diagnosis and management of non-erosive, reflux disease - the Vevey NERD Consensus Group. Digestion 2009; 80: 74-88.

6. Fass R, Sifrim D. Management of heartburn not responding to proton pump inhibitors. Gut 2009; 58: 295-309.

7. Fass R. Functional heartburn: what it is and how to treat it. Gastrointest Endosc Clin N Am 2009; 19: 23-33.

8. Galmiche JP, Clouse RE, Bálint A et al. Functional esophageal disorders. Gastroenterology 2006; 130: 1459-1465.

9. Savarino E, Zentilin P, Tutuian R et al. The role of nonacid reflux in NERD: lessons learned from impedance-pH monitoring in 150 patients off therapy. Am J Gastroenterol 2008; 103: 2685-2693.

10. Farré R, Fornari F, Blondeau K et al. Acid and weakly acidic solutions impair mucosal integrity of distal exposed and proximal non-exposed human oesophagus. Gut 2010; 59: 164-169.

11. Farré R, De Vos R, Geboes K et al. Critical role of stress in increased oesophageal mucosa permeability and dilated intercellular spaces. Gut 2007; 56: 1191-1197.

12. Chua YC, Aziz Q. Perception of gastro-oesophageal reflux. Best Pract Res Clin Gastroenterol 2010; 24: 883-891.

13. Fass R, Tougas G. Functional heartburn: the stimulus, the pain, and the brain. Gut 2002; 51: 885-892.

14. Guarino MP, Cheng L, Ma J, et al. Increased TRPV1 gene expression in esophageal mucosa of patients with non-erosive and erosive reflux disease. Neurogastroenterol Motil 2010; 22: 746-751.

15. Aziz Q, Andersson JLR, Valind S et al. Identification of human brain loci processing esophageal sensation using positron emission tomography. Gastroenterology 1997; 113: 50-59.

16. Frobert O, Arendt-Nielsen L, Bak P, Funch-Jensen P, Bagger JP. Oesophageal sensation assessed by electrical stimuli and brain evoked potentials--a new model for visceral nociception. Gut 1995; 37: 603-609.

17. Frokjaer JB, Andersen SD, Gale J, Arendt-Nielsen L, Gregersen H, Drewes AM. An experimental study of viscero-visceral hyperalgesia using an ultrasound-based multimodal sensory testing approach. Pain 2005; 119: 191-200.

18. Lu HC, Hsieh JC, Lu CL et al. Neuronal correlates in the modulation of placebo analgesia in experimentally-induced esophageal pain: a 3T-fMRI study. Pain 2010; 148: 75-83.

19. Pedersen J, Reddy H, Funch-Jensen P, Arendt-Nielsen L, Gregersen H, Drewes AM. Cold and heat pain assessment of the human oesophagus after experimental sensitisation with acid. Pain 2004; 110: 393-399.

20. Strigo IA, Bushnell MC, Boivin M, Duncan GH. Psychophysical analysis of visceral and cutaneous pain in human subjects. Pain 2002; 97: 235-246.

21. Kern M, Hofmann C, Hyde J, Shaker R. Characterization of the cerebral cortical representation of heartburn in GERD patients. Am J Physiol Gastrointest Liver Physiol 2004; 286: G174-G181.

22. Wang K, Duan LP, Zeng XZ, Liu JY, Weng XC. Differences in cerebral response to esophageal acid stimuli and psychological anticipation in GERD subtypes - An fMRI study. BMC Gastroenterol 2011; 11: 28.

23. Mayer EA, Aziz Q, Coen S et al. Brain imaging approaches to the study of functional GI disorders: a Rome working team report. Neurogastroenterol Motil 2009; 21: 579-596.

24. Peyron R, Laurent B, García-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin 2000; 30: 263-288.

25. Dworkin RH, O'Connor AB, Backonja M et al. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007; 132: 237-251.

26. Jackson JL, O'Malley PG, Tomkins G, Balden E, Santoro J, Kroenke K. Treatment of functional gastrointestinal disorders with antidepressant medications: a meta-analysis. Am J Med 2000; 108: 65-72.

27. Mertz H, Fass R, Kodner A, Yan-Go F, Fullerton S, Mayer EA. Effect of amitriptyline on symptoms, sleep, and visceral perception in patients with functional dyspepsia. Am J Gastroenterol 1998; 93: 160-165.

28. Morgan V, Pickens D, Gautam S, Kessler R, Mertz H. Amitriptyline reduces rectal pain related activation of the anterior cingulate cortex in patients with irritable bowel syndrome. Gut 2005; 54: 601-607.

29. Fornari F, Gruber AC, Lopes AB, Cecchetti D, Barros SGS. [Symptom's questionnaire for gastroesophageal reflux disease]. Arq Gastroenterol 2004; 41: 263-267.

30. Velanovich V, Vallance SR, Gusz JR, Tapia FV, Harkabus MA. Quality of life scale for gastroesophageal reflux disease. J Am Coll Surg 1996; 183: 217-224.

31. Hamilton M. The assessment of anxiety states by rating. Br J Med Psychol 1959; 32: 50-55.

32. Beck AT, Ward CH, Mendelson M, Mock J, Erbaug J. An inventory for measuring depression. Arch Gen Psychiatry 1961; 4: 561-571.

33. Beck AT, Steer RA, Garbin MG. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. Clin Psychol Rev 1988; 8: 77-100.

34. Lipp M, Guevara A. Validação empírica do inventário de sintomas de stress. Estudos de Psicologia 1994; 11: 43-49.

35. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991; 14: 540-545.

36. Liell TP, Tomiozzo Jr JC, Denti F, de Lima LA, Fornari F. Determination of pH turning point with pH mapping of the gastroesophageal junction: an alternative technique to orientate esophageal pH monitoring. Dis Esophagus 2011; 24: 305-311.

37. Cox RW, Hyde JS. Software tools for analysis and visualization of fMRI data. NMR Biomed 1997; 10: 171-178.

38. De Almeida JRC, Phillips ML, Cerqueira CT et al. Neural activity changes to emotional stimuli in healthy individuals under chronic use of clomipramine. J Psychopharmacol 2010; 24: 1165-1174.

39. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception I: The neural basis of normal emotion perception. Biol Psychiatry 2003; 54: 504-514.

40. Lee H, Kim JH, Min BH et al. Efficacy of venlafaxine for symptomatic relief in young adult patients with functional chest pain: a randomized, double-blind, placebo-controlled, crossover trial. Am J Gastroenterol 2010; 105: 1504-1512.

41. Bingel U, Lorenz J, Schoell E, Weiller C, Büchel C. Mechanisms of placebo analgesia: rACC recruitment of a subcortical antinociceptive network. Pain 2006; 120: 8-15.

42. Watson A, El-Deredy W, Iannetti GD et al. Placebo conditioning and placebo analgesia modulate a common brain network during pain anticipation and perception. Pain 2009; 145: 24-30.

43. Adachi K, Furuta K, Miwa H et al. A Study on the Efficacy of Rebamipide for Patients with Proton Pump Inhibitor-Refractory Non-Erosive Reflux Disease. Dig Dis Sci 2012; 57: 1609-1617.

44. Miwa H, Inoue K, Ashida K et al. Randomised clinical trial: efficacy of the addition of a prokinetic, mosapride citrate, to omeprazole in the treatment of patients with non-erosive reflux disease - a double-blind, placebo-controlled study. Aliment Pharmacol Ther 2011; 33: 323-332.

45. Hartono JL, Qua CS, Goh KL. Non-erosive reflux disease (NERD), symptomatic and asymptomatic erosive reflux disease (ERD): from hypersensitive to hyposensitive esophagus. Dig Dis Sci 2011; 56: 90-96.

46. Hartono JL, Mahadeva S, Goh KL. Anxiety and depression in various functional gastrointestinal disorders: Do differences exist? J Dig Dis 2012; 13: 252-257.

47. Lee KJ, Kwon HC, Cheong JY, Cho SW. Demographic, clinical, and psychological characteristics of the heartburn groups classified using the Rome III criteria and factors associated with the responsiveness to proton pump inhibitors in the gastroesophageal reflux disease group. Digestion 2009; 79: 131-136.

48. Oh JH, Kim TS, Choi MG et al. Relationship between Psychological Factors and Quality of Life in Subtypes of Gastroesophageal Reflux Disease. Gut Liver 2009; 3: 259-265.

49. Maizels M, McCarberg B. Antidepressants and antiepileptic drugs for chronic non-cancer pain. Am Fam Physician 2005; 71: 483-490.

50. van Soest EM, Dieleman JP, Kuipers EJ. The effect of anticholinergic agents on gastro-oesophageal reflux and related disorders. Expert Opin Drug Saf 2008; 7:173-180.

51. Phaosawasdi K, Malmud LS, Tolin RD, Stelzer F, Applegate G, Fisher RS. Cholinergic effects on esophageal transit and clerarence. Gastroenterology 1981; 81:915-920

52. Miller L, Dai Q, Vegesna A et al. A missing sphincteric component of the gastro-oesophageal junction in patients with GORD. Neurogastroenterol Motil 2009; 21:813-e52

TABLES

Table 1. Demographic characteristics of the study sample ($n = 20$) [median (25%-75%

interquartile range)]

Characteristics	Descriptive Statistics
Women (%)	75
Men (%)	25
Age (years) §	36.1 ± 9.3
Caucasian (%)	95
Weight (kg)	65.0 (56.0-80.7)
Height (cm)	164.5 (160.5-170.0)
BMI (kg/m^2)	23.4 (21.1-27.8)
Helicobacter pylori + (%)	35
Basal GERD-SQ	12.5 (9.2-16.0)
Basal anxiety score	10.5 (4.5-22.5)
Presence of anxiety (%)	35
Basal depression score	6.5 (3.0-15.0)
Presence of depression (%)	45
Presence of stress (%)	40

BMI: Body mass index;

GERD-SQ: Gastroesophageal reflux disease symptoms questionnaire.

§ mean ± standard deviation.

Table 2. Comparison of fMRI brain activation (%) between the treatments ($n = 20$) [median (25%-75% interquartile range)]

MRI activation	Nortriptyline	Placebo
Hippocampus	-2.7 (-6.0 to 0.5)*	-0.04 (-2.3 to 1.9)
Cingulate	-4.2 (-8.8 to -0.1)**	-0.6 (-1.8 to 3.0)
Insula	-2.4 (-4.8 to -0.6)†	-0.2 (-1.5 to 1.5)
Caudate	-3.0 (-5.1 to -0.01)†	0.48 (-1.9 to 3.1)
Prefrontal cortex	-1.9 (-4.5 to -0.1)††	-0.3 (-2.5 to 2.3)

fMRI: functional magnetic resonance imaging.

* $p = 0.006$ vs. placebo

** $p = 0.017$ vs. placebo

† $p = 0.029$ vs. placebo

†† $p = 0.050$ vs. placebo

Comparisons performed with Wilcoxon test.

Table 3. Comparison of clinical outcomes between the treatments (*n* = 20) [median (25%-75% interquartile range)]

Clinical variable	Nortriptyline	Placebo
Heartburn rating during fMRI	4.0 (1.2-9.2)	3.0 (1.0-9.5)
Decrease of heartburn rating	2.5 (0.2-8.7)	2.5 (0-11.2)
GERD-SQ	8.0 (5.5-11.0)	7.0 (5.0-10.7)
Decrease of GERD-SQ	4.5 (1.5-7.7)	5.0 (3.2-7.0)
Days with heartburn	6.5 (4.0-10.2)	8.5 (3.5-12.5)
Antacid pills	2.0 (0.5-10.0)	3.0 (0-4.2)
Anxiety score	6.0 (2.2-14.2)	8.0 (3.5-12.7)
Presence of anxiety (%)	20	10
Decrease of anxiety score	2.5 (2.0-8.7)	3.5 (0.2-6.0)
Depression score	5.0 (1.0-11.7)	4.5 (2.0-9.7)
Presence of depression (%)	35	25
Decrease of depression score	0 (0-4.0)	1.5 (- 1.0-4.7)
Presence of stress (%)	15	25

GERD-SQ: Gastroesophageal reflux disease symptoms questionnaire;

fMRI: functional magnetic resonance imaging.

No significant difference was found between treatments.

Comparisons of quantitative variables performed with Wilcoxon test.

Comparisons of qualitative variables with McNemar test.

Table 4. Comparison of side effects between the treatments ($n = 18$)

Clinical variable	Nortriptyline	Placebo
Sleepiness score*	6.4 ± 3.5	5.6 ± 3.5
Insomnia (%)	16.7	11.1
Headache (%)	22.2	33.3
Nausea (%)	16.7	16.7
Abdominal pain (%)	33.3	38.9
Constipation (%)	11.1	5.6
Xerosthomia (%)	11.1	0

* Mean ± standard deviation.

No significant difference was found between treatments.

Comparison of sleepiness score performed with paired t test.

Comparisons of qualitative variables with McNemar test.

FIGURE LEGENDS

Figure 1. Study protocol. **(A)** Patients were randomly allocated to placebo or nortriptyline treatment for three weeks. At the end of this period there was a three week washout, followed by crossover for three weeks of the alternate treatment. Assessment of symptoms and measurement of brain activation during esophageal infusion by functional magnetic resonance imaging (fMRI) were performed before beginning the trial and at the end of each treatment phase (arrows). **(B)** Each session of fMRI is described as indicated: after obtaining an anatomical MRI mapping, a series of fMRI signals were recorded during 20 minutes of esophageal infusion. This period was composed by alternate parts, namely: water, acid solution (HCl), water and acid solution.

Figure 2. Predefined global spline waveform model for brain response to esophageal acid infusion during functional resonance imaging (fMRI).

FIGURAS DO ARTIGO 2

Figure 1

A

Clinical + fMRI evaluations

Clinical + fMRI evaluations

Clinical + fMRI evaluations

| Nortriptyline | Placebo |

| Placebo | Nortriptyline |

Baseline — Week 3 — Week 6 — Week 9

B

Anatomical mapping

Water perfusion (pH 6,5) HCl perfusion (pH 1,5) Water perfusion (pH 6,5) HCl perfusion (pH 1,5)
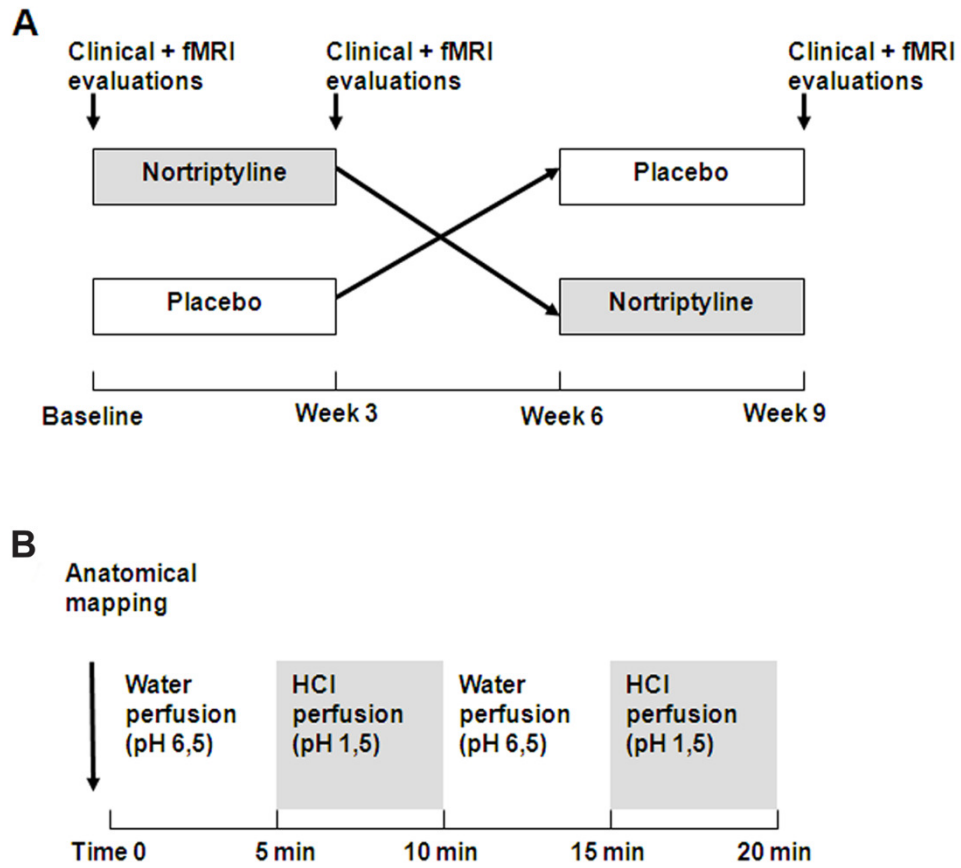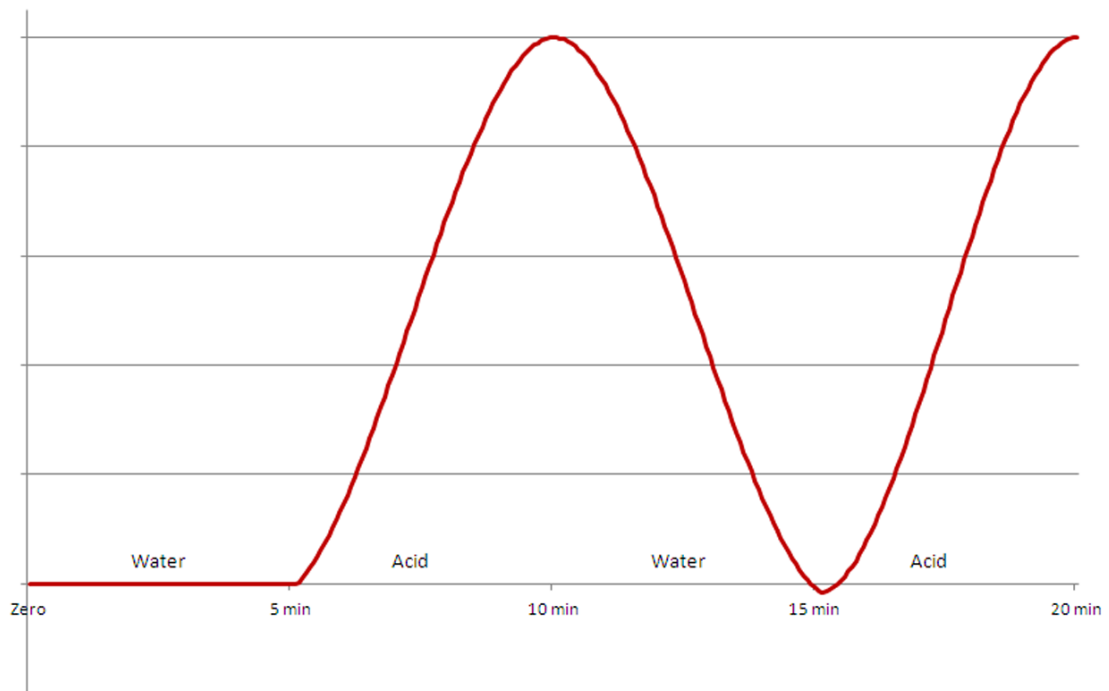
Time 0   5 min   10 min   15 min   20 min

Figure 2

CONCLUSÕES E PERSPECTIVAS

Resultados interessantes emergiram do presente trabalho: o papel da estimulação ácida repetida do esôfago como fator sensibilizador para a pirose na DRGE; a ineficácia da nortriptilina como tratamento para a doença, muito embora com efeitos sobre a ativação encefálica maiores que o placebo; o aperfeiçoamento de um protocolo de investigação da resposta encefálica ao estímulo ácido esofágico. Ressalte-se, também, a criação do Laboratório de Pesquisa da Faculdade de Medicina da UPF, dotado de capacidade para procedimentos com a metodologia ELISA.

Contudo, algumas perguntas de cunho clínico não puderam ser adequadamente respondidas, em parte pelo tamanho amostral pequeno, calculado para os desfechos de RMf. Restam dúvidas acerca do papel de aspectos psicológicos na modulação dos sintomas da DRGE, da avaliação estratificada de homens e mulheres, das respostas em pacientes refratários aos IBPS e com o uso de medicamento antidepressivo e ansiolítico de perfil diferente da nortriptilina.

Além disso, o contínuo aprendizado sobre a RMf poderá resultar na produção de dados cada vez mais verossímeis acerca da resposta encefálica a estímulos. Dentre as ideias estão: a inclusão de controles para comparação com pacientes (já em andamento); o estudo específico de pacientes com sobrepeso ou obesidade; a complementação da investigação com RMf com dados de impedanciopHmetria; a obtenção de dados de conectividade entre áreas encefálicas. Tais estudos, na mesma linha de pesquisa inaugurada pelo presente projeto, estão sendo planejados e/ou conduzidos pelos colegas José Carlos Tomiozzo Jr e Marcelo Ribeiro, sob a orientação do Prof. Fernando Fornari.

Enfim, se trabalho significativo foi ora realizado, há muito ainda por se fazer.

ORÇAMENTO

Materiais/ Procedimentos	Custo/unidade (R$)	Quantidade	Total (R$)
Folha A4 *	15,00 / pacote	3	45,00
Escalas psicométricas *	25,00	60	1.500,00
Cateter de pHmetria **	200,00	20	4.000,00
RNMf ¥	1.000,00	80	80.000,00
Soluções de perfusão	5,50	80	440,00
Antidepressivo §	Doação	40 kits	-
Placebo ¶	Doação	40 kits	-
Refeição (Nutridrink®)	8,40	60	504,00
Frascos p/ armazenar soro *	2,00	140	280,00
Equipamento ELISA ‡	25.000,00	1	25.000,00
Kits p/ cortisol sérico ‡	2.256,00 (valor médio)	5	13.236,00
Material p/coleta de sangue ¥	1.000,00	1	1.000,00
Freezer ‡	1.899,00	1	1.899,00
Computador ‡	1.500,00	1	1.500,00
Centrífuga ‡	3.000,00	1	3.000,00
Material de bancada ‡			1.000,00
TOTAL			*133.404,00*

* Disponível

** Cortesia da Endopasso, Passo Fundo - RS

¥ Cortesia da Clínica Kozma, Passo Fundo - RS

§ Disponibilizado pela indústria farmacêutica Novartis.

¶ Disponibilizado pela farmácia Natupharma, Passo Fundo - RS

‡ Financiado pelo Edital Universal do CNPq.

ANEXOS

Anexo 1: Questionário de sintomas na DRGE (modificado)

Nome: _____ Data: _____

Para cada pergunta marque um **X** no número que achar mais parecido com os seus sintomas, de acordo com o que você sentiu nas **últimas 3 semanas**. O significado dos números é:

0 : não sinto.

1 : sinto mas não me incomoda.

2 : sinto e me incomoda, mas não todos os dias.

3 : sinto e me incomoda todos os dias.

4 : sinto e isto atrapalha o que eu faço durante o dia.

5 : sinto e os sintomas são muito ruins (não me deixam fazer nada).

Perguntas:

1	**Quanto o incomoda a sua azia?**	**1**	**2**	**3**	**4**	**5**
2	**Sente azia quando está deitado?**	**1**	**2**	**3**	**4**	**5**
3	**Sente azia quando está em pé?**	**1**	**2**	**3**	**4**	**5**
4	**Sente azia após as refeições?**	**1**	**2**	**3**	**4**	**5**
5	**A azia altera seus hábitos de alimentação?**	**1**	**2**	**3**	**4**	**5**
6	**A azia acorda você durante o sono?**	**1**	**2**	**3**	**4**	**5**

Anexo 2: Inventário de depressão de Beck
 (versão revisada em 1979)

Nome:_____ Data: _____

"Este questionário consiste em 21 grupos de afirmações. Depois de ler cuidadosamente cada grupo, faça um círculo em torno do número (0, 1, 2 ou 3) próximo à afirmação, em cada grupo, que descreve melhor a maneira que você tem se sentido na última semana, incluindo hoje. Tome o cuidado de ler todas as afirmações, em cada grupo, antes de fazer a sua escolha".

1.   0 – Não me sinto triste
        1 – Eu me sinto triste
        2 – Estou sempre triste e não consigo sair disto.
        3 – Estou tão triste ou infeliz que não consigo suportar.

2.   0 – Não estou especialmente desanimado com relação ao futuro.
        1 – Eu me sinto desanimado com relação ao futuro.
        2 – Acho que nada tenho a esperar.
        3 – Acho o futuro sem esperança e tenho a impressão de que as coisas não podem melhorar.

3.   0 – Não me sinto um fracasso.
        1 – Acho que fracassei mais do que uma pessoa comum.
        2 – Quando olho para trás, na minha vida, tudo o que posso ver é um monte de fracassos.
        3 – Acho que, como pessoa, sou um completo fracasso.

4.   0 – Tenho tanto prazer em tudo como antes.
        1 – Não sinto mais prazer nas coisas como antes.
        2 – Não encontro um prazer real em mais nada.
        3 – Estou insatisfeito ou aborrecido com tudo.

5.   0 – Não me sinto especialmente culpado.
        1 – Eu me sinto culpado grande parte do tempo.
        2 – Eu me sinto culpado na maior parte do tempo.
        3 – Eu me sinto sempre culpado.

6.   0 – Não acho que esteja sendo punido.
        1 – Acho que posso ser punido.
        2 – Creio que serei punido.
        3 – Acho que estou sendo punido.

7.   0 - Não me sinto decepcionado comigo mesmo.
        1 – Estou decepcionado comigo mesmo.
        2 – Estou enjoado de mim.
        3 – Eu me odeio.

8.   0 – Não me sinto, de qualquer modo, pior que os outros.
      1 – Sou crítico em relação a mim por minhas fraquezas ou erros.
      2 – Eu me culpo sempre por minhas falhas.
      3 – Eu me culpo por tudo de mal que acontece.

9.   0 – Não tenho quaisquer idéias de me matar.
      1 – Tenho idéias de me matar, mas não as executaria.
      2 – Gostaria de me matar.
      3 – Eu me mataria se tivesse oportunidade.

10.   0 – Não choro mais do que o habitual.
      1 – Choro mais agora do que costumava.
      2 – Agora, choro o tempo todo.
      3 – Costumava ser capaz de chorar, mas agora não consigo, mesmo que o queira.

11.   0 – Não sou mais irritado agora do que já fui.
      1 – Fico aborrecido ou irritado mais facilmente do que costumava.
      2 – Atualmente me sinto irritado o tempo todo.
      3 – Não me irrito mais com as coisas que costumava me irritar.

12.   0 – Não perdi o interesse pelas outras pessoas.
      1 – Estou menos interessado pelas outras pessoas do que costumava estar.
      2 – Perdi a maior parte do meu interesse pelas outras pessoas.
      3 – Perdi todo o meu interesse pelas outras pessoas.

13.   0 – Tomo decisões tão bem como antes.
      1 – Adio as tomadas de decisões mais do que costumava.
      2 – Tenho mais dificuldade em tomar decisões do que antes.
      3 – Não consigo mais tomar decisões.

14.   0 – Não acho que minha aparência esteja pior do que costumava ser.
      1 – Estou preocupado por estar parecendo velho ou sem atrativos.
      2 – Acho que há mudanças permanentes na minha aparência que me fazem parecer sem atrativos.
      3 – Acredito que pareço feio.

15.   0 – Posso trabalhar tão bem quanto antes.
      1 – Preciso de um esforço extra para fazer alguma coisa.
      2 – Tenho que me esforçar muito para fazer alguma coisa.
      3 – Não consigo mais fazer trabalho algum.

16.   0 – Consigo dormir tão bem quanto o habitual.
      1 – Não durmo tão bem quanto costumava.
      2 – Acordo uma ou duas horas mais cedo que habitualmente e tenho dificuldade em voltar a dormir.
      3 – Acordo várias horas mais cedo do que costumava e não consigo voltar a dormir.

17.   0 – Não fico mais cansado do que o habitual.
       1 – Fico cansado com mais facilidade do que o habitual.
       2 – Sinto-me cansado ao fazer qualquer coisa.
       3 – Estou cansado demais para fazer qualquer coisa.

18.   0 – Meu apetite não está pior do que o habitual.
       1 – Meu apetite não é tão bom quanto costumava ser.
       2 – Meu apetite está muito pior agora.
       3 – Não tenho mais nenhum apetite.

19 .   0 – Não tenho perdido muito peso, se é que perdi algum recentemente.
       1 – Perdi mais de dois quilos e meio.
       2 – Perdi mais de cinco quilos.
       3 – Perdi mais de sete quilos.
       Estou tentando perder peso de propósito, comendo menos: ( ) Sim ( ) Não

20.   0 – Não estou mais preocupado com minha saúde do que o habitual.
       1 – Estou preocupado com problemas físicos, tais como dores, indisposição no estômago ou prisão de ventre.
       2 – Estou muito preocupado com problemas físicos e é difícil pensar em outra coisa.
       3 – Estou tão preocupado com problemas físicos que não consigo pensar em qualquer outra coisa.

21.   0 – Não notei qualquer mudança recente no meu interesse por sexo.
       1 – Estou menos interessado por sexo do que costumava estar.
       2 – Estou muito menos interessado em sexo atualmente.
       3 – Perdi totalmente o interesse por sexo.

Anexo 3: Escala de Avaliação de Ansiedade de Hamilton (HAM-A)

Nome: _____ Data: _____

0 = ausente

1 = intensidade leve

2 = intensidade média

3 = intensidade forte

4 = intensidade máxima

1.Humor Ansioso Inquietação, temor ao pior, apreensão quanto ao presente ou futuro, maus pressentimentos, irritabilidade.	
2. Tensão Sensação de tensão, fadiga, reações de sobressalto, choro fácil, tremores, sensação de cansaço, incapacidade de relaxar, agitação	
3. Medos Do escuro, de estranhos, de ficar sozinho, de animais de grande porte, do trânsito, de multidões.	
4. Insônia Dificuldade de adormecer, sono interrompido, sono insatisfatório, fadiga ao acordar, sonhos penosos, pesadelos, terror noturno.	
5. Dificuldades Intelectuais Dificuldade de concentração, falhas de memória.	
6. Humor deprimido Perda de interesse, oscilação de humor, depressão, despertar precoce.	
7. Somatizações motoras Dores musculares, rigidez muscular, contrações espásticas, contrações involuntárias, ranger de dentes, voz insegura.	
TOTAL PARCIAL	

8. Somatizações sensoriais Ondas de frio ou calor, sensações de fraqueza, visão borrada, sensação de picadas, formigamento, sensações auditivas, zumbidos	
9. Sintomas cardiovasculares Taquicardia, palpitações, dores no peito, sensação de desmaio, sensação de extra-sístotoles.	
10. Sintomas respiratórios Pressão no peito ou aperto no peito, dispnéia, respiração suspirosa, sensação de sufocação.	
11. Sintomas gastrintestinais Deglutição difícil, aerofagia, dispepsia, sensação de plenitude gástrica, dor pré- ou pós-prandial, pirose, meteorismo, náuseas, vômitos, sensação de vazio gástrico, diarréia, constipação, cólicas.	
12. Sintomas geniturinários Polaciúria, urgência miccional, amenorréia, frigidez, diminuição da libido.	
13. Sintomas neurovegetativos Boca seca, palidez, tendência à sudorese, tonturas, cefaléia de tensão.	
14. Comportamento durante a entrevista Tenso, pouco à vontade, inquieto, agitação das mãos (mexer, retorcer, tremores), franzir a testa, face tensa.	
TOTAL GERAL	

:

Anexo 4: Inventário de sintomas de stress de Lipp para adultos

Nome: _____ Data: _____

A) Marque com um (X) os sintomas que tem experimentado nas últimas 24 horas

( ) Mãos e pés frios

( ) Boca seca

( ) Nó no estômago

( ) Aumento de sudorese (do suor)

( ) Tensão muscular

( ) Aperto da mandíbula / ranger os dentes

( ) Diarréia passageira

( ) Insônia (dificuldade para dormir)

( ) Taquicardia (coração acelerado, palpitações)

( ) Hiperventilação (respirar fundo e rápido)

( ) Hipertensão arterial (pressão alta) súbita e passageira

( ) Mudança de apetite

( ) Aumento súbito de motivação

( ) Entusiasmo súbito

( ) Vontade súbita de iniciar novos projetos

B) Marque com um (X) os sintomas que tem experimentado na última semana

( ) Problemas com a memória

( ) Mal-estar generalizado, sem causa específica

( ) Formigamento das extremidades

( ) Sensação de desgaste físico constante

( ) Mudança de apetite

( ) Aparecimento de problemas dermatológicos (de pele)

( ) Hipertensão arterial (pressão alta)

( ) Cansaço constante

( ) Aparecimento de úlcera

( ) Tontura / sensação de estar flutuando

( ) Sensibilidade excessiva

( ) Dúvida quanto a si próprio

( ) Pensar constantemente em um só assunto. Se possível citar: qual?

( ) Irritabilidade excessiva

( ) Diminuição da libido (do apetite sexual)

C) Marque com um (X) os sintomas que tem experimentado no último mês

( ) Diarréia frequente

( ) Dificuldades sexuais

( ) Insônia (dificuldade para dormir)

( ) Náuseas

( ) Tiques (cacoetes)

( ) Hipertensão arterial continuada (pressão alta)

( ) Problemas dermatológicos (de pele)

( ) Mudança extrema de apetite

( ) Excesso de gases

( ) Tontura freqüente

( ) Impossibilidade de trabalhar

( ) Irritabilidade sem causa aparente

( ) Pesadelos

( ) Angústia – Ansiedade diurna

( ) Sensação de incompetência em todas as áreas

( ) Hipersensibilidade emotiva

( ) Vontade de fugir de tudo

( ) Perda do senso de humor

( ) Apatia, depressão ou raiva prolongada

( ) Cansaço excessivo

( ) Pensar / falar constantemente em um só assunto. Se possível citar: qual?

_____

Anexo 5: Escala de sonolência de Epworth

Qual possibilidade de você cochilar ou adormecer nas seguintes situações?

0 - nenhuma chance de cochilar

1 - pequena chance de cochilar

2 - moderada chance de cochilar

3 - alta chance de cochilar

Situações	Chance de cochilar: 0 a 3
1. Sentado e lendo.	
2. Vendo televisão.	
3. Sentado em lugar público sem atividades Como sala de espera, cinema, teatro, igreja.	
4. Como passageiro de carro, trem ou metro andando por 1 hora sem parar.	
5. Deitado para descansar à tarde	
6. Sentado e conversando com alguém	
7. Sentado após uma refeição sem álcool	
8. No carro parado por alguns minutos no durante trânsito	
Total	

Anexo 6: Termo de Consentimento Livre e Esclarecido

Você está sendo convidado para participar da pesquisa entitulada "*Efeito da nortriptilina na pirose e em sua representação cortical em pacientes com doença do refluxo gastroesofágico não erosiva*"**,** realizada pelos pesquisadores Fernando Fornari e Cassiano Mateus Forcelini do curso de Medicina da Universidade de Passo Fundo (UPF)**.** Você foi escolhido por apresentar sintomas da doença do refluxo gastroesofágico.

Sua participação não é obrigatória. A qualquer momento você pode desistir de participar e retirar seu consentimento. Sua recusa não trará nenhum prejuízo em sua relação com o pesquisador, (ou com a entidade vinculada) ou para o seu atendimento e tratamento. O objetivo desta pesquisa é avaliar o efeito do antidepressivo nortriptilina na percepção do sintoma pirose (azia) ao nível do sistema nervoso central.

Sua participação nesta pesquisa será em **quatro etapas (na clínica Kozma):**

Primeira: Será realizada entrevista pelos Drs. Fernando Fornari e Cassiano Mateus Forcelini, aonde você será solicitado a responder a quatro questionários sobre sintomas da doença do refluxo e sobre como se sente emocionalmente, o que levará aproximadamente 20 minutos. Caso você achar que alguma pergunta cause algum constrangimento a você, não precisa responder. O próximo passo será a colocação de um abocath (cânula plástica de fino calibre) em uma veia do antebraço, para coleta de pequenas amostras de sangue durante o exame de ressonância. Você poderá sentir uma dor passageira no antebraço, semelhante a que ocorre numa coleta para exame de sangue. A seguir, será realizado um procedimento para medir a distância entre o nariz e a válvula que existe na junção do esôfago com o estômago, através da passagem de uma sonda flexível pelo nariz e pela garganta. Para isto seu nariz será amortecido com um gel anestésico. Esta sonda permanecerá durante o tempo em que se realizará um exame de ressonância nuclear magnética do sistema nervoso da cabeça, com duração de 80 minutos. Para este exame será necessário deitar-se na maca do equipamento, e permanecer imóvel pelo período do exame. Durante este exame será pingado no esôfago uma quantidade pequena de água e de líquido acidificado, através da sonda posicionada pelo nariz. Serão realizadas algumas perguntas sobre a ocorrência de azia. Haverá um intervalo no qual você tomará 200 ml de uma dieta com gosto de chocolate, através de um canudinho. Após o fim da ressonância, a sonda do nariz e o abocath do antebraço serão retirados, e você será capaz de se levantar sozinho e voltar para casa normalmente.

Segunda: Todo o protocolo deverá ser repetido em alguns dias, sem a administração da dieta. Depois, você também receberá 21 cápsulas para serem tomados durante 21 dias (1 cápsula por dia, sempre entre 8 e 9 horas da noite). Este remédio poderá ser um antidepressivo ou um "placebo", que é uma cápsula sem efeito (vazia). Você receberá um papel para anotar os horários e dias em que sentir azia, além de qualquer outro sintoma que julgue incomodativo durante os próximos 21 dias.

Terceira: Será realizada ao término dos 21 dias do tratamento. O procedimento será igual ao da primeira etapa, com exceção do exame de ressonância, que será mais rápido (30 minutos). Ao ser liberado da clínica, você será orientado a permanecer entre 21 e 28 dias sem tratamento algum, levando uma vida normal. Você receberá um papel para anotar os horários e dias em que sentir azia, além de qualquer outro sintoma que julgue incomodativo nesse intervalo. Também receberá 21 cápsulas para serem tomados durante 21 dias (1 cápsula por dia, sempre entre 8 e 9 horas da noite) após o período sem tratamento. Este remédio poderá ser um antidepressivo ou um "placebo", que é uma cápsula sem efeito (vazia).

Quarta: No último dia do tratamento, será realizado o mesmo procedimento de ressonância da cabeça da segunda etapa.

Ao participar, os riscos para você serão mínimos. Você poderá sentir desconforto no nariz e na garganta quando realizar a passagem da sonda flexível pelo nariz. Poderá também sentir mal-estar, boca seca, e tonturas quando utilizar os tratamentos de 21 dias.

Você não será beneficiado(a) diretamente pela pesquisa, mas você estará ajudando a entender se o uso de antidepressivo pode trazer benefício para pessoas que sentem azia. As informações obtidas através dessa pesquisa serão confidenciais, isto é, só os pesquisadores saberão o que foi respondido e o seu nome não será divulgado em nenhum momento, sendo mantido sigilo sobre sua participação. As suas respostas não serão divulgadas de modo que permitam a sua identificação. Você não será recompensado(a) financeiramente pela sua participação.

Ao assinar este documento, você estará concordando em participar da pesquisa e que entendeu os objetivos, riscos e benefícios da sua participação e todas as informações que lhe foram prestadas pelos pesquisadores. Você receberá uma cópia deste termo onde consta o telefone e o endereço dos pesquisadores, podendo tirar suas dúvidas sobre a pesquisa e sua participação, a qualquer momento.

Pesquisadores:

Nome_____

Assinatura_____Telefone_____

_____

Nome_____

Assinatura_____Telefone_____

_____

Participante:

Nome_____

Assinatura_____

O presente Termo de Consentimento Livre e Esclarecido foi elaborado de acordo com a Res. CNS 196/96 e foi aprovado pelo Comitê de Ética em Pesquisa da Universidade de Passo Fundo.

O participante pode entrar em contato com o Comitê de Ética em Pesquisa da Universidade de Passo Fundo pelo telefone (0xx54) 3316-8370.