Universidade Estadual de Londrina

ELIS CAROLINA DE SOUZA FATEL

A INGESTÃO DE SUCO DE CRANBERRY ACRESCENTA EFEITOS BENÉFICOS SOBRE A ATIVIDADE DA DOENÇA E BIOMARCADORES INFLAMATÓRIOS EM PACIENTES COM ARTRITE REUMATOIDE SUPLEMENTADOS COM ÓLEO DE PEIXE

Londrina
2017

ELIS CAROLINA DE SOUZA FATEL

A INGESTÃO DE SUCO DE CRANBERRY ACRESCENTA EFEITOS BENÉFICOS SOBRE A ATIVIDADE DA DOENÇA E BIOMARCADORES INFLAMATÓRIOS EM PACIENTES COM ARTRITE REUMATOIDE SUPLEMENTADOS COM ÓLEO DE PEIXE

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde, do Centro de Ciências da Saúde, da Universidade Estadual de Londrina para obtenção do título de Doutor
Orientador: Prof. Dr.Isaias Dichi

Londrina
2017

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

FATEL, ELIS CAROLINA DE SOUZA .
A INGESTÃO DE SUCO DE CRANBERRY ACRESCENTA EFEITOS BENÉFICOS SOBRE A ATIVIDADE DA DOENÇA E BIOMARCADORES INFLAMATÓRIOS EM PACIENTES COM ARTRITE REUMATOIDE SUPLEMENTADOS COM OLEO DE PEIXE / ELIS CAROLINA DE SOUZA FATEL. - Londrina, 2017.
122 f. : il.

Orientador: Isaias Dichi.
Tese (Doutorado em Ciências da Saúde) - Universidade Estadual de Londrina, Centro de Ciências da Saúde, Programa de Pós-Graduação em Ciências da Saúde, 2017.
Inclui bibliografia.

1. Artrite Reumatoide - Tese. 2. Óleo de Peixe - Tese. 3. Cranberry - Tese. I. Dichi, Isaias . II. Universidade Estadual de Londrina. Centro de Ciências da Saúde. Programa de Pós-Graduação em Ciências da Saúde. III. Título.

ELIS CAROLINA DE SOUZA FATEL

A INGESTÃO DE SUCO DE CRANBERRY ACRESCENTA EFEITOS BENÉFICOS SOBRE A ATIVIDADE DA DOENÇA E BIOMARCADORES INFLAMATÓRIOS EM PACIENTES COM ARTRITE REUMATOIDE SUPLEMENTADOS COM ÓLEO DE PEIXE

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde, do Centro de Ciências da Saúde, da Universidade Estadual de Londrina para obtenção do título de Doutor.

BANCA EXAMINADORA

_____
Orientador: Prof. Dr. Isaias Dichi
Universidade Estadual de Londrina - UEL

_____
Profa. Dra. Andréa Name Colado Simão
Universidade Estadual de Londrina - UEL

_____
Profa. Dra. Edna Maria Vissoci Reiche
Universidade Estadual de Londrina - UEL

_____
Profa. Dra. Lúcia Helena da Silva Miglioranza
Universidade Estadual de Londrina - UEL

_____
Prof. Dr. Marcell Alysson Batisti Lozovoy
Universidade Estadual de Londrina - UEL

Londrina, 12 de janeiro de 2017

À minha amada mãe Carmelita de S. Fatel e a minha querida Tia Anísia Honorato de Souza (In memoriam) ...exemplos de mulheres...

AGRADECIMENTOS

Agradeço primeiramente a Deus por sempre me abençoar e por cumprir todas as promessas...

Ao meu querido esposo Helder Calsavara Ferreira, por todo apoio, por me acompanhar nas viagens até Londrina, por me ajudar nas coletas e pelas palavras de força e carinho. Sem você tudo teria sido muito mais difícil.

À minha querida e amada mãe, que sempre esteve comigo, que nunca mediu esforços para subsidiar e incentivar meus estudos, pelo exemplo de mulher, profissional e professora que é.

À minha segunda mãe, Tia Dió (Anísia H. de Souza), que auxiliou meus pais na minha criação, dedicando sua vida por nós. Sem você minha tia, eu jamais chegaria até aqui. Saudades eternas!!!

Ao meu Pai, a minha irmã Ellen e ao meu cunhado Henrique que me apoiaram durante estes quatro anos. Obrigada por me acolherem sempre com muito carinho e por torcerem sempre por mim.

Ao meu querido orientador Dr. Isaias Dichi, pela paciência, pelos conselhos, pela valiosa orientação e pela coordenação do projeto. Agradeço pela amizade demonstrada nestes 15 anos de convivência!

A minha querida Dra. Andréa Name Colado Simão, pela paciência, pelas orientações e auxílios durante as coletas, exemplo de pesquisadora e mulher.

Às minhas queridas colegas de projeto Flávia Troncon Rosa e Tatiana Mayumi Veiga Iriyoda que me auxiliaram, durante todo projeto, com muita alegria e dedicação. Sem vocês este projeto não seria possível.

A todos os professores, servidores e bolsistas do Setor de Imunologia Clínica do Laboratório de Análises Clínicas do Hospital Universitário da Universidade Estadual de Londrina, pelo auxílio durante estes 4 anos de pesquisa, em especial ao Professor Dr. Marcell Alysson Batisti Lozovoy e às acadêmicas Daniela Frizon Alfieri e Tamires Flauzino. Muito obrigada por estarem comigo nos momentos das coletas e por me auxiliarem durante todo processo de análises laboratoriais.

À Universidade Estadual de Londrina, professores e servidores do Programa de Pós-Graduação em Ciências da Saúde pela minha formação e crescimento, durantes estes 4 anos.

Aos diretores, coordenadores acadêmicos, coordenadores e professores do Curso de Nutrição da Universidade Federal da Fronteira Sul, por me incentivarem a concluir esta pós-graduação.

FATEL, ECS. **A ingestão de suco de cranberry acrescenta efeitos benéficos sobre a atividade da doença e biomarcadores inflamatórios em pacientes com artrite reumatoide suplementados com óleo de peixe**. 2017. 122f. Tese (Doutorado em Ciências da Saúde) – Universidade Estadual de Londrina, Londrina, 2017.

RESUMO

Introdução: A artrite reumatoide (AR) afeta milhões de pessoas no mundo e é considerada uma doença multissistêmica crônica, cuja causa ainda não é completamente conhecida. De forma geral, o tratamento da doença deve ter como objetivo aliviar a dor, manter ou melhorar a capacidade funcional do paciente, prevenindo, assim, as incapacidades, e melhorar a sua qualidade de vida. Nos últimos anos tem-se observado o papel importante das adipocinas, proteínas secretadas pelos adipócitos, na patogênese da AR e estudos epidemiológicos e clínicos demonstraram a eficácia da suplementação de óleo de peixe (OP) nas doenças inflamatórias. Já o consumo de suco de cranberry tem sido associado a uma redução dos biomarcadores de risco de doenças cardiovasculares com efeitos benéficos no estresse oxidativo, dislipidemia e biomarcadores inflamatórios em indivíduos saudáveis, em pacientes com diabetes mellitus tipo 2 e síndrome metabólica. **Objetivos**: descrever o papel das principais adipocinas (leptina, resistina, visfatina e adiponectina) envolvidas na fisiopatologia da AR e verificar se a ingestão de suco de cranberry poderia melhorar parâmetros clínicos e laboratoriais da atividade da doença em pacientes com AR usando suplementos de OP. **Métodos:** 1^0) Revisão de literatura com busca de artigos científicos nas principais bases de dados com as principais adipocinas envolvidas na fisiopatologia da AR; 2^0) estudo de intervenção que incluiu 62 pacientes com AR. Os pacientes foram selecionados de acordo com critérios de classificação do Colégio Americano de Reumatologia/Liga Europeia contra o Reumatismo (ACR/EULAR). O primeiro grupo (n = 21) foi orientado a manter a sua dieta habitual; o segundo grupo (n = 21) recebeu 3 g / dia de ômega 3 (n-3) e o terceiro grupo (n = 20) consumiu 3 g / dia de n-3 e 500 mL / dia de suco de cranberry de baixa caloria. O estado de atividade da doença foi determinado, por um médico reumatologista, utilizando o *Disease Activity Score 28 (*DAS28-PCR). Os marcadores inflamatórios avaliados foram a proteína C reativa de alta sensibilidade serica (hsPCR) e o fator reumatóide (FR) foram medidos usando o ensaio imunoturbidimétrico. Os níveis de anti-CCP foram testados utilizando o imunoensaio de quimiluminescência por micropartículas. Níveis de interleucina 6 (IL-6) foram medidos por ensaio de imunoabsorção enzimática (ELISA). Os níveis plasmáticos de adiponectina e leptina foram medidos utilizando uma enzima ligada por ensaio imunoenzimático (ELISA). Velocidade de Hemossedimentação (VHS) foi obtida por método cinético-fotométrica automatizado. Os dados categóricos foram analisados com o teste do qui-quadrado. Para verificar as alterações da linha de base (mudanças intra-grupo) foi utilizado o teste de Wilcoxon. O teste de Kruskal-Wallis, com teste post hoc Dunn foi realizado para comparar os valores de base e diferenças entre os grupos de tratamento (mudanças inter-grupo). Os resultados foram considerados signicativos quando $p < 0.05$. **Resultados:** O grupo que consumiu óleo de peixe e cranberry apresentou uma diminuição nos valores de VHS (p = 0,033) e PCR (p = 0,002). Além disso, esse grupo apresentou uma redução em vários outros parâmetros relacionados à atividade da doença, como os níveis de DAS28-PCR (p = 0,001), adiponectina (0,021) e IL-6 (p = 0,045). Entretanto, o grupo que consumiu óleo de peixe também apresentou uma diminuição no *escore* de atividade da doença DAS28PCR (p = 0,045) e de adiponectina (p = 0,024), mas não na VHS, PCR e IL-6. O grupo controle mostrou uma redução nos níveis de adiponectina (p = 0,017). **Conclusões**: 1^a) As implicações das alterações das adipocinas na fisiopatologia e progressão da AR ainda não são totalmente esclarecidas, porém, paradoxalmente, a adiponectina parece ter uma ação deletéria sobre as articulações de pacientes com AR. 2^a) O estudo experimental confirmou o efeito benéfico do consumo de óleo de peixe sobre a atividade da doença em pacientes com AR. Além disso, a hipótese original subjacente a este estudo, de que o suco de cranberry iria adicionar efeitos benéficos para o óleo de peixe foi confirmada tanto na diminuição da atividade da doença como na redução dos biomarcadores inflamatórios.

Palavras-chave: Artrite reumatoide, adipocinas, ácidos graxos n-3 de óleo de peixe, cranberry, poilifenóis, DAS 28

FATEL, ECS. **Cranberry juice intake adds beneficial effects on disease activity and inflammatory biomarkers in patients with rheumatoid arthritis supplemented with fish oil**. 2017. 122f. Tese (Doutorado em Ciências da Saúde) – Universidade Estadual de Londrina, Londrina, 2017.

ABSTRACT

Introduction: Rheumatoid arthritis (RA) affects millions of people worldwide and is considered a chronic multisystemic disease whose causes are not completely known. In general, the main objective of rheumatoid arthritis treatment is to improve the quality of life of patients by relieving pain, maintaining or improving functional capacity and thus preventing disability. In recent years the role of adipocytokines, the proteins secreted by the adipocytes, has been reported as an important pathophysiological mechaniism in patients with RA, whereas the epidemiological and clinical studies on the beneficial effects of fish oil in RA patients have been known for many years. On the other hand, cranberry juice consumption has been associated with a reduction in disease biomarkers of cardiovascular diseases, such as oxidative stress, dyslipidemia and inflammatory status in healthy individuals, type 2 diabetes mellitus and metabolic syndrome. **Objectives:** To describe the role of key adipokines (leptin, resistin, visfatin and adiponectin) in the pathophysiology of RA and to verify whether cranberry juice intake could improve clinical and laboratory parameters of disease activity in patients with RA using fish oil supplements. **Methods:** 1^0) Literature review with the search for scientific articles in the main data bases in relation to the pathophysiological mechanisms of adipocytokines in patients with RA; 2^0) Intervention study which included 62 patients with RA. Patients were selected according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification. The first group (n=21) was only to maintain their usual diet; the second group (n=21) received 3 g/d of fish oil *n*-3 fatty acids and the third group (n=20) consumed 3 g/d of fish oil *n*-3 fatty acids and 500 mL/d of low calorie cranberry juice. Disease activity status was determined using the DAS28-C-reactive protein (CRP). After fasting for 12h, the patients underwent the following laboratory blood analysis: Serum high sensitivity CRP (hsCRP) and rheumatoid factor (RF) were measured using immunoturbidimetric assay. Anti – citrullinated cyclic peptide antibodies (anti-CCP) levels were assayed using the chemiluminescence microparticle immunoassay. IL-6 levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA). Plasma levels of adiponectin and leptin were measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Erythrocyte sedimentation rate (ESR) was obtained by automated kinetic-photometric method. Categorical data were analyzed with chi-square test. The Wilcoxon matched pairs test was performed to verify changes from baseline (intra-group changes). The Kruskal–Wallis with post hoc Dunn's test was performed to compare baseline values and differences across treatment groups (inter-group changes). The results were considered significant when $p < 0.05$. **Results:** The group which consumed fish oil and cranberry showed a decrease in ESR (p=0.033) and CRP (p= 0.002) values. In addition, this group had a reduction in several other parameters related to disease activity, such as DAS28CRP (p=0.001), adiponectin (0.021), and IL-6 levels (p=0.045). In the meantime the group which only consumed fish oil also showed a decrease in DAS28 (p=0.045) and adiponectin levels (p=0.024), but not in ESR, CRP and IL-6. At last, the control group showed a reduction in adiponectin levels (p=0.017). **Conclusion** 1^a) The implications of adipocytokines changes in RA pathophysiology are not completely clear yet, but paradoxically adiponectin seems to have a deleterious action in the joints of RA patients.
2^a) The present study confirmed previous reports on disease activity of fish oil in patients with RA. In addition, the original hypothesis which underlies this study, that cranberry juice would add beneficial effects to fish oil was confirmed both in the robust findings of decreasing disease activity and also by inflammatory biomarkers results.

Key Word: Rheumatoid arthritis, adipokines, fish oil n-3 fatty acids, cranberry, polyphenols, DAS 28.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

AA	Ácido araquidônico
ACR	*American College of Rheumatism*
AdipoQ	Adiponectina
AgRP	*Agouti-related peptide*
AGT	Angiotensinogênio
ALA	Ácido alfa linolênico
Anti-CCP	Anticorpos antipeptídeo citrulinado cíclico
AR	Artrite reumatoide
ASC	*Adipose-derived stem cells*
BMI	M*ass index*
CDAI	*Clinical Disease Activity Index*
CRP	*C-reactive protein*
CVD	C*ardiovascular diseases*
DAS 28	*Disease Activity Score 28*
DHA	Ácido docosahexaenóico
DMARD	*Anti rheumatic drugs*
EPA	Ácido eicosapentaenóico
ESH	*Erythrocyte sedimentation rate*
EULAR	*European League Against Rheumatology*
FR	Fator reumatóide
HAQ	*Health Assessment Questionnaire*
HDL	*High- Density Lipoprotein*
HMW	*High molecular weight*
hsPCR	Proteína C Reativa de alta sensbilidade
IL	Interleucina
IMC	Índice de massa corpórea
Kcal	Quilocaloria
KJ	Quilo Joule
LA	Ácido linoleico
LDL	*Low -Density Lipoprotein*
LMW	*Low molecular weight*
LT	Leucotrieno

MCP 1	*Proteína Quimioatraente a monócitos-1*
MetS	*Metabolic syndrome*
MMP	*Matrix metalloproteinase*
N-3	Ômega-3
N-6	Ômega-6
NF-KB	*Factor nuclear kappa B*
NO	*Nitric oxide*
PAI-1	Inibidor de ativação do plasminogénio 1
PCR	Proteína C reativa
PG	Prostaglandina
PPARγ	*Peroxisome Proliferator-Activated Receptor Gamma*
PUFAs	*Polyunsaturated Fatty Acids*
RA	*Rheumatoid Arthritis*
RA-FLS	*Fibroblast-like synoviocytes*
SDAI	*Simplified Disease Activity Index*
SFRP5	*Secreted Frizzled-Related Protein 5*
SM	Síndrome metabólica
Th	T *helper*
TNF-α	*Tumor necrosis factor alpha*
VHS	Velocidade de hemossedimentação

SUMÁRIO

SUMÁRIO

1 INTRODUÇÃO

1.1 Artrite Reumatoide

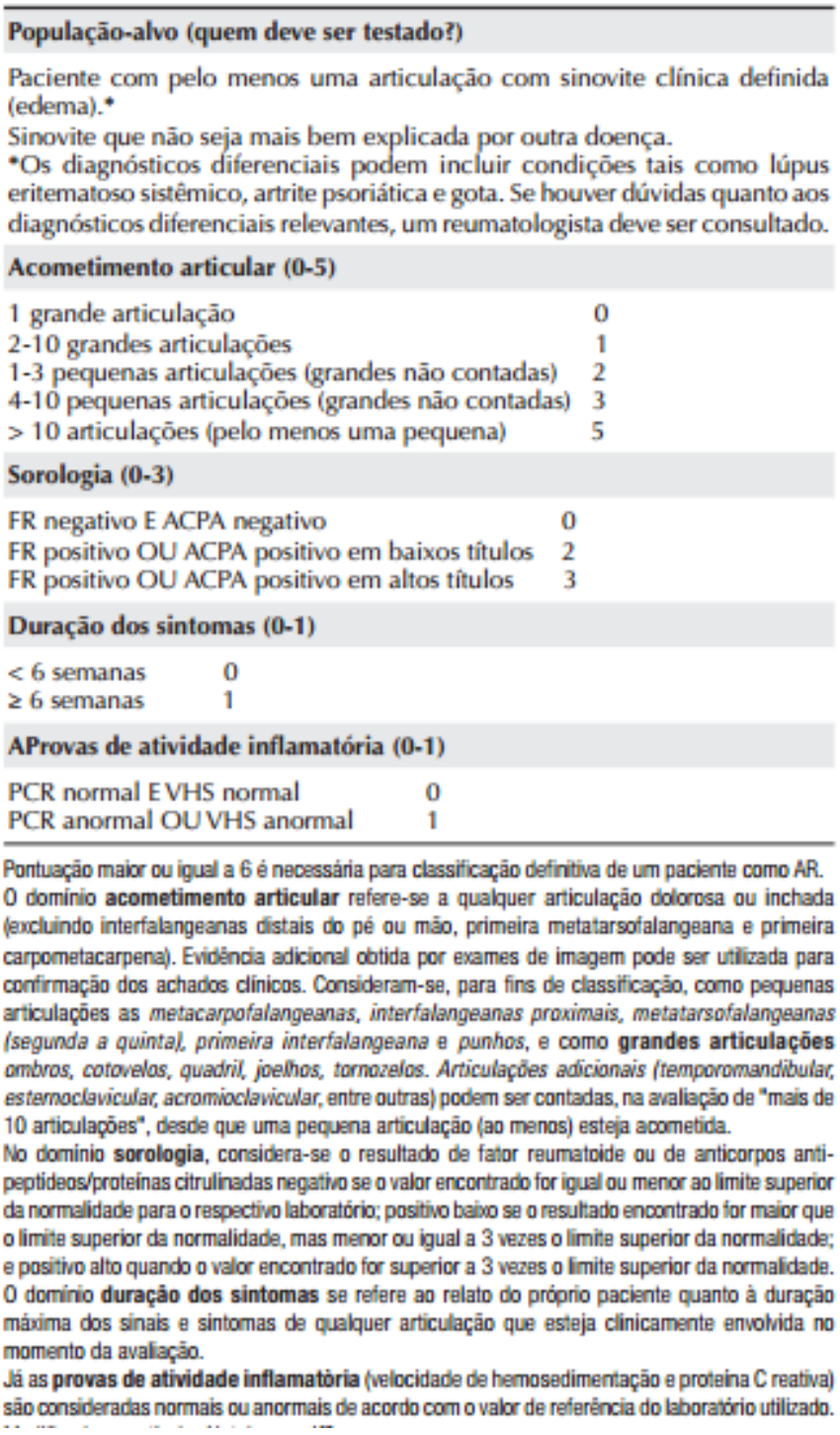
A artrite reumatoide (AR) é uma doença inflamatória crônica caracterizada por hiperplasia sinovial, destruição progressiva das articulações (NEUMANN et al., 2010; MCINNES; SCHETT, 2011; KUMAR et al., 2016) e produção de autoanticorpos fator reumatoide (FR) e anticorpo antipeptídeo citrulinado cíclico (Anti-CCP) (SCHELLEKENS et al.,2000). É considerada uma doença multissistêmica crônica, cuja causa ainda não está totalmente esclarecida. No entanto as evidências apontam para uma interação de fatores genéticos, imunológicos e ambientais (LIPSKY, 1998; IWASAKI; YAMAMOTO, 2016)

A AR afeta milhões de pessoas no mundo e apresenta prevalência de 0,5 a 1,0% (LAWRENCE et al., 1998; MYASOEDOVA et al., 2010). A prevalência encontrada no Brasil mostrou-se semelhante em amostras populacionais (MARQUES NETO et al., 1993; MOTA et al., 2011). Além disso, 86,0% dos pacientes atendidos com AR em ambulatórios de serviços universitários, em São Paulo, eram mulheres entre a quarta e quinta década (LOUZADA-JUNIOR et al., 2007).

O diagnóstico da AR é realizado considerando-se dados clínicos e exames complementares. As manifestações mais frequentes da AR são envolvimento articular, queixas de dor, inchaço e limitação dos movimentos das articulações acometidas; acometimento poliarticular (mais de 4 articulações); acometimento dos punhos e articulações metacarpofalangeanas e interfalangeanas proximais; artrite simétrica e aditiva e rigidez matinal caracterizada por enrijecimento e sensação de inchaço percebida, em especial, pela manhã, com duração de mais de 1 hora (WOOLF, 2003; MOTA et al., 2011). Além disso, em pacientes mais graves, observam-se sintomas gerais como febre, astenia, fadiga, mialgia, perda ponderal e manifestações extra articulares, que incluem quadros cutâneos, oculares, pleuropulmonares, cardíacos, hematológicos, neurológicos e osteometabólicos. Estes pacientes podem apresentar sorologia positiva para FR ou anti-CCP e nódulos reumatoides (TURESSON et al., 2007; GOELDNER et al., 2011).

Inicialmente, a classificação da AR era baseada nos critérios introduzidos pelo Colégio Americano de Reumatologia (ACR), em 1987, que não apresentavam boa performance na AR inicial. Por isso, tornou-se necessário o estabelecimento de novos critérios de classificação para a AR, com enfoque na fase precoce da doença. Os novos critérios classificatórios para AR 2010 estabelecidos em conjunto pelo ACR e pela Liga Europeia contra o Reumatismo (EULAR) (ACR/EULAR) (MOTA et al., 2011), estão apresentados na figura 1.

Figura 1 - Critérios classificatórios para AR 2010 ACR/EULAR



População-alvo (quem deve ser testado?)	
Paciente com pelo menos uma articulação com sinovite clínica definida (edema).*	
Sinovite que não seja mais bem explicada por outra doença.	
*Os diagnósticos diferenciais podem incluir condições tais como lúpus eritematoso sistêmico, artrite psoriática e gota. Se houver dúvidas quanto aos diagnósticos diferenciais relevantes, um reumatologista deve ser consultado.	
Acometimento articular (0-5)	
1 grande articulação	0
2-10 grandes articulações	1
1-3 pequenas articulações (grandes não contadas)	2
4-10 pequenas articulações (grandes não contadas)	3
> 10 articulações (pelo menos uma pequena)	5
Sorologia (0-3)	
FR negativo E ACPA negativo	0
FR positivo OU ACPA positivo em baixos títulos	2
FR positivo OU ACPA positivo em altos títulos	3
Duração dos sintomas (0-1)	
< 6 semanas	0
≥ 6 semanas	1
AProvas de atividade inflamatória (0-1)	
PCR normal E VHS normal	0
PCR anormal OU VHS anormal	1

Pontuação maior ou igual a 6 é necessária para classificação definitiva de um paciente como AR.
O domínio **acometimento articular** refere-se a qualquer articulação dolorosa ou inchada (excluindo interfalangeanas distais do pé ou mão, primeira metatarsofalangeana e primeira carpometacarpena). Evidência adicional obtida por exames de imagem pode ser utilizada para confirmação dos achados clínicos. Consideram-se, para fins de classificação, como pequenas articulações as *metacarpofalangeanas, interfalangeanas proximais, metatarsofalangeanas (segunda a quinta), primeira interfalangeana* e *punhos*, e como **grandes articulações** *ombros, cotovelos, quadril, joelhos, tornozelos. Articulações adicionais (temporomandibular, esternoclavicular, acromioclavicular*, entre outras) podem ser contadas, na avaliação de "mais de 10 articulações", desde que uma pequena articulação (ao menos) esteja acometida.
No domínio **sorologia**, considera-se o resultado de fator reumatoide ou de anticorpos anti-peptídeos/proteínas citrulinadas negativo se o valor encontrado for igual ou menor ao limite superior da normalidade para o respectivo laboratório; positivo baixo se o resultado encontrado for maior que o limite superior da normalidade, mas menor ou igual a 3 vezes o limite superior da normalidade; e positivo alto quando o valor encontrado for superior a 3 vezes o limite superior da normalidade.
O domínio **duração dos sintomas** se refere ao relato do próprio paciente quanto à duração máxima dos sinais e sintomas de qualquer articulação que esteja clinicamente envolvida no momento da avaliação.
Já as **provas de atividade inflamatória** (velocidade de hemosedimentação e proteína C reativa) são consideradas normais ou anormais de acordo com o valor de referência do laboratório utilizado.

Fonte: Mota et al. (2011).

Após o diagnóstico, Mota et al. (2011), reforçam que é de extrema importância caracterizar os parâmetros que serão úteis para acompanhar a atividade da doença, tais como escalas visuais da dor pelo paciente, da atividade de doença pelo paciente e pelo médico, número de articulações dolorosas e edemaciadas, instrumentos de avaliação da capacidade

funcional, como o *Health Assessment Questionnaire (*HAQ), provas inflamatórias (velocidade de hemossedimentação (VHS) e/ou Proteína C Reativa (PCR)), fadiga, duração da rigidez matinal, radiografia de mãos, punhos e pés e índices de qualidade de vida (SCOTT et al., 1991; VAN DER HEIJDE et al., 1992; GOLDSMITH et al., 1993; FELSON et al., 1993; BOERS et al., 1994).

Com esses parâmetros foi possível criar e validar alguns índices para avaliar a atividade da doença (figuras 2 e 3). Os principais índices são: o índice de atividade de doença - DAS28 (*Disease Activity Score* 28); o índice simplificado de atividade de doença - SDAI (*Simplified Disease Activity Index*) e o índice clínico de atividade de doença - CDAI (*Clinical Disease Activity Index*) (VAN DER HEIJDE et al., 1993; VAN GESTEL; HAAGSMA; VAN RIEL, 1998; SMOLEN et al., 2003; SIEGEL; ZHEN, 2005; ALETAHA et al., 2005; ALETAHA et al., 2009).

Figura 2 - Cálculo para valor dos índices de atividade da doença.

Elementos	SDAI	CDAI	DAS28 (com 4 variáveis)
Contagem de articulações edemaciadas	(0-28) Soma simples	(0-28) Soma simples	Raiz quadrada da soma simples
Contagem de articulações dolorosas	(0-28) Soma simples	(0-28) Soma simples	Raiz quadrada da soma simples
Reagentes de fase aguda	PCR (0,1 - 10 mg/dL)	—	VHS 2-100 mm ou PCR 0,1-10 mg/dL transformação logarítmica
Avaliação global saúde (Paciente)		—	0-100 mm
Avaliação de atividade de doença (Paciente)	(0-10 cm)	(0-10 cm)	—
Avaliação de atividade de doença (Avaliador)	(0-10 cm)	(0-10 cm)	—
Índice total (Variação do índice)	Soma simples (0,1-86)	Soma simples (0-76)	Requer inserir o número na calculadora (0,49-9,07)

SDAI: Índice simplificado de atividade de doença; CDAI: índice clínico de atividade de doença; DAS28: índice de atividade de doença (28 articulações); PCR: proteína C reativa; VHS: velocidade de hemossedimentação. Assumindo uma variação entre 2 e 100 mm/h para a VHS e entre 0,1 e 10 mg/dL para a PCR.

Fonte: Mota et al. (2011).

Figura 3 - Pontes de corte par avaliar a atividade da doença.

Índice	Estado da atividade de doença	Pontos de corte
SDAI	Remissão	≤ 5
	Baixa	> 5 e ≤ 20
	Moderada	> 20 e ≤ 40
	Alta	> 40
CDAI	Remissão	≤ 2,8
	Baixa	≤ 10
	Moderada	> 10 e ≤ 22
	Alta	> 22
DAS28	Remissão	≤ 2,6
	Baixa	> 2,6 e ≤ 3,2
	Moderada	> 3,2 e ≤ 5,1
	Alta	> 5,1

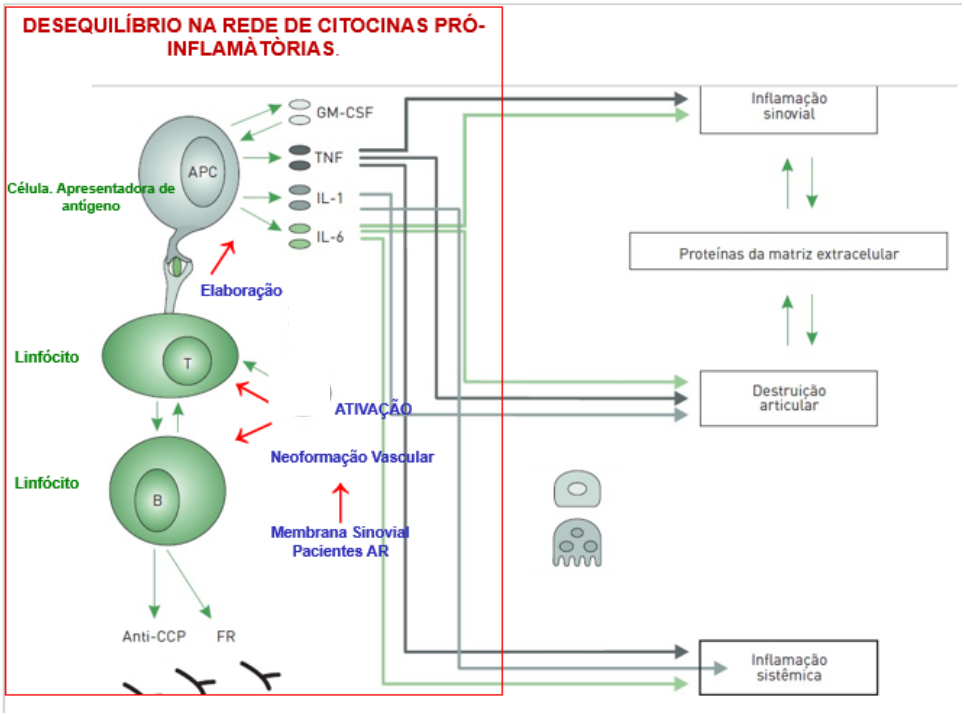
SDAI: Índice simplificado de atividade de doença; CDAI: índice clínico de atividade de doença; DAS28: índice de atividade de doença (28 articulações); modificado a partir de Aletaha *et al.*[83]

Fonte: Mota el al. (2011)

1.2 Inflamação e Artrite Reumatoide

As citocinas desempenham um papel importante no processo de inflamação, destruição articular, e nas comorbidades associadas em pacientes com AR. Os pacientes com AR possuem células B e T ativadas na membrana sinovial (figura 4), organizadas em estruturas de centro germinativo semelhante, células plasmáticas, mastócitos e macrófagos ativados, particularmente todos recrutados por meio de um processo de neovascularização intensa com linfangiogenese associada. As células do tecido hospedeiro (fibroblastos sinoviais, condrócitos e osteoclastos), são envolvidas para manter a perpetuação da inflamação e destruição do osso. Isso tudo é conduzido por uma rede de citocinas. (BRENNAN; MCINNES, 2008).

Figura 4 - Processo inflamatório e destruição articular na AR.



APC, célula apresentadora de antígeno; T, linfócito T; B, linfócito B; FR, fator reumatoide; TNF, fator de necrose tumoral alfa; GM-CSF, fator estimulador de colônia de granulócito-macrófago; IL-1, interleucina-1; IL-6, interleucina-6; IL-17, interleucina-17; anti-CCP, anticorpos antipeptídeos/proteínas cíclicos.

Fonte: Adaptado, 2016 (http://www.medicinanet.com.br.) Adaptado de Fochesatto Filho et al., 2013.

As doenças inflamatórias e autoimunes, tais como AR, podem ser causadas por um desequilíbrio na rede de citocinas. As citocinas pró-inflamatórias, tais como o fator de necrose tumoral (TNF-α), interleucina (IL) -1, IL-6 e IL-17 induzem a inflamação das articulações e a

destruição de osso e cartilagem através da ativação de macrófagos, fibroblastos como sinoviócitos, células T *helper* (Th) e osteoclastos (KANEKO et al., 2016).

Esta inflamação persistente pode provocar outras manifestações, com comprometimento sistêmico, envolvendo quase todos os sistemas e órgãos, com graves complicações e morbidades. As principais complicações são anemia, aterosclerose, doença vascular e osteoporose e as principais comorbidades são: síndrome de Sjögren, fibrose pulmonar intersticial difusa, vasculite necrosante de pequenas artérias, que podem agravar a disfunção social e gerar morte prematura (BRANIMIR ANIĆ; MIROSLAV MAYER, 2014; NURMOHAMED; HESLINGA; KITAS, 2015). Esta inflamação pode desenvolver uma lesão articular irreversível, gerar deficiência física, além da redução da expectativa de vida (SEMERANO et al., 2016).

Além disso, estudo revela altos índices de síndrome metabólica (SM) diagnosticada nestes pacientes, como realizado em um hospital universitário do Nordeste Brasileiro com prevalências de SM acima de 50,0% nos pacientes avaliados (OLIVEIRA et al., 2016).

Estudos sugerem que o estado pró-inflamatório na AR pode contribuir para o desenvolvimento da SM (BORGES et al., 2007; CHUNG et al., 2008).

A SM é um conjunto de distúrbios cardiometabólicos que resultam do aumento da obesidade. Os principais elementos da SM são a resistência à insulina, obesidade central, dislipidemia e hipertensão, aumentando, assim, o risco de doenças cardiovasculares. Pacientes com doenças reumáticas, tais como AR, possuem maior prevalência de risco de doenças cardiovasculares (PICERNO et al., 2015; SKEOCH; BRUCE, 2015; NAKAJIMA, 2016). Este risco ainda é maior quando a obesidade se faz presente nestes pacientes.

Um estudo relatou que a prevalência de obesidade foi associada com o aumento da incidência e gravidade de AR (SCRIVO et al., 2013) e que a crescente prevalência de obesidade podia ser responsável por grande parte do aumento da incidência de AR (CROWSON et al., 2013). Confirmando esta última hipótese, pesquisadores demonstraram em uma meta-análise que um aumento no índice de massa corporal (IMC) contribuía para um maior risco de desenvolvimento de AR (QIN et al., 2015). Recentemente, um estudo demostrou que os pacientes com AR com diagnóstico de obesidade tinham parâmetros de maior atividade da doença (ELLABBAN et al., 2016).

Nos últimos anos, estudos demonstram que pode haver uma ligação entre alteração de secreção de adipocinas pró-inflamatórias presentes na obesidade e na SM com as doenças cardiovasculares e doenças reumáticas (ABELLA et al., 2014) e que durante o curso da AR, as manifestações clínicas que constituem a SM devem ser monitoradas (KEREKES et al., 2014).

1.2.1 Adipocinas e Artrite Reumatoide

O tecido adiposo é um componente estrutural de muitos órgãos, como, por exemplo, as articulações, trato intestinal e pele (NEUMANN et al., 2011). É um tipo de tecido com múltiplas funções (TRAYHURN; WOOD, 2004; NEUMANN et al., 2011). Como o armazenamento de lipídeos, funções endócrinas e secretoras, além da termogênese e proteção das vísceras contra choques mecânicos. Ressalta-se neste contexto seu papel como órgão endócrino, por sintetizar e secretar as adipocinas, proteínas secretadas pelos adipócitos, e desempenhando importantes funções na fisiopatologia da resistência à insulina, processo inflamatório e aterogênese (TRAYHURN; WOOD, 2004). Estas funções possibilitam uma grande interação deste tecido com outros órgãos e células adiposas (WAJCHENBERG, 2000).

As adipocinas desempenham funções imunológicas, cardiovasculares, metabólicas e endócrinas. As adipocinas envolvidas nas funções imunológicas são a IL-6, TNF-α e fatores do complemento B e C3 e D (adipsina). Estas são capazes de modular a inflamação ativa e o sistema imune inato (NEUMANN et al., 2011). Em relação ao desempenho cardiovascular, podem-se citar as moléculas do eixo renina/angiotensina e o inibidor de ativação do plasminogênio (PAI-1). Já entre as moléculas com funções metabólicas destacam-se as relacionadas à homeostasia energética, como os ácidos graxos livres, tais como adiponectina (AdipoQ), resitina, visfatina e *Agouti-related peptide* (AgRP). Em relação às funções endócrinas, os autores destacam o importante papel da leptina na regulação do armazenamento de energia (COSTA; DUARTE, 2006).

Há fortes evidências de que as adipocinas possuem funções exógenas relacionada à patogênese da obesidade, da SM e das doenças imunoinflamatórias (FANTUZZI, 2005; HUTCHESON, 2015).

As adipocinas pró-inflamatórias descritas na literatura são: leptina, resistina, visfatina, IL-6, TNF-α, adipsina e angiotensinogênio (AGT). Já as adipocinas anti-inflamatórias são: AdipoQ, IL-10 e a *Secreted Frizzled-Related Protein* 5 ou SFRP5 (SIPPEL et al., 2014).

Ressalta-se que as principais adipocinas relacionadas a estudos com AR são a leptina, AdipoQ, visfatina e resistina (BARBOSA; RÊGO; ANTÔNIO, 2012), além da adipocina denominada Chemerin (FATIMA et al., 2014), Lipocalin2, amilóide no soro A3, vaspin, omentina e adipsina (DEL PRETE; SALVI; SOZZANI, 2014).

Apesar dos estudos, nos últimos anos demonstrarem as implicações das adipocinas na fisiopatologia de doenças autoimunes, incluindo AR, ainda não é claro seu papel na patogênese da progressão da doença (DEL PRETE; SALVI; SOZZANI, 2014)

Neste contexto, as adipocinas leptina e AdipoQ merecem destaque, visto os

inúmeros estudos realizados demonstrando suas ações na patogênese da AR. Estudos com a leptina vêm demonstrando o importante papel desta adipocina na patogênese da AR (TOUSSIROT et al., 2015; SCOTECE et al., 2014; SKALSKA; KONTNY, 2016). Foi descoberta em 1994 (ZHANG et al., 1994) e é produzida no tecido adiposo branco (GUIMARÃES et al., 2007). Considerada uma adipocina pró-inflamatória, a leptina estimula a produção de citocinas pró-inflamatórias, tais como TNF-α, IL-6 e espécies reativas de oxigênio, induz a produção de quimiocinas CC por macrófagos e altera o equilíbrio Th1/Th2 (DEL PRETE; SALVI; SOZZANI, 2014). Apesar de ser considerada uma adipocina pró-inflamatória por induzir a produção de citocinas pró-inflamatórias, mais estudos são necessários para elucidar seu real efeito na patogênese da AR.

A AdipoQ é uma proteína de 244 aminoácidos produzida e secretada, principalmente, pelos adipócitos (SCHERER et al., 1995; GARAULET et al., 2007). Apesar de ser considerada uma adipocina anti-inflamatória na patogênese da obesidade, diabetes melittus tipo 2, aterosclerose e SM, (ROBINSON; PRINS; VENKATESH, 2011; OHASHI; OUCHI; MATSUZAWA, 2012; FANTUZZI, 2013; ABELLA et al., 2014), estudos têm demonstrado que na patogênese da AR, esta adipocina desempenha um papel pró-inflamatório (GILES et al., 2009; BROCHU-GAUDREAU et al., 2010; FERRACCIOLI; GREMESE, 2011; GILES; VAN DER HEIJDE; BATHON, 2011; SCOTECE et al., 2012; CHEN et al., 2013).

1.3 Tratamento da AR

O tratamento do paciente com AR deve ter como objetivo aliviar a dor, manter ou melhorar a capacidade funcional do paciente, prevenindo, assim, as incapacidades e melhorar a qualidade de vida dos mesmos (CALABRÒ et al., 2016)**.** O tratamento pode ser dividido em medicamentoso e não medicamentoso. Os medicamentos utilizados para o tratamento da AR são anti-inflamatórios não hormonais; corticosteroides e drogas antirreumáticas modificadoras da doença, além da terapia biológica (AMERICAN COLLEGE OF RHEUMATOLOGY SUBCOMMITTEE ON RHEUMATOID ARTHRITIS GUIDELINES, 2002). Já o tratamento não medicamentoso inclui repouso, apoio psicológico, educação do paciente e de sua família, utilização de terapia ocupacional e abordagens cirúrgicas (MOTA et al., 2011) e terapias alternativas complementares, como ioga, acupuntura, massagem terapêutica *e tai chi* (KUMAR et al., 2016).

Apesar dos vários tratamentos existentes, muitos pacientes não apresentam boa resposta terapêutica. Por isso, novas terapias são urgentemente necessárias (SMOLEN et al., 2016). Neste contexto, terapias não invasivas como as intervenções nutricionais pela suplementação alimentar, podem ser consideradas como uma boa estratégia coadjuvante na

AR.

1.3.1 Intervenção nutricional e Artrite Reumatoide

1.3.1.1 Óleo de peixe

Os ácidos graxos essenciais (não sintetizados pelos humanos) foram descobertos em 1929 (O. BURR, 1981; KINSELLA, 1991) por George Burr e Mildred Brurr pelo do achado de uma nova deficiência nutricional. O casal descreveu em seu estudo com ratos que quando as gorduras foram eliminadas da dieta por vários meses, mas a quantidade de alimento não foi alterada, os ratos desenvolveram pele escamosa e caudas inflamadas. As patas traseiras avermelhavam e às vezes edemaciavam. Os animais perderam pele ao redor da face e garganta com o aparecimento de feridas. Com a continuação da dieta sem gordura, os animais começaram a perder peso e, dentro de três ou quatro meses morreram. Quando foram autopsiados, os autores observaram que os rins dos animais e o trato urinário apresentavam sinais significativos de danos. Os pesquisadores demostraram que as vitaminas adicionadas não ajudaram os animais a se recuperar da síndrome, mas que a adição de apenas três gotas de banha foi suficiente para ajudar o animal a se recuperar. Demostraram também que o ácido linoleico n-6 era um ácido graxo poli-insaturado essencial (*Polyunsaturated Fatty Acids* (PUFAS)) e que era necessário apenas em pequenas quantidades para manutenção da saúde. Esta pesquisa os levou a identificar o ácido linoleico como nutriente essencial (O. BURR, 1981; SMITH e MUKHOPADHYAY, 2012).

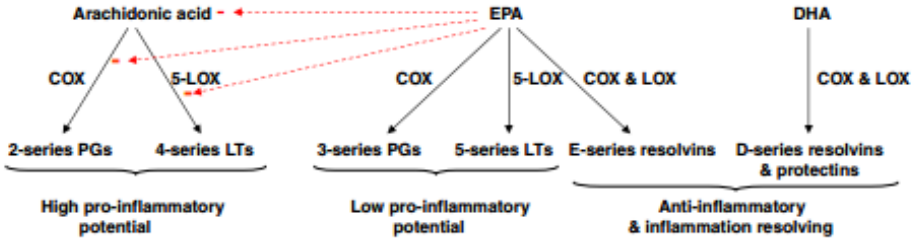
Durante muitos anos os pesquisadores da área acreditaram que o ácido linoleico era o único ácido graxo essencial. Em meados da década de 1990, a Organização Mundial de Saúde determinou que a fórmula infantil deveria conter uma distribuição de ácidos graxos mais parecida com o leite humano, que, além de ácidos graxos n-6, continha outros PUFAS de cadeia longa tais como o ômega-3 (n-3) (SMITH e MUKHOPADHYAY, 2012).

Os ácidos graxos n-3 e n-6 consistem de PUFAS contendo de 18 a 22 carbonos (SUÁREZ-MAHECHA et al., 2002). Os principais ácidos graxos n-3 são: o ácido alfa linolênico (ALA) 18:3, o ácido eicosapentaenoico (EPA) 20:5 e o ácido docosahexaenoico (DHA) 22:6, enquanto os principais n-6 são o ácido linoleico (LA) 18:2 e o ácido araquidônico (AA) 20:4 (KINSELLA; LOKESH; STONE, 1990; MAYSER et al., 1998; SUÁREZ-MAHECHA et al., 2002).

Entre as fontes de ácidos graxos essenciais, o óleo de peixe merece destaque, pois possui quantidades significativas de EPA e DHA, sendo esses ácidos graxos os principais na proteção da saúde (HARRIS, 1999). Estudo clínico demonstrou a eficácia da suplementação

de óleo de peixe nas doenças inflamatórias (BARBOSA et al., 2003), e em particular na AR (BERBERT et al., 2005; JAMES; PROUDMAN; CLELAND, 2010; MILES; CALDER, 2012; PARK et al., 2013; RAJAEI et al., 2016). O principal mecanismo de ação dos PUFAS n-3 é a inibição dos metabólicos do AA, em especial o leucotrieno (LT) B4 produzido por meio da via lipoxigenase (figura 5). Salienta-se que a suplementação com óleo de peixe inibe tanto a produção de leucotrieno LTB4 quanto a de citocinas pró-inflamatórias, como IL-1 e TNF-α (JAMES; GIBSON; CLELAND, 2000; KOLAHI et al., 2010). Além disso, n-3 reduz a produção de outros eicosanoides como a prostaglandina (PG) E2 e enzimas de degradação da cartilagem (CLELAND et al., 2006), diminuindo, assim a inflamação (RONTOYANNI et al., 2012).

Figura 5 - Síntese e ações de mediadores lipídicos produzidos a partir de ácido araquidônico, EPA e DHA.

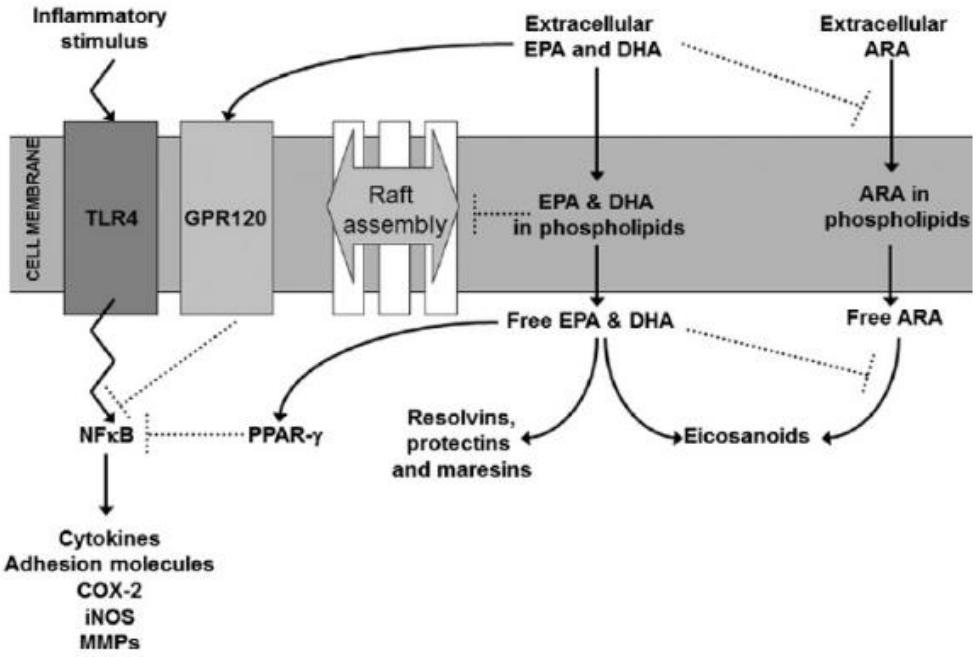


COX, cyclooxygenase; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin.

Fonte: Calder, (2011)

O n-3 é capaz de inibir parcialmente certo número de eventos que envolvem a inflamação, incluindo a quimiotaxia de leucócitos, a expressão de moléculas de adesão molecular e interações adesivas de leucócitos-endotélio; além disso, o n-3 pode inibir o fator de transcrição nuclear pró-inflamatório NFκB em vários tecidos, mediado pelo receptor-γ ativado pelo *Peroxisome Proliferator-Activated Receptor Gamma* (PPARγ) e muitas outras proteínas sinalizadoras (figura 6) (CALDER, 2015).

Figura 6 - Representação da inter-relação entre as principais ações anti-inflamatórias de ácidos graxos n-3.



ARA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GPR, G-protein coupled receptor; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; NF κ B, nuclear factor κ B, PPAR, peroxisome proliferator activated receptor. Dotted lines indicate inhibition.

Fonte: Calder ,2015

Apesar dos mecanismos pelos quais os n-3 exercem os seus efeitos benéficos ainda não serem totalmente conhecidos (NORLING; PERRETTI, 2013), os benefícios mais evidentes da utilização de PUFAS n-3 de óleo de peixe são a redução da dor articular e do edema, a diminuição da duração da rigidez matinal e a melhora da avaliação global da dor e da atividade da doença (BERBERT et al., 2005; MILES; CALDER, 2012), e possível redução no consumo de analgésicos (RAJAEI et al., 2016). Por isso, o óleo de peixe pode representar um valioso apoio ao tratamento farmacológico tradicional da AR (CLELAND et al., 2006; SALES; OLIVIERO; SPINELLA, 2008a; SALES; OLIVIERO; SPINELLA, 2008b; JAMES; PROUDMAN; CLELAND, 2010). Outra ação do óleo de peixe está relacionada à redução do risco cardiovascular. Esse risco cumpre ressaltar, tem aumentado em pacientes com AR (PROUDMAN; CLELAND; JAMES, 2008).

1.3.1.2 Cranberry

Cranberry (V*accinium macrocarpon*) é uma planta ericácea cultivada na América do Norte desde o início do século 19, sendo originária do norte dos Estados Unidos e Canadá (MARCHAND et al., 2013). O cranberry foi utilizado, inicialmente, por índios, para tratar infecções do trato urinário (GUAY, 2009; MARCHAND et al., 2013). Os nativos norte-americanos utilizavam este fruto para tratar feridas, indigestão, higiene oral e dentária, além de pedras nos rins e outros problemas urinários (DUTHIE et al., 2005; DESSÌ; ATZEI; FANOS, 2011). Os principais componentes do cranberry são os flavonoides, catequinas, triterpenóides, ácidos orgânicos, vitaminas (A e C), carboidratos, sais minerais e taninos tais como as proantocianidinas (DESSÌ; ATZEI; FANOS, 2011). Pode ser consumido como frutas secas, molhos ou suco (MARCHAND et al., 2013).

Em estudo realizado em 2001, que avaliou o teor de fenóis totais em 20 frutas mais consumidas na dieta americana, observou-se que o cranberry apresentou maior teor de fenóis totais, ficando muito à frente da uva vermelha (VINSON et al., 2001).

Estudos, como realizado por Dessì et al. (2011), vêm demonstrando a ação benéfica da ingestão do suco de cranberry na prevenção de infecções do trato urinário. Apesar dos benefícios na profilaxia de infecções urinárias recorrentes serem evidentes, os pesquisadores alertam que ainda não é clara qual a dosagem correta a utilizar (PINA et al., 2011). Em estudo recente, pesquisadores demonstraram que o consumo de suco de cranberry reduziu o número de episódios clínicos de infecção do trato urinário em mulheres com uma história recente da doença (MAKI et al., 2016). Em outro estudo, observaram que, entre as mulheres submetidas a cirurgia ginecológica eletivas e benignas, com cateterismo urinário, o uso de cápsulas de extrato de cranberry durante o período pós-operatório reduziu em 50% a taxa de infecção do trato urinário (FOXMAN et al., 2015).

Simão et al. (2013) realizaram uma pesquisa com objetivo de avaliar o efeito da ingestão do suco de cranberry de baixa caloria em biomarcadores metabólicos e inflamatórios em pacientes com SM. Observaram que após o consumo de 700 mL de suco, por 60 dias, houve aumento significativo dos níveis de AdipoQ e de ácido fólico, diminuição da homocisteína e redução dos níveis de lipoperoxidação e oxidação de proteínas, porém não foram observadas alterações significativas nas citocinas pró-inflamatórias TNF-α, IL-1 e IL-6, concluindo que o consumo de suco de cranberry auxiliou na melhora de vários fatores de risco cardiovasculares (SIMÃO et al., 2013).

A ingestão de suco de cranberry pode ocasionar a melhora de vários fatores de risco de doenças cardiovasculares, em adultos, incluindo triglicerídeos circulantes, PCR, glicose, resistência à insulina e pressão arterial diastólica (NOVOTNY et al., 2015). A ingestão de

polifenóis oriundos do cranberry pode desempenhar um papel importante na promoção de marcadores anti-inflamatórios entre os consumidores de suco de cranberry, pois uma pesquisa realizada recentemente demostrou que os consumidores deste coquetel tinham níveis significativamente mais baixos de PCR (DUFFEY; SUTHERLAND, 2015). Já em relação à função vascular, Rodriguez-Mateos e colaboradores, concluíram que pela alta presença de compostos bioativos, em especial os polifenóis, o suco de cranberry pode melhorar a função vascular em homens saudáveis (RODRIGUEZ-MATEOS et al., 2016).

Outra pesquisa realizada recentemente demonstrou que os componentes presentes no cranberry podem ser úteis, como agente terapêutico modulador, para prevenir ou tratar artropatias inflamatórias da articulação temporomandibular. Os pesquisadores observaram que estes componentes inibiram a IL-1β e a produção de fator de crescimento endotelial vascular em todas as linhas celulares (TIPTON; CHRISTIAN; BLUMER, 2016).

2 JUSTIFICATIVA

Assim, considerando:

1°) A alta prevalência da AR, suas sequelas articulares e seus efeitos sobre a qualidade de vida dos pacientes;

2°) O papel ainda não totalmente definido sobre as adipocinas na fisiopatologia da AR;

3°) Os efeitos benéficos da suplementação com óleo de peixe em pacientes com AR;

4°) Os efeitos anti-inflamatórios do suco de cranberry em algumas doenças;

5°) A inexistência de artigos que tenham verificado a ação do suco de cranberry em pacientes com AR;

Colocamos, a seguir, os objetivos do presente trabalho.

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar se o consumo de suco de cranberry de baixa caloria poderia ter efeito aditivo à suplementação com óleo de peixe sobre a atividade da doença e o processo inflamatório em pacientes com artrite reumatoide.

3.2 Objetivos Específicos

Investigar o papel das adipocinas na fisiopatologia da AR;

Avaliar o efeito da ingestão do suco de cranberry associado ao uso de óleo de peixe nos marcadores inflamatórios e atividade da doença em pacientes com AR;

Avaliar parâmetros clínicos e laboratoriais de pacientes com AR antes e após 90 dias do uso de óleo de peixe associado ao suco de cranberry.

4 METODOLOGIA

4.1 Revisão da Literatura

Para a revisão da literatura foram utilizadas as seguintes bases de dados: *U.S. National Library of Medicine PUBMED*, *Science Direct*, e *Scientific Electronic Library Online*.

4.2 Delineamento do Estudo

Esse projeto é um estudo clínico, de intervenção nutricional, envolvendo pacientes com AR, atendidos no ambulatório de Reumatologia do Hospital Universitário de Londrina, Paraná, Brasil

4.3 Pacientes

Foram incluídos no estudo 62 pacientes (50 mulheres e 12 homens) com AR. O diagnóstico de AR foi definido de acordo com os critérios do (ACR de 1987 e/ou novos critérios classificatórios ACR/EULAR 2010 (MOTA et al., 2011). Informações sobre os fatores de estilo de vida e história médica foram obtidos em avaliação clínica. (APÊNDICE A).

Nenhum dos participantes do estudo apresentou doenças cardíacas, tireóideas, renais, hepáticas, gastrointestinais ou oncológicas, e nenhum estava recebendo terapia de reposição de estrogênio, drogas hipolipemiantes ou hipoglicemiantes ou suplementos antioxidantes.

Os critérios utilizados para a divisão dos grupos foram as variáveis sexo e atividade da doença e medicamentos utilizados para o tratamento da doença. Como critério de inclusão optou-se por pacientes que não faziam uso de imunobiológicos inibidores de TNF- α.

Os pacientes com AR foram divididos em três grupos: pacientes que não usaram nenhuma suplementação, definidos como grupo controle (Grupo C, n = 21), pacientes que fizeram ingestão de óleo de peixe, definidos como grupo óleo de peixe (Grupo OP, n = 21) e pacientes que fizeram ingestão de óleo de peixe e suco de cranberry de baixa caloria (Grupo Cr+OP, n=20).

O grupo C foi instruído a manter sua dieta habitual; o grupo OP foi instruído a manter sua dieta habitual e fazer a ingestão de 3g de ácidos graxos n-3 por dia (10 cápsulas); o grupo Cr+OP foi instruído a manter sua dieta habitual, e fazer ingestão de 3g ácidos graxos n-3 (10 cápsulas) e 500 mL de suco de cranberry de baixa caloria por dia. Cada cápsula de óleo de peixe contém 300 mg de ácidos graxos n-3 (180 mg de EPA e 120 mg de DHA). A composição de nutrientes de 200 ml de suco de cranberry é de: 84 KJ (20 kcal); 0 g de proteína; 5 g de

hidrato de carbono; 0 g de lipídeos, 0 g de fibra; 30 mg de sódio; 60 mg de vitamina C (figuras 7 e 8).

Figura 7 - Certificado de análise das cápsulas de óleo de peixe utilizadas no estudo fornecidas pela empresa Ad Oceanum Indústria e Comércio, Ltda, Grande Florianópolis, Santa Catarina, Brasil.

TESTE FÍSICO-QUÍMICO	**ESPECIFICAÇÃO**	**MÉTODO ANÁLISE**	**RESULTADO**
Descrição	Cápsula mole de cor natural, forma oblonga. Contém líquido oleoso de cor amarelo transparente, livre de material estranho. Com bom brilho, selagem e dureza.	GRA924500 R 2	Conforme
Peso Bruto	Informativo	GRA924500 R 2	1541,28 mg/caps
Peso Médio Conteúdo	Médio=1000,00 mg / caps Máx.=1075,00 mg / caps Mín.= 925,00 mg / caps RSD <6,0%	GRA924500 R 2	994,70 mg/caps 0,4%
Desintegração	Máximo 45 minutos	GRA924500 R 2	08 minutos
Teor de EPA	Mínimo 180,0 mg/caps	GRA924500 R 2	183,0 mg/cáps
Teor de DHA	Mínimo 120,0 mg/caps	GRA924500 R 2	122,4 mg/cáps
Mercúrio	Máximo 0,01ppm	GRA924500 R 2	<0,005ppm
Chumbo	Máximo 0,80ppm	GRA924500 R 2	<0,1ppm
Cádmio	Máximo 1,00ppm	GRA924500 R 2	<0,01ppm
Arsênio	Máximo 1,00ppm	GRA924500 R 2	<0,1ppm
TESTE MICROBIOLOGICO	**ESPECIFICAÇÃO**	**MÉTODO ANÁLISE**	**RESULTADO**
Contagem total de aeróbios	≤ 1000 UFC/g	GRA924500 R 2	< 10 UFC/g
Bolores e leveduras	≤ 100 UFC/g	GRA924500 R 2	< 10 UFC/g
E. coli	Ausente	GRA924500 R 2	Ausente
S.aureus	Ausente	GRA924500 R 2	Ausente
P. aeruginosa	Ausente	GRA924500 R 2	Ausente
Salmonella SP	Ausente	GRA924500 R 2	Ausente

Fonte: Ad Oceanum Indústria e Comércio, Ltda

Figura 8 - Composição nutricional do suco de cranberry normocalórico e de baixa caloria, Empresa Juxx, Barueri, São Paulo, Brasil.

Cranberry

DADOS NUTRICIONAIS DATOS NUTRICIONALES		
Porção de 200ml (um copo) / Porción de 200ml (un vaso)		
Quantidade por porção / Cantidad por Porción		**%VD(*) %VD(*)**
Valor energético / Valor energetico 110kcal=460kJ		5
Carboidratos / Carbohidratos	26g	9
Proteínas / Proteínas	0g	0
Gorduras Totais / Grasas Totales	0g	0
Gorduras Saturadas / Grasas Saturadas	0g	0
Gorduras Trans / Grasas Trans	0g	**
Fibra Alimentar / Fibras Alimentícias	0g	0
Sódio / Sódio	30mg	1
Vitamina C / Vitamina C	60mg	130

Cranberry Zero

DADOS NUTRICIONAIS DATOS NUTRICIONALES		
Porção de 200ml (um copo) / Porción de 200ml (un vaso)		
Quantidade por porção / Cantidad por Porción		**%VD(*) %VD(*)**
Valor energético / Valor energetico 20kcal=84kJ		
Carboidratos / Carbohidratos	5g	
Proteínas / Proteínas	0g	0
Gorduras Totais / Grasas Totales	0g	0
Gorduras Saturadas / Grasas Saturadas	0g	0
Gorduras Trans / Grasas Trans	0g	
Fibra Alimentar / Fibras Alimentícias	0g	0
Sódio / Sódio	30mg	1
Vitamina C / Vitamina C	60mg	130

Fonte: Empresa Juxx

4.4 Controle da Adesão ao Tratamento

Algumas medidas foram tomadas para otimizar e avaliar a adesão do paciente. As cápsulas de óleo de peixe bem como o suco de cranberry foram entregues no início da pesquisa e após 45 dias de experimento. Neste segundo momento, realizou-se a contagem das cápsulas e das caixas de suco para verificar se a ingestão estava correta. Repetiu-se este procedimento ao final dos 90 dias de experimento.

Além disso, foram realizadas entrevistas telefônicas para avaliar se os pacientes estavam usando corretamente os suplementos e para evitar mudanças no estilo de vida.

4.5 Medidas Antropométricas

A circunferência abdominal foi medida (em centímetros) no início e ao término do estudo, por meio de fita milimetrada, inextensível, posicionada horizontalmente no ponto médio entre a margem inferior da última costela e a crista ilíaca, com o indivíduo ereto, abdômen relaxado, braços ao lado do corpo, peso igualmente sustentado pelas pernas e pés

separados 25 a 30 cm.

4.6 Atividade da Doença

Os pacientes foram selecionados de acordo com critérios de classificação do ACR/EULAR (ALETAHA et al., 2010), e o estado de atividade da doença foi determinada utilizando a DAS28-PCR (FRANSEN J, WELSING P M, DE KEIJZER R M, 2004), conforme a equação descrita no site da DAS ("DAS28 - *Home of the Disease activity score* and DAS28", [s.d.]), por um único reumatologista. O DAS28 considera a contagem de articulações inchadas e dolorosas, estado geral de saúde (GH; avaliação do paciente da atividade da doença usando uma escala visual analógica de 100 mm em locais onde 0 = melhor, 100 = pior), além de níveis de PCR (mg/L).

Segundo a DAS 28, os pacientes podem ser categorizados em quatro grupos, ou seja, grupo de remissão: DAS28 ≤ 2,6; grupo de baixa atividade da doença: 2.6 <DAS28 ≤ 3,2; grupo com atividade da doença moderada 3,2 <DAS 28 ≤ 5,1; e grupo com alta atividade da doença: DAS28> 5.1 (PREVOO et al., 1995).

4.7 Avaliação Bioquímica

Após jejum de 12h, os pacientes foram submetidos a seguinte análise de sangue laboratorial: PCR de alta sensibilidade (hsPCR) e FR foram medidos usando o ensaio imunoturbidimétrico (*C8000 Architect Abbott Laboratories, Abbott Park, IL, USA*). Anti-CCP foram testados utilizando o imunoensaio de quimiluminescência por micropartículas (*Architect, Abbott Laboratory, Abbott Park, IL, USA*).

Níveis IL-6 foram medidos por ensaio de imunoabsorção enzimática (ELISA) usando um imunoensaio de ELISA comercial (*Ready-Set Go! Set, e-Bioscience, San Diego, California, USA*). Os níveis plasmáticos de AdipoQ e leptina foram medidos utilizando ensaio imunoenzimático (ELISA) (*Duo Set, R&D System*, Minneapolis, UM, USA). VHS foi obtida por método cinético-fotométrico automatizado (*Ves-Matic CUBE 30, DIESSE, Siena, Italy*).

4.8 Análise Estatística

Os dados categóricos foram analisados com o teste do qui-quadrado. Os resultados estão apresentados em número absoluto. Para verificar as alterações da linha de base (mudanças intra-grupo) foi utilizado o teste de Wilcoxon. O teste de Kruskal-Wallis, com teste *post hoc* Dunn foi realizado para comparar os valores de base e diferenças entre os grupos de tratamento (mudanças inter-grupo). Os dados estão expressos como medianas e percentis

25 a 75th. Os resultados foram considerados significativos quando p <0,05. Um programa de análise estatística *Graph Pad Prism* Versão 4.0, (San Diego, CA, USA) foi usado para as avaliações.

4.9 Aspectos Éticos

Este estudo foi realizado de acordo com as orientações definidas na Declaração de Helsinque e do Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, Paraná, Brasil que aprovou todos os procedimentos (ANEXO A). Todos pacientes deram ciência e assinaram o termo de consentimento livre e esclarecido, antes do início da pesquisa (APÊNDICE B).

5 ARTIGOS CIENTÍFICOS REFERENTES AO TRABALHO

Para o cumprimento dos objetivos propostos por este trabalho, foram desenvolvidos dois artigos científicos:

1 - Título: *Adipokines in Rheumatoid Arthritis*, submetido à Revista Brasileira de Reumatologia (ISNN:0482-5004); Qualis B2. Fator de impacto: 0,859

2 - Título: *Cranberry juice adds beneficial effects on disease activity and inflammatory biomarkers in patients with rheumatoid arthritis supplemented with fish oil*, submetido à revista British Journal of Nutrition (ISNN:) 0007-1145; Qualis A2. Fator de impacto: 3,311.

5.1 ARTIGO: ADIPOKINES IN RHEUMATOID ARTHRITIS

Adipokines in rheumatoid arthritis

Elis Carolina de Souza Fatel, M.Sc[a]., Flávia Troncon Rosa, Ph.D.[b], Andréa Name Colado Simão, Ph.D.[c], and Isaias Dichi, MD, Ph.D.[d]

[a]Postgraduate Program, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil; [b] Postgraduate Program, Experimental Pathology, State University of Londrina, Londrina, Paraná, Brazil; [c] Department of Pathology, Clinical Analysis and Toxicology, University of Londrina, Londrina, Paraná, Brazil; [d]Department of Internal Medicine - University of Londrina, Londrina, Paraná, Brazil.

None of the authors had any conflict of interest in relation to this study.

Corresponding author: Isaias Dichi, MD, PhD. Department of Internal Medicine. Robert Koch Avenue n. 60, Cervejaria, University of Londrina. Londrina, Paraná, Brazil. CEP: 86038-440 Tel: (55) 43 3371 2234 E-mail: dichi@sercomtel.com.br

ABSTRACT

Rheumatoid arthritis (RA) affects millions of people worldwide and is considered a chronic multisystem disease whose causes are not completely known. In general, the main objective of RA treatment is to improve the quality of life of patients by relieving pain, maintaining or improving functional capacity, preventing thus, disability. In recent years the role of adipokines in the pathogenesis of RA has been discussed but results are still conflicting. Although results from some studies have shown the implications of adipokines in the pathophysiology of autoimmune diseases, including RA, their role in the pathogenesis of disease progression is not clear. Thus, this review aimed to describe the role of key adipokines (leptin, resistin, visfatin and adiponectin) in the pathophysiology of RA, given the high prevalence of this disease and the important social impact caused by this chronic disabling disease.

Key words: Adipokines, Rheumatoid Arthritis, Cytokines.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic multisystem disease whose causes are not completely known.[1] This disease presents a variety of systemic manifestations being the persistent inflammatory synovitis the most typical feature, compromising peripheral joints in a symmetric distribution. Mateen et al. (2016)[2] highlight RA as a disease characterized by the presence of rheumatoid factor (RF) and anti–citrullinated cyclic peptide (ACPA). These authors reinforce that cytokines such as Tumor Necrosis Factor α (TNF-α), IL (interleucin) -1 and IL-17 have an important role in the pathophysiology of RA since serum concentrations of these substances may indicate the severity of the disease.

The diagnosis of RA must be at earlier stages of disease and treatment should aim to relieve pain, maintain or improve functional capacity, preventing thus, disability, and improving patients quality of life.[3]

Barbosa et al. (2012)[4] reported the important role of mediators synthesized in adipose tissue, named adipokines, in RA. Hutcheson (2015)[5] points out that knowledge about adiposity has changed and currently it appears as an important regulator of several key processes, including inflammation. Furthermore, adipokines have hormonal action supporting the regulation of appetite and glucose metabolism, and some of them such as leptin, resistin, adiponectin and visfatin have been associated to RA development. However, the results are still conflicting.

Thus, this review aimed to describe the role of key adipokines (leptin, resistin, visfatin and adiponectin) in the pathophysiology of RA, given the high prevalence of this disease and the important social impact caused by chronic disabling diseases of the articular system.

METHODS

The present review performed a search for references in the following electronic databases: U.S. National Library of Medicine PUBMED, Periódicos Capes, Science Direct, and Scientific Electronic Library Online written in English, Portuguese or Spanish. The search was limited to articles performed in human beings (clinical studies, randomized controlled studies, controlled clinical studies, multicentric studies and meta-analysis) which have been published between January 2010 and April 2016; classical articles outside from this period of time were also used. The crossing of the following descriptors was used to accomplish this review: Rheumatoid Arthritis and Adipokines, Leptin, Resistin, Visfatin, and Adiponectin.

ADIPOKINES

Adipose tissue is a multifunctional tissue responsible for lipid storage, thermogenesis, structural components and support of many organs such as joints, gastrointestinal tract and skin and also described as secretory and endocrine functions.[6] It is noteworthy in this context its role as an endocrine organ by synthesizing and secreting adipokines, which play an important role in the pathophysiology of insulin resistance, inflammation and atherogenesis.[7, 8]

Leptin

Leptin is an adipokine produced in white adipose tissue. Discovered in 1994[9], it is an *Ob* gene product[10], cloned and sequenced in mice and considered the adipokine responsible for the regulation of energy metabolism and homeostasis, as well as neuroendocrine functions.[11] It also assists the immunity and inflammation control through its receptor.[12] Thus, leptin is responsible for the regulation of various biological processes, being involved in the pathophysiology of many diseases. Leptin is considered a proinflammatory adipokine since it stimulates production of cytokines such as TNF-α, IL-6 and reactive oxygen species, induces the production of CC chemokines by macrophages and alters the Th1 / Th2 profile.[12]

Paz-Filho et al. (2012)[13] described molecular mechanisms and proinflammatory systemic effects of leptin. It acts through its Ob receptor triggering inflammatory responses together with infectious and inflammatory stimuli of cytokines such as IL-1, lipopolysaccharide (LPS), and TNF-α, which may, in turn, increase levels of leptin. The interaction between leptin and inflammation are bidirectional, but all proinflammatory since cytokines increases the synthesis and release of leptin, which in turn perpetuates the cycle of inflammation.

Leptin showed a significant effect on increasing the expression of Th1 cytokines. Experimental studies on mice demonstrated that these animals showed less severe stages of the disease with low levels of IL-1β and TNF-α in the synovial fluid and reduction in T-cell proliferative response induced by antigen.[13] However, clinical studies revealed paradoxical results about the effects of endogenous leptin in protecting joints in severe forms of erosive RA in humans.[14]

In the last two decades, several studies have described the action of leptin in RA,[15] leading researchers near to assume the hypothesis that this hormone has a key role in rheumatic diseases. [16] Thus, leptin levels may be a risk factor for the pathogenesis of RA.[17]

Olama et al. (2012)[14] evaluated the ratio of synovial and serum leptin in patients with RA and found that the local utilization of leptin at the joint cavity has a protector role against the destructive course of RA. Rho et al. (2010)[18] also examined the hypothesis that the

adipokines could influence insulin resistance and coronary atherosclerosis in patients with RA. Leptin was positively associated with insulin resistance assessed by Homeostasis Model Assessment - Insulin Resistance (HOMA-IR), even after adjusting for age, race, sex, body mass index (BMI), traditional cardiovascular risk factors and inflammation mediators. Targońska-Stepniak et al. (2010)[19] assessed leptin levels in patients with RA and demonstrated positive correlation between leptin levels and Disease Activity Score (DAS28). Yoshino et al. (2011)[20] found leptin levels significantly higher in RA patients compared to controls, and this adipokine correlated positively with C-reactive protein (CRP) levels, suggesting that leptin can act as a proinflammatory in this disease. On the other hand, Kontunen et al. (2011)[21] showed that leptin levels were increased only in patients with RA and concomitant diagnosis of metabolic syndrome (MetS).

Kang et al. (2013)[22] demonstrated that TNF-α was positively associated to leptin and the latter was associated with various metabolic risk factors, including insulin resistance. Bustos Rivera-Bahena et al. (2015)[23] evidenced that circulating levels of leptin correlate positively with clinical activity of RA, regardless of BMI. However, Xibille-Friedmann et al. (2015)[24] concluded that in a shortterm, basal levels of leptin may predict disease activity independent of BMI. However, when submitted to treatment, this only occurred in patients with normal body weight.

Tian et al. (2014)[25] reported a review in which 23 studies were analyzed. The following results were obtained: 13 studies showed increased leptin levels; 8 studies did not demonstrate any significant difference and 2 had reduced leptin levels when compared to control subjects. Therefore, the majority of the studies have found higher levels of leptin in patients with RA, showing a possible role in the regulation of joint damage, and suggested that more studies are needed to understand the mechanisms of action of this adipokine. A meta-analysis conducted by Lee e Bae (2016)[26] confirmed these data showing that circulating levels of leptin were significantly higher in patients with RA with a positive correlation between this hormone and RA activity.

Despite the evidence demonstrated, some studies do not corroborate these associations. Allam e Radwan (2012)[27] and Abdalla et al. (2014)[28] found that although serum leptin level was significantly higher in RA patients than in control group, there was no correlation with clinical and laboratory markers of disease activity. Mirfeizi et al. (2014)[29] also stated that leptin has no effect on the process of joint damage in RA patients. Oner et al. (2015)[30] did not find any correlation between disease activity and serum leptin levels, indicating that this adipokine is not a good biomarker to monitor inflammation in RA.

Thus, leptin seems to have a role in the pathophysiology of RA and comorbidities

associated, such as obesity and MetS. Leptin is considered a proinflammatory adipokine by the great majority of authors who suggest a predominantly deleterious action on the joint. Only one survey showed that increased levels of leptin can act as a protective factor against the destructive course of RA[24].

Table 1 summarizes the main findings of leptin in RA patients.

Insert Table 1

Resistin

Isolated in rodents, resistin was first described in 2001. It is a cysteine–rich protein, compounded by 108 amino acids, called resistin-like molecules (RELMs) also known as FIZZ 332.[31] In humans, it is originated mainly from circulating monocytes and macrophages.[32]

It was initially correlated to the pathogenesis of insulin resistance in obesity and some cardiovascular diseases (CVD) but now is also considered an important link between obesity and inflammation.[33] Resistin has been found in areas of inflammation and seems to be mediated by IL-6 and TNF- α.[34]

Due to its implication in inflammatory processes, the involvement of resistin in the pathogenesis of RA has been investigated. Kassem et al. (2010)[35] studied whether there is a role of resistin in the pathogenesis of RA by investigating possible correlations between resistin concentration in serum and synovial fluid with disease activity and radiographic joint damage. The authors' results supported the hypothesis that resistin is involved in the pathogenesis of RA and suggested serum resistin as a good marker of prognosis of the disease in RA patients. Yoshino et al. (2011)[20] found that the level of resistin in serum did not differ between RA patients and healtty controls, but observed that serum resistin were positively associated with CRP levels in RA patients, suggesting a proinflammatory action of this adipokine.

Kontunen et al. (2011)[21] reported that high levels of resistin are associated with RA, regardless of the presence of MetS. Fadda et al. (2013)[36] compared resistin levels in serum and synovial fluid of patients with RA and osteoarthritis and found higher levels in patients with RA. This result indicates a possible role of resistin in the pathogenesis of inflammatory rheumatic diseases. The high levels of this adipokine in the synovial fluid could suggest a worst prognosis for progression of RA, but the authors point out that more studies are needed to confirm whether resistin is a good marker to evaluate the progression of this disease. Kang et al. (2013)[22] reinforced this hypothesis. The authors found an association between resistin levels and inflammatory markers in patients with RA. Recently, Bustos Rivera-Bahena et al. (2015)[23] demonstrated that resistin levels correlated positively with clinical manifestations of

disease activity in patients with RA, albeit of patient BMI. Huang et al. (2015)[37] in a meta-analysis concluded that serum resistin levels were significantly higher in RA patients compared to control group.

However, some authors did not show significant associations between serum resistin and HOMA-IR (Rho et al., 2010)[18], nor differences between serum and synovial fluid resistin levels between RA patients and controls. Al-Kady et al. (2010)[38] after studying the levels of resistin in of RA patients found no significant differences in resistin levels between RA patients and controls. Hammad et al. (2014)[39] also found no correlation between serum levels of resistin with clinical or laboratory markers in RA patients.

The pro inflammatory action of resistin was observed in most studies of patients with RA, which suggest that this adipokine is a good marker to assess the progression of this disease.

Table 2 summarizes the main findings of resistin in RA patients.

Insert table 2

Visfatin

Also known as pre-B-cell colony-enhancing factor (PBEF) or Nampt,[40] visfatin is a protein with molecular weight of 52 kDa, first described by Samal et al. (1994).[41] It is primarily found in liver, bone marrow and muscle tissue, but also produced by adipose tissue and secreted by macrophage.[42] Its production is influenced by TNF-α, IL-6, toll-like receptor (TLR) and chemokines.[43] Stofkova (2010)[44] reports that visfatin may contribute to inflammatory processes, triggering production of cytokines and activation of nuclear fator K B (NF-κB). Thus, some studies have suggested some relation between this adipokine and the pathogenesis of type 2 diabetes mellitus and obesity[45] and increased cardiovascular risk. [46]

Other studies have demonstrated a correlation between serum and synovial fluid levels of visfatin and the pathogenesis of RA.[40,47,48,12] This adipokine can act as a regulator of inflammation and the destruction process of joints[34] and induce stimulation of great quantities of chemokines[49], thus possibly contributing to the inflammatory state of RA. However, its association to disease activity is not yet fully known.[50]

Alkady et al. (2011)[51] showed that visfatin levels correlated with disease activity and may be involved in the progression of RA. Khalifa et al. (2013)[52] suggested that visfatin has a role in the pathogenesis of RA, and it may be considered as a marker of the disease and the radiographic bone lesion score. Therefore, it can be a potential therapeutic target for RA. El-Hini et al. (2013)[53] demonstrated positive and significant correlation between visfatin and

insulin resistance and also with serum cholesterol, LDL-cholesterol and triglycerides. Additionally, the disease activity score was positively correlated with visfatin.

Sglunda et al. (2014)[54] observed that visfatin levels in serum were significantly higher in RA patients compared to healthy individuals and suggested that reduction in visfatin concentrations could reduce disease activity in patients at early stage of RA. They also found positive association between this adipokine and elevated levels of total cholesterol, but not with the atherogenic index. Mirfeizi et al. (2014)[29] found that serum levels of visfatin in RA patients with radiographic joint damage were significantly higher than in patients without joint damage.

Nonetheless, Rho et al. (2010)[18] did not show any evidence between visfatin and insulin resistance or coronary atherosclerosis in patients with RA and Meyer et al. (2013)[55] did not verify any correlation between serum levels of visfatin and radiographic progression of the disease.

Table 3 summarizes the main findings of visfatin in RA patients.

Insert table 3.

Adiponectin

Adiponectin is an anti-inflammatory adipokine compounded by 244 amino acids and is produced and secreted mainly by adipocytes. [56,57] Studies suggest that monomeric form of adiponectin appears to occur only in adipocytes, but there are three plasma circulating isoforms of adiponectin: trimmers (low molecular weight, LMW), hexamers (middle molecular weight, MMW) and multimers (high molecular weight, HMW). [57] The receptors are AdipoR1and AdipoR2, respectively present at skeletal muscles and liver. [58]

Several studies have demonstrated the role of this important anti-inflammatory cytokine in obesity, type 2 diabetes mellitus, atherosclerosis and MetS, being the highest levels a protective factor for these diseases. [59,60,34,61]

Paradoxically, in the pathogenesis of RA adiponectin seems to have proinflammatory effects in the joints, because its ability to stimulate the secretion of inflammatory mediators[62] and may also be associated to disease activity.[51] Scotece et al. (2012)[63] described the major effects of increased synovial and circulating levels of adiponectin in RA. They concluded that adiponectin in synovial fibroblasts induced prostaglandin (PG) E2, IL-6, IL-8, MMP-1 and MMP-13; in human chondrocytes induced nitric oxide (NO), IL-6, matrix metalloproteinase (MMP) -3, MMP-9, MCP-1 and IL-8 and promoted inflammation by increasing TNF-α, IL-6 and IL-8.

Krysiak et al. (2012)[64] suggested that these different actions can be explained by

different mechanisms: LMW adiponectin has anti-inflammatory activities, while the HMW adiponectin has pro-inflammatory activities. However, Frommer et al. (2012)[65] showed a proinflammatory and destructive role of all isoforms of adiponectin in patients with RA, suggesting a much more harmful than beneficial action of adiponectin in chronic inflammatory diseases. Several studies evidenced association of adiponectin in radiographic progression of RA.[66,67] Thus, serum adiponectin levels could be a good biomarker to evaluate the early stages of disease progression.[55] However, this association was not mediated by the selective effect of HMW adiponectin. [68] Recently, Skalska and Kontny (2016)[17] observed that HMW and MMW adiponectins potentially stimulated the secretion of rheumatoid adipose-derived stem cells (ASC) in patients with RA, but did not exert a strong impact on ASC towards fibroblast-like synoviocytes (RA-FLS) and peripheral blood mononuclear cells.

Furthermore, Rho et al. (2010)[18] found no association between adiponectin levels and insulin resistance or coronary artery calcium score. Yoshino et al. (2011)[20] also observed higher levels of adiponectin in serum of RA patients, but it was negatively associated with CRP. Bustos Rivera-Bahena et al (2015)[23] evidenced no association between adiponectin and disease activity and Chennareddy et al. (2016)[69] reported that despite serum levels of adiponectin are higher in RA patients than in controls there was no correlation with disease activity, duration, BMI and waist-to-hip ratio.

Despite the protective effect of adiponectin in the pathogenesis of obesity, diabetes mellitus, atherosclerosis, and Mets, it is unclear whether this effect is reproduced in RA. Several studies emphasize that adiponectin appears to play a pro inflammatory role in the pathogenesis of RA, particularly in the joints, by stimulating the secretion of inflammatory mediators. In this scenario, it highlights the importance of developing new research elucidating the actual role of adiponectin in the pathogenesis of RA.

Table 4 summarizes the main findings of adipnectin in RA patients.

Insert Table 4

DRUGS ACTION ON ADIPOKINES LEVELS IN PATIENTS WITH RA

Some researchers evaluated the action of disease-modifying anti rheumatic drugs (DMARD) in serum levels of adipokines in patients with RA. Klaasen et al. (2012)[70] reported that visfatin levels decreased after treatment with adalimumab, an TNF-α drug. The authors also described that 16 weeks of treatment with adalimumab, and two weeks with high doses of prednisolone resulted in a reduction in resistin levels associated to a decrease in DAS28, CRP and ESR. Kim et al. (2014)[50] reported that after six months of DMARD and TNF-α drug

treatment, only adiponectin was significantly increased in patients who responded to treatment. Manrique-Arija et al (2016)[71] showed improvement in inflammatory and lipid profile and decreased resistin levels after DMARD treatment.

Hence, the complex pathophysiology of RA together with the innumerable cells that contribute to the process of joint destruction begin to be evaluated from the standpoint of adipokines and the action of drugs on them. However there is still a long way to a better understanding of their role in RA.

CONCLUSION

In recent years, it has been studied the importance of adipokines in the pathogenesis of RA; however the results are still conflicting and the exact role of adipose tissue in RA is not yet fully understood. Despite studies have been demonstrating the implications of adipokines in the pathophysiology of RA, it is not yet clear their role in the progression of disease. It is noteworthy the complex pathophysiology of this disease, thus requiring better knowledge about the mechanisms of action of these adipokines in RA as well as the changes that drugs can promote in the circulating levels of these adipokines in these patients.

REFERENCES

1. LIPSKY PE. Artrite Reumatoide. In: Medicina interna de Harrison. 14th ed. Rio de Janeiro; 1998. p. 1996–7.
2. Mateen S, Zafar A, Moin S, Khan AQ, Zubair S. Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. Clin Chim Acta. 2016;455:161–71.
3. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Guidelines for the management of rheumatoid arthritis: 2002 Update. Arthritis Rheum [Internet]. 2002 Feb [cited 2016 Aug 1];46(2):328–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11840435
4. Barbosa VDS, Rêgo J, Antônio N. Possível papel das adipocinas no lúpus eritematoso sitêmico e na artrite reumatoide. Rev Bras Reumatol. 2012;52(2):278–87.
5. Hutcheson J. Adipokines influence the inflammatory balance in autoimmunity. Cytokine. 2015;75(2):272–9.
6. Neumann E, Frommer KW, Vasile M, Müller-Ladner U. Adipocytokines as driving forces in rheumatoid arthritis and related inflammatory diseases? Arthritis Rheum. 2011;63(5):1159–69.

7. Freitas Lima LC, Braga V de A, do Socorro de França Silva M, Cruz J de C, Sousa Santos SH, de Oliveira Monteiro MM, et al. Adipokines, diabetes and atherosclerosis: An inflammatory association. Front Physiol. 2015;6(NOV):1–15.
8. Dichi I, Simão ANC. Metabolic syndrome: new targets for an old problem. Expert Opin Ther Targets [Internet]. 2012 Feb 7 [cited 2016 Sep 28];16(2):147–50. Available from: http://www.tandfonline.com/doi/full/10.1517/14728222.2012.648924
9. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature [Internet]. 1994 Dec 1 [cited 2016 Aug 1];372(6505):425–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7984236
10. Guimarães DED, Sardinha FL de C, Mizurini D de M, Carmo M das GT do. Adipocitocinas: uma nova visão do tecido adiposo. Rev Nutr [Internet]. 2007 Oct [cited 2016 Aug 1];20(5):549–59. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-52732007000500010&lng=pt&nrm=iso&tlng=pt
11. Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim S, et al. Leptin in human physiology and pathophysiology. AJP Endocrinol Metab. 2011;301:567–84.
12. Del Prete A, Salvi V, Sozzani S. Adipokines as potential biomarkers in rheumatoid arthritis. Mediators Inflamm. 2014;2014:1–12.
13. Paz-Filho G, Mastronardi C, Franco CB, Wang KB, Wong M-L, Licinio J. Leptin: molecular mechanisms, systemic pro-inflammatory effects, and clinical implications. Arq Bras Endocrinol Metabol [Internet]. 2012;56(9):597–607. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23329181
14. Olama SM, Senna MK, Elarman M. Synovial/serum leptin ratio in rheumatoid arthritis: the association with activity and erosion. Rheumatol Int [Internet]. 2012 Mar [cited 2016 Aug 1];32(3):683–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21140264
15. Toussirot É, Michel F, Binda D, Dumoulin G. The role of leptin in the pathophysiology of rheumatoid arthritis. Life Sci [Internet]. 2015 Nov [cited 2016 Aug 1];140:29–36. Available from: http://linkinghub.elsevier.com/retrieve/pii/S002432051500257X
16. Scotece M, Conde J, López V, Lago F, Pino J, Gómez-Reino JJ, et al. Adiponectin and leptin: New targets in inflammation. Basic Clin Pharmacol Toxicol. 2014;114(1):97–102.
17. Skalska U, Kontny E. Adiponectin Isoforms and Leptin Impact on Rheumatoid Adipose

Mesenchymal Stem Cells Function. Stem Cells Int. 2016;2016:1–7.

18. Rho YH, Chung CP, Solus JF, Raggi P, Oeser A, Gebretsadik T, et al. Adipocytokines, insulin resistance, and coronary atherosclerosis in rheumatoid arthritis. Arthritis Rheum. 2010;62(5):1259–64.
19. Targońska-Stepniak B, Dryglewska M, Majdan M. Adiponectin and leptin serum concentrations in patients with rheumatoid arthritis. Rheumatol Int. 2010;30:731–7.
20. Yoshino T, Kusunoki N, Tanaka N, Kaneko K, Kusunoki Y, Endo H, et al. Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. Intern Med [Internet]. 2011;50(4):269–75. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=medl&NEWS=N&AN=21325757
21. Kontunen P, Vuolteenaho K, Nieminen R, Lehtimäki L, Kautiainen H, Kesäniemi Y, et al. Resistin is linked to inflammation, and leptin to metabolic syndrome, in women with inflammatory arthritis. Scand J Rheumatol [Internet]. 2011 [cited 2016 Aug 1];40(4):256–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21453187
22. Kang Y, Park H-J, Kang M-I, Lee H-S, Lee S-W, Lee S-K, et al. Adipokines, inflammation, insulin resistance, and carotid atherosclerosis in patients with rheumatoid arthritis. Arthritis Res Ther [Internet]. 2013 [cited 2016 Aug 1];15(6):1–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24245495
23. Bustos Rivera-Bahena C, Xibillé-Friedmann DX, González-Christen J, Carrillo-Vázquez SM, Montiel-Hernández JL. Peripheral blood Leptin and Resistin levels as clinical activity biomarkers in Mexican Rheumatoid Arthritis patients. Reumatol Clin. 2015;(xx):3–6.
24. Xibille-Friedmann DX, Ortiz-Panozo E, Bustos Rivera-Bahena C, Sandoval-Rios M, Hernandez-Gongora SE, Dominguez-Hernandez L, et al. Leptin and adiponectin as predictors of disease activity in rheumatoid arthritis. Clin Exp Rheumatol. 2015;33(4):471–7.
25. Tian G, Liang J-N, Wang Z-Y, Zhou D. Emerging role of leptin in rheumatoid arthritis. Clin Exp Immunol [Internet]. 2014;177(3):557–70. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4137840&tool=pmcentrez&rendertype=abstract
26. Lee YH, Bae S-C. Circulating leptin level in rheumatoid arthritis and its correlation with disease activity: a meta-analysis. Z Rheumatol [Internet]. 2016 Jan 28 [cited 2016 Aug 1];1–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26820722

27. Allam A, Radwan A. The relationship of serum leptin levels with disease activity in Egyptian patients with rheumatoid arthritis. Egypt Rheumatol. 2012;34(4):185–90.
28. Abdalla M, Effat D, Sheta M, Hamed WE. Serum Leptin levels in Rheumatoid arthritis and relationship with disease activity. Egypt Rheumatol. 2014;36(1):1–5.
29. Mirfeizi Z, Noubakht Z, Rezaie AE, Jokar MH, Sarabi ZS. Plasma levels of leptin and visfatin in rheumatoid arthritis patients; is there any relationship with joint damage? Iran J Basic Med Sci [Internet]. 2014 Sep [cited 2016 Aug 1];17(9):662–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25691942
30. Oner SY, Volkan O, Oner C, Mengi A, Direskeneli H, Tasan DA. Serum leptin levels do not correlate with disease activity in rheumatoid arthritis. Acta Reum Port [Internet]. 2015 [cited 2016 Aug 1];40(1):50–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25342093
31. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature [Internet]. 2001 Jan 18 [cited 2016 Aug 1];409(6818):307–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11201732
32. Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. J Clin Endocrinol Metab [Internet]. 2003 Oct [cited 2016 Aug 1];88(10):4848–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14557464
33. Codoñer-Franch P, Alonso-Iglesias E. Resistin: Insulin resistance to malignancy. Clin Chim Acta. 2015;438:46–54.
34. Abella V, Scotece M, Conde J, López V, Lazzaro V, Pino J, et al. Review Article Adipokines , Metabolic Syndrome and Rheumatic Diseases. J Immunol Researc. 2014;2014:1–15.
35. Kassem E, Mahmoud L, Salah W. Study of Resistin and YKL-40 in Rheumatoid Arthritis . J Am Sci. 2010;6(10):1004–12.
36. Fadda SMH, Gamal SM, Elsaid NY, Mohy AM. Resistin in inflammatory and degenerative rheumatologic diseases: Relationship between resistin and rheumatoid arthritis disease progression. Z Rheumatol. 2013;72(6):594–600.
37. Huang Q, Tao S-S, Zhang Y-J, Zhang C, Li L-J, Zhao W, et al. Serum resistin levels in patients with rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis. Clin Rheumatol [Internet]. 2015;1713–20. Available from:

http://link.springer.com/10.1007/s10067-015-2955-5

38. Al-kady EA, Ahmed HM, Tag L, Adel M, Al-Kady EA. Adipocytokines: Adiponectin, Resistin and Visfatin in Serum and Synovial Fluid of Rheumatoid Arthritis Patients and Their Relation to Disease Activity. Med J Cairo Univ. 2010;78(2):723–9.
39. Hammad MH, Nasef S, Musalam D, Ahmed MM, Osman I, Hammad MH. Resistin , an adipokine , its relation to inflammation in Systemic Lupus Erythematosus and Rheumatoid Arthritis. MIDDLE EAST J Intern Med. 2014;7(September):1–7.
40. Bao JP, Chen WP, Wu LD. Visfatin: a potential therapeutic target for rheumatoid arthritis. J Int Med Res [Internet]. 2009;37(6):1655–61. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20146863
41. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and Characterization of the cDNA Encoding a Novel Human Pre-B-Cell Colony-Enhancing. Mol Cell Biol. 1994;14(2):1431–7.
42. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science [Internet]. 2005 Jan 21 [cited 2016 Aug 1];307(5708):426–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15604363
43. Kerekes G, Nurmohamed MT, González-Gay MA, Seres I, Paragh G, Kardos Z, et al. Rheumatoid arthritis and metabolic syndrome. Nat Rev Rheumatol [Internet]. 2014 Nov [cited 2016 Aug 1];10(11):691–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25090948
44. Stofkova A. Resistin and visfatin: regulators of insulin sensitivity, inflammation and immunity. Endocr Regul [Internet]. 2010 Jan [cited 2016 Aug 1];44(1):25–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20151765
45. Haider DG, Schindler K, Schaller G, Prager G, Wolzt M, Ludvik B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. J Clin Endocrinol Metab [Internet]. 2006 Apr [cited 2016 Aug 1];91(4):1578–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16449335
46. Romacho T, Sánchez-ferrer CF, Peiró C. Review Article Visfatin / Nampt : An Adipokine with Cardiovascular Impact. Mediators Inflamm. 2013;2013:1–16.
47. Naguib A, Elsawy N, Aboul-enein F, Hossam N. The relation between serum visfatin levels and cardiovascular involvement in rheumatoid arthritis. Alexandria J Med [Internet]. 2011;47(2):117–24. Available from: http://dx.doi.org/10.1016/j.ajme.2011.07.005\nhttp://linkinghub.elsevier.com/retrieve/pii

/S2090506811000479

48. Gómez R, Suarez A, Villalvilla A, Herrero-Beaumont G, Largo R, Young DA. Visfatin: a new player in rheumatic diseases. Immunometabolism [Internet]. 2013;1:10–5. Available from: http://www.degruyter.com/view/j/immun.2013.1.issue/immun-2013-0002/immun-2013-0002.xml
49. Meier FMP, Frommer KW, Peters MA, Brentano F, Lefèvre S, Schröder D, et al. Visfatin/pre-B-cell colony-enhancing factor (PBEF), a proinflammatory and cell motility-changing factor in rheumatoid arthritis. J Biol Chem. 2012;287(34):28378–85.
50. Kim KS, Choi HM, Ji HI, Song R, Yang HI, Lee SK, et al. Serum adipokine levels in rheumatoid arthritis patients and their contributions to the resistance to treatment. Mol Med Rep. 2014;9(1):255–60.
51. Alkady EAM, Ahmed HM, Tag L, Abdou MA. Adiponectin, Resistin und Visfatin in Serum und Gelenkflüssigkeit bei Patienten mit rheumatoider Arthritis. Z Rheumatol [Internet]. 2011;70(7):602–8. Available from: http://link.springer.com/10.1007/s00393-011-0834-2
52. Khalifa, Ibrahim A, Abdelfattah A. Relation between serum visfatin and clinical severity in different stages of rheumatoid arthritis. Egypt Rheumatol Rehabil. 2013;40(1):1–8.
53. El-Hini SH, Mohamed FI, Hassan AA, Ali F, Mahmoud A, Ibraheem HM. Visfatin and adiponectin as novel markers for evaluation of metabolic disturbance in recently diagnosed rheumatoid arthritis patients. Rheumatol Int. 2013;33(9):2283–9.
54. Sglunda O, Mann H, Hulejová H, Kuklová M, Pecha O, Pleštilová L, et al. Decreased circulating visfatin is associated with improved disease activity in early rheumatoid arthritis: Data from the PERAC cohort. PLoS One. 2014;9(7):1–5.
55. Meyer M, Sellam J, Fellahi S, Kotti S, Bastard J-P, Meyer O, et al. Serum level of adiponectin is a surrogate independent biomarker of radiographic disease progression in early rheumatoid arthritis: results from the ESPOIR cohort. Arthritis Res Ther. 2013;15(6):1–13.
56. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem [Internet]. 1995 Nov 10 [cited 2016 Aug 1];270(45):26746–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7592907
57. Garaulet M, Hernández-Morante JJ, de Heredia FP, Tébar FJ. Adiponectin, the controversial hormone. Public Health Nutr [Internet]. 2007 Oct [cited 2016 Aug 1];10(10A):1145–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17903323
58. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, et al. Targeted

disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med [Internet]. 2007 Mar [cited 2016 Aug 1];13(3):332–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17268472

59. Ohashi K, Ouchi N, Matsuzawa Y. Anti-inflammatory and anti-atherogenic properties of adiponectin. Biochimie. 2012;94(10):2137–42.
60. Fantuzzi G. Adiponectin in inflammatory and immune-mediated diseases. Cytokine [Internet]. 2013 Oct [cited 2016 Aug 1];64(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23850004
61. Simão TNC, Lozovoy MAB, Simão ANC, Oliveira SR, Venturini D, Morimoto HK, et al. Reduced-energy cranberry juice increases folic acid and adiponectin and reduces homocysteine and oxidative stress in patients with the metabolic syndrome. Br J Nutr [Internet]. 2013 Nov 11 [cited 2016 Sep 20];110(10):1885–94. Available from: http://www.journals.cambridge.org/abstract_S0007114513001207
62. Chen X, Lu J, Bao J, Guo J, Shi J, Wang Y. Adiponectin: A biomarker for rheumatoid arthritis? Cytokine Growth Factor Rev. 2013;24(1):83–9.
63. Scotece M, Conde J, G??mez R, L??pez V, Pino J, Gonz??lez A, et al. Role of adipokines in atherosclerosis: Interferences with cardiovascular complications in rheumatic diseases. Mediators Inflamm. 2012;2012:1–14.
64. Krysiak R, Handzlik-Orlik G, Okopien B. The role of adipokines in connective tissue diseases. Eur J Nutr. 2012;51(5):513–28.
65. Frommer KW, Schäffler A, Büchler C, Steinmeyer J, Rickert M, Rehart S, et al. Adiponectin isoforms: a potential therapeutic target in rheumatoid arthritis? Ann Rheum Dis [Internet]. 2012 Oct [cited 2016 Aug 1];71(10):1724–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22532632
66. Giles JT, van der Heijde DM, Bathon JM. Association of circulating adiponectin levels with progression of radiographic joint destruction in rheumatoid arthritis. Ann Rheum Dis [Internet]. 2011 Sep [cited 2016 Aug 1];70(9):1562–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21571734
67. Klein-Wieringa IR, Van Der Linden MPM, Knevel R, Kwekkeboom JC, Van Beelen E, Huizinga TWJ, et al. Baseline serum adipokine levels predict radiographic progression in early rheumatoid arthritis. Arthritis Rheum. 2011;63(9):2567–74.
68. Klein-Wieringa IR, Andersen SN, Herb-Van Toorn L, Kwekkeboom JC, Van Der Helm-Van Mil AHM, Meulenbelt I, et al. Are baseline high molecular weight adiponectin levels associated with radiographic progression in rheumatoid arthritis and osteoarthritis? J Rheumatol. 2014;41(5):853–7.

69. Chennareddy S, Kishore Babu K V., Kommireddy S, Varaprasad R, Rajasekhar L. Serum adiponectin and its impact on disease activity and radiographic joint damage in early rheumatoid arthritis – A cross-sectional study. Indian J Rheumatol. 2016;11(2):82–5.

70. Klaasen R, Herenius MMJ, Wijbrandts CA, de Jager W, van Tuyl LH, Nurmohamed MT, et al. Treatment-specific changes in circulating adipocytokines: a comparison between tumour necrosis factor blockade and glucocorticoid treatment for rheumatoid arthritis. Ann Rheum Dis [Internet]. 2012;71(9):1510–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3414229&tool=pmcentrez&rendertype=abstract

71. Manrique-Arija S, Ureña I, Valdivielso P, Rioja J, Jiménez-Núñez FG, Irigoyen M V., et al. Insulin resistance and levels of adipokines in patients with untreated early rheumatoid arthritis. Clin Rheumatol. 2016;35(1):43–53.

Table 1 – Studies investigating the association of leptin and rheumatoid arthritis in humans

Authors	Study design	Subjects	Results/outcomes
Rho et al. (2010)[18]	Cross-sectional study evaluating correlation between HOMA-IR and serum adipokine levels.	169 RA patients	Positive correlation between serum leptin and insulin resistance.
Targońska-Stepniak et al. (2010)[19]	Cross-sectional study evaluating correlation between disease activity and serum adipokine levels.	80 RA patients	Positive correlation between serum leptin and DAS28.
Yoshino et al. (2011)[20]	Case-control study evaluating correlation between inflammation markers and serum adipokine levels.	141 RA patients 146 controls without RA	Positive correlation between serum leptin and CRP.
Kontunen et al. (2011)[21]	Cross-sectional study evaluating correlation between serum adipokines levels and markers of inflammation and MetS.	54 RA patients, 20 with MetS	Increased levels of serum leptin observed only in patients with MetS.
Olama et al. (2012)[14]	Case-control study evaluating differences between serum leptin and synovial/serum leptin ratio.	40 RA patients 30 controls without RA	Inverse correlation between leptin concentration and protection of joints in severe RA.
Allam and Radwan (2012)[27]	Case-control study evaluating correlation between serum leptin levels and disease activity.	37 RA patients 34 controls without RA	No correlation between leptin levels and disease activity.

Kang et al. (2013)[22]	Cross-sectional study evaluating correlation between adipokine levels, inflammation markers, insulin resistance and atherosclerosis.	192 RA patients	Positive correlation between serum leptin and TNF-α and metabolic risk, including insulin resistance
Mirfeizi et al. (2014)[29]	Cross-sectional study evaluating correlation between adipokine levels and radiographic joint damage.	54 RA patients (29 with erosion and 25 without erosion)	No differences in serum leptin between the two groups.
Abdalla et al. (2014)[28]	Case-control study evaluating correlation between serum leptin levels and clinical manifestations of disease activity.	60 RA patients 30 healthy controls	No correlation between leptin levels and clinical and laboratorial markers of disease activity.
Bustos Rivera-Bahena et al. (2015)[23]	Cross-sectional study evaluating correlation between adipokine levels and disease activity.	121 RA patients	Positive correlation between serum leptin and disease activity.
Xibille-Friedmann et al. (2015)[24]	Cohort study evaluating if baseline levels of adipokines may predict disease activity or response to treatment.	127 RA patients after 6 months of follow up; 91 after 1 year of follow up; 52 after 2 years of follow up	Positive correlation between serum leptin and prevention of disease activity progression.
Oner et al. (2015)[30]	Case-control study evaluating correlation between serum leptin levels and disease activity.	106 RA patients 52 healthy controls 37 osteoarthritis patients	No correlation between serum leptin and disease activity.

Lee and Bae (2016)[26]	Meta-analysis evaluating correlation between serum leptin levels and disease activity.	13 studies: 648 RA patients 426 controls without RA	Leptin levels significantly higher in RA patients and weak positive correlation between leptin levels and disease activity.

RA = rheumatoid arthritis; HOMA-IR = homeostatic model assessment-insulin resistance; DAS28 = Disease Activity Score-28; CRP = C-reactive protein; TNF-α = tumor necrosis factor-α; Metabolic Syndrome = MetS.

Table 2 - Studies investigating the association of resistin and rheumatoid arthritis in humans

Authors	Study design	Subjects	Results/outcomes
Kassem et al. (2010)[35]	Case-control study evaluating correlation between serum and synovial resistin and inflammation markers, disease activity and radiographic joint damage.	30 RA patients 15 healthy controls	Significant correlation between serum resistin levels and CRP, ESR, rheumatoid factor and disease activity. Also considered a good prognostic marker of RA.
Rho et al. (2010)[18]	Cross-sectional study evaluating correlation between HOMA-IR and serum adipokine levels.	169 RA patients	No significant correlation between serum resistin and insulin resistance.
Al-Kady et al. (2010)[38]	Case-control study evaluating correlation between serum and synovial liquid adipokines and disease activity.	70 RA patients 30 controls	No differences between groups in serum resistin, but it was observed synovial liquid resistin levels significantly higher in patients with active disease.
Yoshino et al. (2011)[20]	Case-control study evaluating correlation between inflammation markers and serum adipokines levels.	141 RA patients 146 controls	No differences in serum resistin between groups, but in RA patients it was positively associated with CRP levels.
Kontunen et al. (2011)[21]	Cross-sectional study evaluating correlation between serum adipokine levels and markers of inflammation and MetS in RA.	54 RA patients, 20 with MetS	Increased levels of resistin were associated with RA irrespective of the presence of MetS.
Fadda et al. (2013)[36]	Case-control study comparing serum and synovial liquid resistin	25 RA patients	Significant correlation between synovial liquid resistin,

	in patients with RA and osteoarthritis.	25 osteoarthritis patients	rheumatoid factor, and ACPA, indicating a bad prognosis of disease.
Kang et al. (2013)[22]	Cross-sectional study evaluating correlation between adipokines levels, inflammation markers, insulin resistance and atherosclerosis.	192 RA patients	Significant correlation between serum resistin and inflammation markers ESR and CRP and disease duration.
Hammad et al. (2014)[39]	Case-control study comparing serum resistin in RA patients and a control group and its association to disease activity.	30 RA patients 30 controls	No correlation between serum resistin levels and clinical and laboratorial markers of disease activity.
Bustos Rivera-Bahena et al. (2015)[23]	Cross-sectional study evaluating correlation between adipokines levels and disease activity.	121 RA patients	Positive correlation between resistin levels and disease activity.
Huang et al. (2015)[37]	Meta-analysis evaluating correlation between serum resistin levels and RA.	8 studies with RA: 620 RA patients 460 controls	Serum resistin levels were significantly higher in patients with RA.

RA = rheumatoid arthritis; CRP = C-reactive protein; TNF-α = tumor necrosis factor-α; ESR = erythrocyte sedimentation rate; ACPA = anti-citrullinated protein antibody; HOMA-IR = homeostatic model assessment-insulin resistance; Metabolic Syndrome = MetS.

Table 3 - Studies investigating the association of visfatin and rheumatoid arthritis in humans.

Authors	Study design	Subjects	Results/outcomes
Rho et al. (2010)[18]	Cross-sectional study evaluating correlation between HOMA-IR and serum adipokine levels.	169 RA patients	No correlation between visfatin levels and IR
Alkady et al. (2011)[51]	Case-control study evaluating correlation between serum and synovial liquid adipokines and disease activity.	70 RA patients 30 controls	Positive correlation between serum visfatin levels and disease activity.
Khalifa et al. (2013)[52]	Case-control study evaluating correlation between serum visfatin and inflammation markers.	60 RA patients 20 controls	Positive correlation between visfatin levels and IL-6, CRP, ERS, TNF-α and DAS-28 in RA.
El-Hini et al. (2013)[53]	Case-control study evaluating metabolic disorder and its association with clinical characteristics of RA patients.	40 RA patients 40 controls	Positive correlations between serum visfatin levels and IR, cholesterol, triglycerides and LDL-C.
Meyer et al. (2013)[55]	Cohort study evaluating serum adipokine levels and radiographic progression of RA.	632 RA patients at early stage of disease and 159 with unspecific arthritis	No correlation between visfatin levels and progression of RA.
Sglunda et al. (2014)[54]	Prospective study evaluating visfatin level and its relationship with disease activity and serum lipids.	40 patients with early, treatment-naïve RA 30 controls	Correlation between visfatin levels and disease activity and reduced levels after treatment.

Mirfeizi et al. (2014)[29]	Cross-sectional study evaluating correlation between serum adipokines levels and radiographic joint damage.	54 RA patients (29 with erosion and 25 without erosion)	The levels of visfatin were higher in patients with radiographic joint damage and dependent on the duration of the disease.

RA = rheumatoid arthritis; IR = insulin resistance; MetS = metabolic syndrome; IL-6 = interleukin-6; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; DAS28 = Disease Activity Score-28; TNF-α = tumor necrosis factor α; LDL-C = low-density lipoprotein cholesterol.

Table 4 – Studies investigating the association of adiponectin and rheumatoid arthritis in humans.

Authors	Study design	Subjects	Results/outcomes
Rho et al. (2010)[18]	Cross-sectional study evaluating correlation between HOMA-IR and serum adipokine levels.	169 RA patients	No correlation between adiponectin and insulin resistance.
Alkady et al. (2011)[51]	Case-control study evaluating correlation between serum and synovial liquid adipokines and disease activity.	70 RA patients 30 controls	Positive correlation between serum and synovial adiponectin levels and disease activity.
Yoshino et al. (2011)[20]	Case-control study evaluating correlation between inflammation markers and serum adipokine levels.	141 RA patients 146 controls	No correlation between serum adiponectin levels and CRP.
Giles et al. (2011)[66]	Prospective study evaluating association of serum adipokine levels with progression of radiographic joint damage in patients with rheumatoid arthritis.	152 RA patients	Positive correlation between serum adiponectin levels and erosive joint destruction.
Klein-Wieringa et al. (2011)[67]	Cohort study evaluating baseline adipokine levels to predict radiographic progression of RA over a period of 4 years.	253 RA patients	Positive correlation between serum levels of adiponectin and radiographic progression of RA.
Meyer et al. (2013)[55]	Cohort study evaluating serum adipokines levels and radiographic progression of RA.	632 RA patients at early stage of disease and 159 with unspecific arthritis	Positive association between serum adiponectin levels and

			radiographic progression of RA at early stage.
Bustos Rivera-Bahena et al (2015)[23]	Cross-sectional study evaluating correlation between adipokines levels and disease activity.	121 RA patients	No correlation between serum adiponectin and clinical activity of RA, but negative correlation with TNFα and positive correlation with IL-1β.
Chennareddy et al. (2016)[69]	Cross-sectional study evaluating the serum concentrations of adiponectin and its impact on disease activity and radiographic joint damage.	43 RA patients 25 controls	Increased levels of serum adiponectin in RA, but no correlation with erosive and non-erosive disease, disease duration, BMI, waist-hip ratio and disease activity.

RA = rheumatoid arthritis; IR = insulin resistance; CRP = C-reactive protein; BMI = body mass index.

5.2 ARTIGO: CRANBERRY JUICE ADDS BENEFICIAL EFFECTS ON DISEASE ACTIVITY AND INFLAMMATORY BIOMARKERS IN PATIENTS WITH RHEUMATOID ARTHRITIS SUPPLEMENTED WITH FISH OIL

E.C.S. Fatel1, F.T. Rosa2, D.F. Alfieri1, T. Flauzino1, B.M. Scavuzzi1, M.A.B. Lozovoy3, T.M.V. Iriyoda4, A.N.C. Simão3, and I. Dichi5.

1Health Science Graduate Program, Universidade Estadual de Londrina - Avenida Robert Koch, 60, Londrina, Paraná, Brazil; 2Department of Nutrition, Centro Universitário Filadélfia Londrina (UNIFIL) - Avenida Juscelino Kubitscheck, 1626, Londrina, Paraná, Brazil; 3Department of Pathology, Clinical Analysis and Toxicology, Universidade Estadual de Londrina - Rodovia Celso Garcia Cid, Km 380, s/n - Campus Universitário, Londrina, Paraná, Brazil; 4Rheumatologist in the University Hospital of Universidade Estadual de Londrina - Avenida Robert Koch, 60, Londrina, Paraná, Brazil; 5Department of Internal Medicine, Universidade Estadual de Londrina - Avenida Robert Koch, 60, Londrina, Paraná, Brazil.

Corresponding author: Isaias Dichi, MD, PhD. Department of Internal Medicine. Avenida Robert Koch n. 60, Cervejaria, Universidade Estadual de Londrina - Londrina, Paraná, Brazil. CEP: 86038-440 Tel: (55) 43 3371 2234 E-mail: dichi@sercomtel.com.br

Cranberry, Fish Oil in Rheumatoid Arthritis

Keywords: Rheumatoid arthritis, fish oil n-3 fatty acids, cranberry, polyphenol, DAS 28

ABSTRACT

The beneficial effect of fish oil in Rheumatoid arthritis (RA) patients is well known. However, the effects of cranberry juice on RA have not been reported. The aim of this study was to verify whether cranberry juice intake could improve clinical and laboratory parameters of disease activity in patients with RA using fish oil supplements. A prospective study with 62 RA patients was performed. Disease activity (DAS28) and several inflammatory markers were analyzed. The first group (control group - C, n=21) maintained their usual diet; the second group (fish oil group, n=21) received 3 g/d of fish oil *n*-3 fatty acids (10 capsules) and the third group (fish oil and low calorie cranberry juice, n=20) consumed 3 g/d of fish oil *n*-3 fatty acids (10 capsules) and 500 mL/d of reduced-energy cranberry juice. In relation to the baseline values, the group receiving fish oil and cranberry showed decreased erythrocyte sedimentation rate (ESR) ($p=0.033$), C-reactive protein (CRP), DAS28 ($p=0.001$), adiponectin ($p=0.021$), and interleucin (IL)-6 levels ($p=0.045$), whereas the group which consumed fish oil showed decreased DAS28 ($p=0.0261$) and adiponectin levels ($p=0.0239$). Differences across treatments showed that the group which consumed fish oil and cranberry had decreases in ESR and CRP ($p < 0.05$) compared with the controls and with the group which consumed fish oil, whereas reduction in DAS28 was verified when the fish oil and cranberry group was compared to the control group. The present study indicated that cranberry juice could add beneficial effects to fish oil, decreasing disease activity and inflammatory biomarkers in RA patients.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that leads to severe joint destruction, affecting millions of people worldwide with prevalence from 0.5 to 1% [1]. Several studies have reported beneficial effects of fish oil n-3 polyunsaturated fatty acids in inflammatory diseases [2,3], and especially in RA [4-7]. A considerable number of anti-inflammatory mechanisms have been attributed to the role of fish oil in inflammatory conditions, such as reduced agonist-induced activation of transcriptional factors nuclear factor-κB (NF-κB) and increased peroxisome proliferator-activated receptor γ (PPARγ); with potential effects on anti-inflammatory and antiatherogenic properties [8]; decreases in inflammatory markers such as tumor necrosis factor α (TNF- α) and interleukin (IL) -1 [9] and in vascular cell adhesion molecule-1, E-selectin, intercellular adhesion molecule-1, IL-6, and IL-8 [10]; decreased synthesis of inflammatory prostaglandins and leukotrienes [11], and a lower prevalence of sub-clinical atherosclerosis [12]. The more evident beneficial effects of n-3 fatty acids in patients with RA are on swollen and painful joints, duration of morning stiffness, global assessment of disease activity and decreasing the production of pro inflammatory mediators [4,6]. Therefore, fish oil has been considered a valuable support to traditional pharmacologic treatment in RA [5,13].

Whereas the impact of inflammatory chronic diseases on mortality has diminished dramatically over the years due to intensive treatment, cardiovascular risk has emerged as one of the most important cause of morbidity and mortality in these patients [14,15]. The pathogenesis of atherosclerosis, the main factor which underlies cardiovascular diseases (CVD), is thought to be mediated, at least partially, by inflammation[16]. Diets rich in fruits and vegetables enhance polyphenolic intake and are protective against CVD. Cranberry *(Vaccinium macrocarpon)* juice is unique among fruit juices because it has a relatively low natural carbohydrate content compared to its high content of vitamins, minerals, and polyphenolic compounds including flavonols (myricetin and quercetin), anthocyanins and proanthocyanidins, which confers to any cranberry-derived products, like juice, a potent antioxidant activity [17]. These phenolic compounds have a wide range of biological effects including the ability to serve as antioxidants, modulate enzyme activity, and regulate gene expression [17]. Cranberry juice consumption has been associated with a reduction of surrogate biomarkers of CVD risks as reported in clinical studies [18-23]. Intervention trials have reported beneficial effects of cranberry in oxidative stress, dyslipidemia, and inflammatory biomarkers in healthy volunteers[22,24] , as well as in patients with type 2 diabetes mellitus [25] and metabolic syndrome (MetS) [26].

In a previous study with MetS patients, our group verified a significant decrease in protein oxidation and lipoperoxidation in individuals receiving reduced- calorie cranberry juice [27]. Beneficial effects on cardiovascular biomarkers, such as adiponectin and homocysteine levels were also shown. To date, we are not aware of any study, which has evaluated the effects of cranberry in patients with RA. Therefore, the purpose of the present study was to verify whether of cranberry juice intake could improve clinical and laboratory parameters of disease activity in patients with RA using fish oil supplements.

METHODS

Subjects

This study included 62 RA patients (50 women and 12 men), aged between 43 and 65 years. Patients with RA were selected from the Rheumatology outpatient clinic of the University Hospital of Londrina, Paraná, Brazil. None of the participants in the study presented heart, thyroid, renal, hepatic, gastrointestinal or oncological diseases, and none were receiving estrogen replacement therapy, hipolipemiant or hypoglicemiant drugs or antioxidant supplements. All the individuals reported that they did not smoke and did not drink alcohol regularly. Patients who were taking antihypertensive drugs were not excluded and were allowed to continue taking the same dose of the drugs. None of the subjects followed a specific diet before the inclusion in the study no use of immunobiological therapy TNF-α inihibitor. The patients were instructed by a nutritionist to maintain their usual diets, alcohol intake, level of physical activity, or other lifestyle factors throughout the intervention period.

Non-compliance was verified in two patients from the control group, two patients from the fish oil group and four patients from the cranberry juice group (Figure 1).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the University of Londrina Paraná, Brazil. Written informed consent was obtained from all subjects/patients. Trial registry name: “Influência da ingestão de óleo de peixe e suco de cranberry de baixa caloria sobre o processo inflamatório e o estresse oxidativo em pacientes com artrite reumatoide”, registration identification number: CAAE: 13426014.6.0000.5231, URL for the registry:
http://aplicacao.saude.gov.br/plataformabrasil/login.jsf;jsessionid=80E997928B85A42C6B74F3420214D98C.server-plataformabrasil-srvjpdf130

Study Design

Patients with RA were assigned to one of three groups into three groups after stratification by age, sex, and waist circumference (WC): the first group (control group (C), n=21) was only directed to maintain their usual diet; the second group (fish oil group (FO), n=21) received 3 g/d of fish oil *n*-3 fatty acids (10 capsules) and the third group (fish oil and low calorie cranberry juice (FOCR), n=20) consumed 3 g/d of fish oil *n*-3 fatty acids (10 capsules) and 500 mL/d of reduced-energy cranberry juice. Opção Ad Oceanum, LTDA, Grande Florianópolis, SC, Brazil, manufacturers of pharmaceutical products provided fish oil capsules and Juxx Company, Barueri, São Paulo, Brazil supplied the cranberry juice.

Each fish oil capsule contained 180 mg of eicosapentaenoic acid (EPA) and 120 mg of docosahexaenoic acid (DHA) originated from sardines. The capsules were given at breakfast, lunch, and dinner. The subjects were recommended to avoid resting after meals to avoid unpleasant effects. The juice was given at lunch and dinner. The subjects were recommended to avoid resting after meals to prevent unpleasant effects. Interviews were performed to assure no change in lifestyle factors throughout the study. The nutrient composition of 200 mL of cranberry juice was as follows: 20 Kcal, 0 g of protein, 5g of carbohydrate, 0 g of lipids, 0 g of fiber, 30 mg of sodium, vitamin C 60 mg, 65.96 mg of proanthocyanidins, total phenolics of 103.5 mg, and 0.12 mg of folic acid. The total antioxidant power of cranberry juice determined by Oxygen Radical Antioxidant Capacity (ORAC) was 183.65 umol TE/mL. Evaluation of clinical and laboratorial parameters was assessed at the beginning of the study and after 90 days.

Steps taken to optimize compliance

Various measures were taken to optimize and to assess patient compliance. Before the begging of each trial, it was assured that the patients understood that they could be allocated to any group. Boxes of fish oil capsules were handed out at the initial interview and at the two later visits. The participants were asked to return the boxes at each visit so the number of capsules taken could be estimated by questioning the patients and by counting the remaining capsules. Boxes of cranberry juice were handed out at the initial interview and at the two visits later. Subjects were asked to bring back any unconsumed juice to assess unmonitored compliance. In addition, telephone interviews were performed to evaluate whether the patients were using correctly the supplements and to avoid lifestyle changes.

Clinical Evaluation

Waist circumference (WC) was measured with a soft tape on standing subjects midway between the lowest rib and the iliac crest. Patients were selected according to

the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria [28], and disease activity status was determined using the DAS28-C-reactive protein (CRP) [29], according to the formula on the DAS website [30], by a single blind rheumatologist. The DAS28-CRP considers 28 tender and swollen joint counts, general health (GH; patient assessment of disease activity using a 100 mm visual analogue scale where 0=best, 100=worst), plus levels of CRP (mg/L). DAS28 values categorize patients into four different groups, namely, remission group: DAS28 ≤ 2.6; low disease activity group: 2.6 < DAS28 ≤ 3.2; moderate disease activity group 3.2 < DAS 28 ≤ 5.1; and high disease activity group: DAS28 > 5.1 [31].

Immunological, and Inflammatory Biomarkers

After fasting for 12h, the patients underwent the following laboratory blood analysis: Serum high sensitivity CRP (hsCRP) and rheumatoid factor (RF) were measured using immunoturbidimetric assay (C8000 Architect Abbott Laboratories, Abbott Park, IL, USA). Anti – citrullinated cyclic peptide antibodis (anti-CCP) levels were assayed using the chemiluminescence microparticle immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA).

IL-6 levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial immunoassay ELISA (Ready-Set Go! Set, e-Bioscience, San Diego, California, USA). Plasma levels of adiponectin and leptin were measured using a sandwich enzyme-linked immunosorbent assay (ELISA), (Duo Set, R&D System, Minneapolis, MN, USA). Erythrocyte sedimentation rate (ESR) was obtained by automated kinetic-photometric method (Ves-Matic CUBE 30, DIESSE, Siena, Italy).

Statistical analysis

Categorical data were analyzed with chi-square test. Absolute number and percentage demonstrated the results. The Wilcoxon matched pairs test was performed to verify changes from baseline (intra-group changes). The Kruskal–Wallis with post hoc Dunn's test was performed to compare baseline values and differences across treatment groups (inter-group changes). Data are expressed as the medians and 25th–75th percentiles. The results were considered significant when $p < 0.05$. The statistical analysis program Graph Pad Prism version 4.0 (San Diego, CA, USA), was used for evaluations.

The sample size was estimated statistically for each group considering a statistical power of 80% and the two-sided significance level of $p < 0.05$. The sample size was calculated in order to detect statistical differences of at least 10% in the evaluated parameters. Cal Stat

program was used for sample size calculation, based on the average and standard deviation for some of the parameters evaluated previously in other studies.

RESULTS

Non-compliance was verified in eight patients, two from the control group, two from the fish oil group and four from the cranberry juice group (Figure 1).

Demographic and clinical characteristics of the patients with RA are shown in table 1. There were no differences between the groups in relation to age, sex, ethnicity, extra articular involvement, physical activity, tabagism, and medications of the patients. Also, ESR, CRP, IL-6 and DAS 28 values were not statistically different at the beginning of the study (data not shown).

Regarding the baseline values (table 2), the group which consumed fish oil and cranberry showed a decrease in ESR ($p=0.033$) and CRP ($p= 0.002$) values. In addition, this group had a reduction in several other parameters related to disease activity, such as DAS28 ($p=0.001$), adiponectin ($p=0.021$), and IL-6 levels ($p=0.045$). In the meantime the group which only consumed fish oil also showed a decrease in DAS28 ($p=0.045$) and adiponectin levels ($p=0.024$), but not in ESR, CRP and IL-6. At last, the control group showed a reduction in adiponectin levels ($p=0.017$). Statistically significant changes in RF, anti-CCP and leptin were not observed in the groups in relation to the baseline values (table 2). Also, there were not significant differences in WC in the groups before and after the study (data not shown). Differences across treatments (table 2) showed that the group which consumed fish oil and cranberry had significant decreases ($p < 0.05$) in ESR and CRP compared with the controls and with the group which only consumed fish oil, whereas reduction in DAS28 was verified when the fish oil and cranberry group was compared to the control group.

DISCUSSION

The main findings of the present study confirmed the hypothesis that the group of RA patients that associated fish oil and cranberry juice would have an additional improvement of disease activity than the group that only consumed fish oil. This effect was shown by the significant decrease in inflammatory biomarkers ESR, CRP and IL-6, and also by a robust significant decrease in disease activity score.

Accordantly to other reports, the current study found similar results with those from other authors who did not find any significant difference using fish oil in relation to the pro-

inflammatory markers ESR [4], CRP [4,33,34] and IL-6 [34]. Classically, arachdonic acid metabolites inhibition, especially leukotriene (LT) B4, is considered as one of the main mechanisms by which fish oil exerts its action, but many other mediators are also decreased, such as proinflammatory cytokines [35]. Therefore, fish oil inhibition of proinflammatory nuclear transcription factor κB in several tissues activating peroxisome proliferator activated receptor-γ and many other signaling proteins is a mechanism which has deserved considerable attention due to the potential possibility of using medications and nutritional interventions, such as fish oil, to regulate these transcription factors mediated pathway [3].

In the current study, adiponectin levels decreased in all groups. This reduction is considered beneficial in RA since this adipocytokine is considered to have proinflammatory effects in RA [36-39], differently from other metabolic diseases, such as obesity, MetS and diabetes mellitus, where it has anti-inflammatory effects [40,41]. Scotece et al. [37] reported that adiponectin induces prostaglandin (PG) E2, IL-6, IL-8, matrix metalloproteinase (MMP)-1 and MMP-13 in human synovial fibroblasts and also induces nitric oxide (NO), IL-6, MMP-3, MMP-9, MCP-1 and IL-8 in human condrocytes which leads to inflammation by increasing TNF-α and IL-6. Frommer et al. [38] showed a destructive role in all adiponectin isoforms in RA patients reinforcing the detrimental action of adiponectin in this chronic inflammatory disease. Unexpectedly, the control group also reduced the adiponectin levels. Although the effect of medications used for RA could be suggested to explain adiponectin reduction in all groups, no study has demonstrated such effect yet. In contrast to the current findings, other studies reported higher adiponectin levels with the treatment with methotrexate and low prednisolone dose in patients with RA [42] or with sulfasalazine, hydroxychloriquine or infliximab [43]. Altogether, these data seem to suggest the need for more cross-sectional and longitudinal studies to clarify the scenario on the role of adiponectin in RA pathophysiology.

We are not aware of any study which has verified the effect of cranberry juice in RA patients. Duffey & Sutherland [44] reported that cranberry juice consumers had lower CRP levels than non-consumers. Other studies with individuals in different conditions, such as healthy subjects [45], type 2 diabetes mellitus [25] and MetS [26] also found a decrease in CRP [25,26,45] and IL-6 values [26] with cranberry juice. However, in a study performed by our group with MetS patients, low-calorie cranberry juice (700 mL/d) given for two months was not able to decrease CRP or IL-6 values [27]. The decrease in inflammatory biomarkers only verified in RA patients who used fish oil associated with cranberry juice, but not isolated, reinforces not only the well-known cranberry antioxidant effect, but also its important anti-inflammatory action [46]. The reduction in pro-inflammatory cytokines is important in RA pathophysiology since their increased levels can indicate disease severity [47]. IL-6 is considered to be the major mediator

of the hepatic acute-phase reaction and is thought to play a central role in the pathogenesis of CVD in patients with insulin resistance and MetS [48]. In addition, inflammation, demonstrated primarily by elevated levels of serum CRP, is thought to be associated with both insulin resistance and MetS [49,50]. Therefore, it is possible to assume that reduced CRP and IL-6 levels verified in the group who consumed both fish oil and cranberry juice could have a potential influence in decreasing CVD risk.

Although ESR can be influenced by confounding factors such as age, sex, fibrinogen levels, hypergammaglobulinemia, RF and anemia, ESR showed a reduction in the group which associated fish oil and cranberry juice. ESR tends to reflect disease activity of the past few weeks, whereas CRP reflects more short-term changes in disease activity. Therefore, the advantage of CRP is that it is more sensitive to short-term changes in disease activity [29].

Studies have reported several mechanisms by which cranberry and components could exert their biological actions. There is accumulating evidence that quercetin, a flavonoid found in large quantities in cranberries, is a potent down-regulator of the nuclear factor-kappa B (NF-kB) pathway [51]. In addition, resveratrol, a polyphenol also present in cranberry juice, has been shown to suppress the expression of inflammatory genes relevant to CVD through the modulation of the NF-kB and JAK/STAT^3 pathways in cultured cells [17]. Numerous genes of inflammatory proteins are under the regulation of NF-kB, including adhesion molecules, IL-6 and TNF-α. Other components like proanthocyanidin, anthocyanidins, hydroxycinnamic acid and acetylsalicylic acid that can be found in cranberries have all been shown to prevent expression of adhesion molecules induced by TNF-α through their inhibitory action on NF-kB activation [23,24,52]. In addition, flavonols, such as quercetin, found in some fruit including berries, have been shown to inhibit cyclooxygenase and lipoxygenase activities [53], enzymes which are released after arachidonic acid stimulus, the initiator of a general inflammatory response.

The following limitations must be considered in the present study: first, the small number of participants, although sample size calculation showed that the number of patients was above of the minimum required; second, the lack of a placebo group, although several studies pointed out the difficulty in obtaining a placebo group in fish oil studies due to the fishy taste the patients have while consuming the product. In addition, many studies with cranberry juice did not use a placebo group [24,54]. Third, the absence of an isolated cranberry juice group does not allow us to have more definite conclusions.

Nevertheless, this study has several strengths. The current study is the first in our knowledge to use concomitantly fish oil and cranberry juice to evaluate disease activity and inflammatory biomarkers in patients with RA. In addition, medications and associated diseases, which could interfere in the results, were rigorously monitored. At last, demographic

and clinical characteristics were similar in the different groups.

In conclusion, the present study confirmed previous reports on disease activity of fish oil in patients with RA. In addition, the original hypothesis which underlies this study, that cranberry juice would add beneficial effects to fish oil was confirmed both in the robust findings of decreasing disease activity and also by inflammatory biomarkers results. These findings may open new avenues to analyze the effects of cranberry in decreasing disease activity in RA patients, but this issue must be confirmed in other studies, and especially with an additional group only consuming cranberry. Overall, this study reinforce the role of fish oil in patients with RA, but also points out the need for more studies with cranberry in this highly prevalent chronic disability disease.

FINANCIAL SUPPORT

This research was supported by the National Council of Brazilian Research – CNPq.

The Juxx Company supplied the cranberry juice. Juxx Company had no role in the design, analysis or writing of this article.

CONFLICT OF INTEREST

None.

AUTHORSHIP

ECSF, FTR and TMVI collected the data, interpreted the results and wrote the manuscript; DFA, TF and MABL collected data, performed cytokine analysis and wrote the manuscript; BMS collected data, performed cranberry analysis and wrote the manuscript; ANCS interpreted the results, designed the study, wrote the manuscript and performed the statistical analysis and ID developed the hypothesis tested in the study, designed the study, interpreted the results and wrote the manuscript.

REFERENCES:

1. Myasoedova E, Crowson CS, Kremers HM *et al*. (2010). Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007. *Arthritis Rheum* **62**,1576–1582.

2. Barbosa DS, Cecchini R, El Kadri MZ, *et al*. (2003). Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty acids. *Nutrition* **19**,837–842.

3. Calder PC (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta* **1851**,469–484.

4. Berbert AA, Kondo CR, Almendra CL, *et al*. (2005). Supplementation of fish oil and olive oil in patients with rheumatoid arthritis. *Nutrition* **21**,131–136.

5. James M, Proudman S, Cleland L (2010). Fish oil and rheumatoid arthritis: past, present and future. *Proc Nutr Soc* **69**,316–323.

6. Miles EA, Calder PC (2012). Influence of marine n-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis. *Br J Nutr* **107,**S171-184.

7. Proudman SM, James MJ, Spargo LD, *et al*. (2015). Fish oil in recent onset rheumatoid arthritis: a randomised, double-blind controlled trial within algorithm-based drug use. *Ann Rheum Dis* **74,**89–95.

8. Jung UJ, Torrejon C, Tighe AP, *et al*. (2008). n-3 Fatty acids and cardiovascular disease: mechanisms underlying beneficial effects. *Am J Clin Nutr* **87**,2003S–9S.

9. Endres S, Ghorbani R, Kelley VE, *et al*. (1989). The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* **320**,265–271.

10. De Caterina R, Liao JK, Libby P (2000). Fatty acid modulation of endothelial activation. *Am J Clin Nutr* **71**,213S–223S.

11. Calder PC (2006). n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* **83**,1505S–1519S.

12. He K, Liu K, Daviglus ML, *et al*. (2008). Intakes of long-chain n-3 polyunsaturated fatty acids and fish in relation to measurements of subclinical atherosclerosis. *Am J Clin Nutr* **88**,1111–1118.

13. Cleland LG, Caughey GE, James MJ, *et al*. (2006). Reduction of cardiovascular risk factors with longterm fish oil treatment in early rheumatoid arthritis. *J Rheumatol* **10,** 1973-1979.

14. Pereira IA, Mota LMH, Cruz BA, *et al*. (2012). Consenso 2012 da Sociedade Brasileira de Reumatologia sobre o manejo de comorbidades em pacientes com artrite reumatoide. Rev Bras Reumatol . *Sociedade Brasileira de Reumatologia* **52**,483–495.

15. Picerno V, Ferro F, Adinolfi A, *et al*. (2015). One year in review: the pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol* **33**,551–558.

16. Ross R (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med* **340**,115–126.

17. McKay DL, Blumberg JB (2007). Cranberries (*Vaccinium macrocarpon*) and cardiovascular disease risk factors. *Nutr Rev* . ;**65**,490–502.

18. Pedersen CB, Kyle J, Jenkinson AM, *et al*. (2000). Effects of blueberry and cranberry juice consumption on the plasma antioxidant capacity of healthy female volunteers. Eur J Clin Nutr . ;**54**,405–408.

19. Vinson JA, Su X, Zubik L, *et al*. (2001). Phenol antioxidant quantity and quality in foods: fruits. *J Agric Food Chem* .;**49**,5315–5321.

20. Porter ML, Krueger CG, Wiebe DA, *et al*. (2001). Cranberry proanthocyanidins associate with low-density lipoprotein and inhibitin vitro Cu2+-induced oxidation. *J Sci Food Agric* **81**,1306–1313.

21. Yan X, Murphy BT, Hammond GB, *et al*. (2002). Antioxidant activities and antitumor screening of extracts from cranberry fruit (Vaccinium macrocarpon). *J Agric Food Chem* **50**,5844–5849.

22. Ruel G, Pomerleau S, Couture P, *et al*. (2005). Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. *Metabolism* **54**,856–861.

23. Ruel G, Couillard C (2007). Evidences of the cardioprotective potential of fruits: the case of cranberries. *Mol Nutr Food Res* **51**,692–701.

24. Ruel G, Pomerleau S, Couture P, *et al*. (2008). Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. *Br J Nutr* **99**,352–359.

25. Lee IT, Chan YC, Lin CW, *et al*. (2008). Effect of cranberry extracts on lipid profiles in subjects with Type 2 diabetes. *Diabet Med* **25**,1473–1477.

26. Basu A, Betts NM, Ortiz J, *et al*. (2011). Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. *Nutr Res* **31**,190–196.

27. Simão TNC, Lozovoy MAB, Simão ANC, *et al*. (2013). Reduced-energy cranberry juice increases folic acid and adiponectin and reduces homocysteine and oxidative stress in patients with the metabolic syndrome. *Br J Nutr* **110**,1885–1894.

28. Aletaha D, Neogi T, Silman AJ, *et al*. (2010). Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* **69**,1580–1588.

29. Fransen J, Welsing P M, Keijzer R M van RPL (2004). Disease activity scores using C-reactive protein: CRP may replace ESR in the assessment of RA disease activity. *Ann Rheum Dis* **62**, Suppl:151.

30. DAS28 - Home of the Disease activity score and DAS28 . Available from: http://www.das-score.nl/das28/en/(accessed November 2016).

31. Prevoo ML, van't Hof MA, Kuper HH, , *et al*. (1995). Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* **38**,44–48.

32. Haffner SM, Miettinen H, Stern MP (1997). The homeostasis model in the San Antonio Heart Study. *Diabetes Care* **20**,1087–1092.

33. Skulas-Ray AC (2015). Omega-3 fatty acids and inflammation: A perspective on the challenges of evaluating efficacy in clinical research. *Prostaglandins Other Lipid Mediat* **116**,104–111.

34. Muldoon MF, Laderian B, Kuan DCH, , *et al*. (2016). Fish oil supplementation does not lower C-reactive protein or interleukin-6 levels in healthy adults. *J Intern Med* **279**,98–109.

35. James MJ, Gibson RA, Cleland LG (2000). Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* **71**:343S–348S.

36. Klein-Wieringa IR, Van Der Linden MPM, Knevel R, *et al*. (2011). Baseline serum adipokine levels predict radiographic progression in early rheumatoid arthritis. *Arthritis Rheum* **63**,2567–2574.

37. Scotece M, Conde J, Gomez R, Lopez V, *et al*. (2012). Role of adipokines in atherosclerosis: Interferences with cardiovascular complications in rheumatic diseases. *Mediators Inflamm* **2012**,1–14.

38. Frommer KW, Schäffler A, Büchler C, *et al*. (2012). Adiponectin isoforms: a potential therapeutic target in rheumatoid arthritis? *Ann Rheum Dis* **71**,1724–1732.

39. Skalska U, Kontny E (2016). Adiponectin Isoforms and Leptin Impact on Rheumatoid Adipose Mesenchymal Stem Cells Function. *Stem Cells Int* **2016**,1–7.

40. Dichi I, Simão ANC (2012). Metabolic syndrome: new targets for an old problem. *Expert Opin Ther Targets* **16**,147–150.

41. Abella V, Scotece M, Conde J, *et al*. (2014). Review Article Adipokines , Metabolic Syndrome and Rheumatic Diseases. *J Immunol Research* **2014**,1–15.

42. Yaşar Bilge NŞ, Kaşifoğlu N, Kaşifoğlu T, *et al*. (2016). The role of methotrexate and low-dose prednisolone on adiponectine levels and insulin resistance in patients with rheumatoid arthritis naïve to disease-modifying antirheumatic drugs. *Int J Rheum Dis* .;**19**,665–671.

43. Engvall I-L, Tengstrand B, Brismar K, , *et al*. (2010). Infliximab therapy increases body fat mass in early rheumatoid arthritis independently of changes in disease activity and levels of leptin and adiponectin: a randomised study over 21 months. *Arthritis Res Ther* **12,**R197.

44. Duffey KJ, Sutherland LA (2015). Adult consumers of cranberry juice cocktail have lower C-reactive protein levels compared with nonconsumers. *Nutr Res* **35**,118–126.

45. Novotny JA, Baer DJ, Khoo C, *et al*. (2015). Cranberry juice consumption lowers markers of cardiometabolic risk, including blood pressure and circulating C-reactive protein, triglyceride, and glucose concentrations in adults. *J Nutr* **145**,1185–1193.

46. Cassidy A, Rogers G, Peterson JJ, *et al*. (2015). Higher dietary anthocyanin and flavonol intakes are associated with anti-inflammatory effects in a population of US adults. *Am J Clin Nutr* **102**,172–181.

47. Mateen S, Zafar A, Moin S, *et al*. (2016). Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. *Clin Chim Acta* **455**,161–171.

48. Yudkin JS, Kumari M, Humphries SE, *et al*. (2000). Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* **148**,209–214.

49. Festa A, D'Agostino R, Howard G, *et al*. (2000). Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* **102**,42–47.

50. Tamakoshi K, Yatsuya H, Kondo T, *et al*. (2003). The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. *Int J Obes Relat Metab Disord* **27**,443–449.

51. Martínez-Flórez S, Gutiérrez-Fernández B, Sánchez-Campos S, *et al*. (2005). Quercetin attenuates nuclear factor-kappa B activation and nitric oxide production in interleukin-1beta-activated rat hepatocytes. *J Nutr* **135**,1359–1365.

52. Youdim KA, McDonald J, Kalt W, *et al*. (2002). Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *J Nutr Biochem* **13**,282–288.

53. Nijveldt RJ, van Nood E, van Hoorn DE, *et al*. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr* **74**,418–425.

54. O'Byrne DJ, Devaraj S, Grundy SM, *et al*. (2002). Comparison of the antioxidant effects of Concord grape juice flavonoids alpha-tocopherol on markers of oxidative stress in healthy adults. *Am J Clin Nutr* **76**,1367–1374.

Figure 1 – Schematic of subject flow and reasons for exclusion

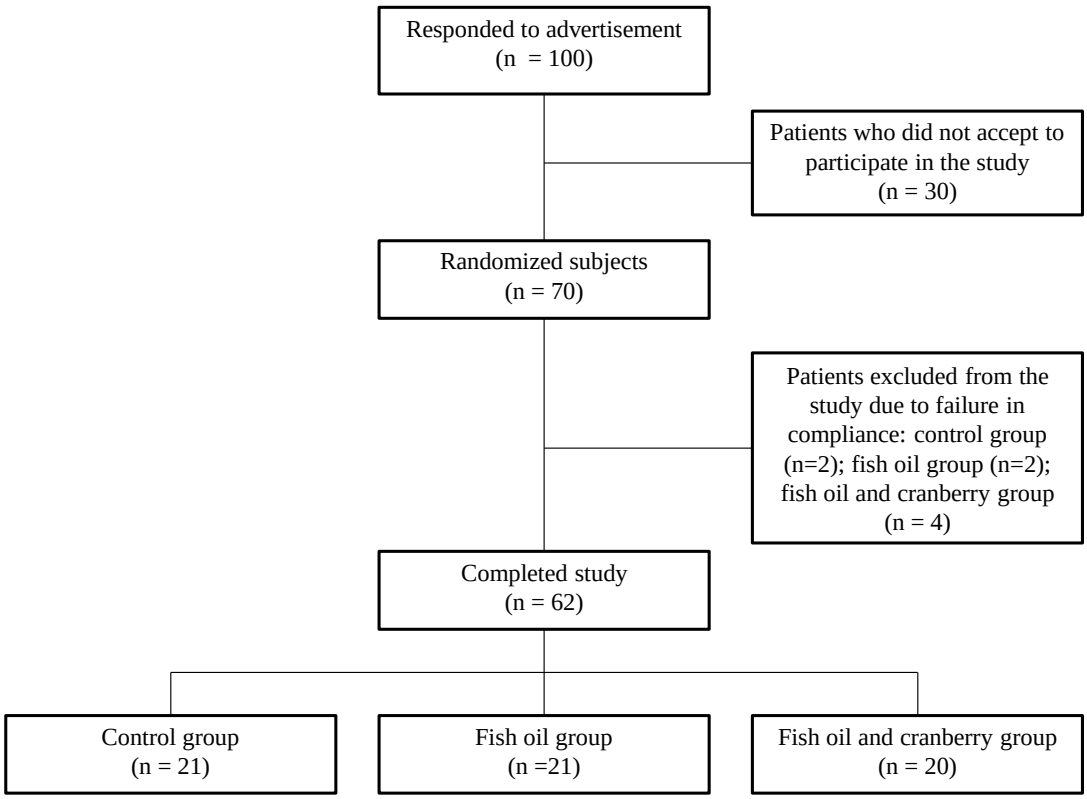


Table 1 – Demographic and clinical characteristics in the control (C), fish oil (FO) and fish oil associated with cranberry (FOCR) groups of patients with rheumatoid arthritis (RA) at the beginning of the study

Characteristics	**C (n=21)**	**FO (n=21)**	**FOCR (n=20)**	**C vs FO**	**C vs FOCR**	**FO vs FOCR**
Age (years)	52 (43-63)	58 (47-64)	58 (47-65)	NS	NS	NS
Sex						
Female	18	17	15	NS	NS	NS
Male	3	4	5			
Ethnicity						
Caucasian	19	18	18	NS	NS	NS
Non caucasian	2	3	2			
Extra articular RA						
Yes	0	1	2	NS	NS	NS
No	21	20	18			
Physical activity						
Yes	5	4	2	NS	NS	
No	16	17	18			NS
Tabagism						
Yes	2	1	1	NS	NS	NS
No	19	20	19			
Prednisone						
Yes	17	17	12	NS	NS	NS
No	4	4	8			
Metotrexate						
Yes	12	12	13	NS	NS	NS
No	9	9	7			
Hydroxychloriquine						

Yes	9	11	7	NS	NS	NS
No	12	10	13			
Leflunomide						
Yes	12	12	12	NS	NS	NS
No	9	9	8			

The Kruskall-Wallis test. Data are shown in median and interquartile range (25% -75%); NS, non-significant

Table 2 – Evaluation of biomarkers related to rheumatoid arthritis disease activity in the control (C), fish oil (FO) and fish oil associated with cranberry (FOCR) groups at the baseline and after 90 days.

Biomarker	C (n=21)			FO (n=21)			FOCR (n=20)		
	T0	T90	P value	T0	T90	P value	T0	T90	P value
ESR (mm/h)*	28.0 (11.5-39.5)	19.0 (13.0-39.5)	NS	21.5 (11.5-39.0)	26.5 (21.0-45.0)	NS	16.0 (10.0-27.0)	11.0 (5.5-29.0)	**0.033**
CRP (mg/L)*	5.5 (2.0-9.5)	3.9 (1.7-11.1)	NS	3.7 (1.9-5.5)	7.1 (3.9-12.6)	NS	3.7 (1.7-7.5)	2.5 (1.9-5.1)	**0.002**
DAS 28 (CRP)#	2.96 (2.55-4.12)	2.77 (2.42-3.52)	NS	3.65 (2.63-4.42)	2.98 (2.47-3.53)	**0.045**	2.57 (2.22-3.21)	1.90 (1.64-2.36)	**0.001**
RF (UI / mL)	15.0 (12.9-47.5)	28.8 (3.9-54.1)	NS	58.1 (15.0-131.2)	50.5 (9.5-83.7)	NS	15.8 (6.6-150.9)	46.0 (34.2-125.5)	NS
Anti-CCP (U/mL)	4.2 (0.5-28.5)	3.9 (0.50-20.0)	NS	21.2 (0.5-136,2)	17.0 (0.5-221.5)	NS	2.0 (0.5-120.0)	2.9 (0.5-100.5)	NS
Adiponectin (pg/ml)	2002 (1462-2548)	1417 (1146-2019)	**0.017**	1577 (1163-2173)	1409 (1025-1821)	**0.024**	2346 (1693-3169)	1958 (1581-3011)	**0.021**
Leptin (ng/ml)	6.53 (2.05-15.88)	5.52 (1.96-11.36)	NS	6.27 (3.48-9.88)	7.51 (3.70-12.46)	NS	9.23 (3.10-16.02)	11.00 (4.88-15.60)	NS
IL-6 (pg/ml)	6.34 (2.74-18.91)	3.25 (1.27-12.99)	NS	5.46 (2.59-15.21)	10.18 (4.29-20.62)	NS	3.24 (1.00-5.51)	1.00 (1.00-2.14)	**0.045**

Data are shown in median and interquartile range (25% -75%). The Wilcoxon test was performed to verify changes from baseline (intra-group changes). The Kruskall-Wallis test was performed to compare differences between the baseline values and across treatment groups (inter-group changes). ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS 28 (CRP), *Disease Activity Score*-28; RF, rheumatoid factor; Anti-CCP, anti-cyclic citrullinated peptide; IL-6, Interleucin 6; NS, non-significant.

* Difference across treatments FOCR vs controls and FOCR vs FO, $p < 0.05$ # Difference across treatments FOCR vs controls, $p < 0.05$.

6 CONCLUSÃO

Apesar dos estudos demonstrarem as implicações das adipocinas na fisiopatologia de doenças autoimunes, incluindo AR, ainda não está claro seu papel na patogênese da progressão da doença ou na atividade da doença.

Este é o primeiro estudo que avalia o efeito da ingestão de óleo de peixe associado ou não ao suco de cranberry de baixa caloria no processo de inflamação, presente na AR, bem como na atividade desta doença

O presente estudo confirmou relatos anteriores sobre a eficácia da suplementação com óleo de peixe sobre a atividade da doença em pacientes com AR. Além disso, a hipótese original subjacente a este estudo, de que o suco de cranberry iria adicionar efeitos benéficos para o óleo de peixe foi confirmada tanto na diminuição da atividade da doença como nos biomarcadores inflamatórios, em especial VHS, PCR e IL-6. Esses achados podem abrir novos caminhos para analisar os efeitos do cranberry sobre a atividade da doença em pacientes com AR.

Ressalta-se a importância de novos estudos que investiguem a ação isolada do suco de cranberry em marcadores inflamatórios e atividade da doença em pacientes com AR.

REFERÊNCIAS

ABELLA, V. et al. Review Article Adipokines , Metabolic Syndrome and Rheumatic Diseases. **Journal of Immunology Research**, v. 2014, p. 1–15, 2014.

ALETAHA, D. et al. Acute phase reactants add little to composite disease activity indices for rheumatoid arthritis: validation of a clinical activity score. **Arthritis research & therapy**, v. 7, n. 4, p. R796-806, 2005.

ALETAHA, D. et al. Perception of improvement in patients with rheumatoid arthritis varies with disease activity levels at baseline. **Arthritis and Rheumatism**, v. 61, n. 3, p. 313–20, 15 mar. 2009.

ALETAHA, D. et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. **Annals of the Rheumatic Diseases**, v. 69, n. 9, p. 1580–8, set. 2010.

AMERICAN COLLEGE OF RHEUMATOLOGY SUBCOMMITTEE ON RHEUMATOID ARTHRITIS GUIDELINES. Guidelines for the management of rheumatoid arthritis: 2002 Update. **Arthritis and Rheumatism**, v. 46, n. 2, p. 328–46, fev. 2002.

BARBOSA, D. S. et al. Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty acids. **Nutrition (Burbank, Los Angeles County, Calif.)**, v. 19, n. 10, p. 837–42, out. 2003.

BARBOSA, V. D. S.; RÊGO, J.; ANTÔNIO, N. Possível papel das adipocinas no lúpus eritematoso sitêmico e na artrite reumatoide. **Revista Brasileira de Reumatologia**, v. 52, n. 2, p. 278–287, 2012.

BERBERT, A. A. et al. Supplementation of fish oil and olive oil in patients with rheumatoid arthritis. **Nutrition (Burbank, Los Angeles County, Calif.)**, v. 21, p. 131–136, 2005.

BERBERT, A. A. **Avaliação da Atividade Inflamatória e da qualidade de vida em pacientes com artrite reumatoide suplementados com ácidos gráxos poliinsaturados ômega-3 e ácido oléico**. [s.l.] Universidade Estadua de Londrina, 2002.

BOERS, M. et al. World Health Organization and International League of Associations for Rheumatology core endpoints for symptom modifying antirheumatic drugs in rheumatoid arthritis clinical trials. **The Journal of Rheumatology. Supplement**, v. 41, p. 86–9, set.

1994.

BORGES, P. K. DE O. et al. Prevalence and characteristics associated with metabolic syndrome in Japanese-Brazilians with and without periodontal disease. **Cadernos de Saude Publica**, v. 23, n. 3, p. 657–68, mar. 2007.

BRANIMIR ANIĆ; MIROSLAV MAYER. Pathogenesis of rheumatoid arthritis. **Reumatizam**, v. 61, n. 2, p. 19–23, 2014.

BRENNAN, F. M.; MCINNES, I. B. Evidence that cytokines play a role in rheumatoid arthritis. **The Journal of Clinical Investigation**, v. 118, n. 11, p. 3537–45, nov. 2008.

BROCHU-GAUDREAU, K. et al. Adiponectin action from head to toe. **Endocrine**, v. 37, n. 1, p. 11–32, 2010.

CALABRÒ, A. et al. One year in review 2016: novelties in the treatment of rheumatoid arthritis. **Clinical and Experimental Rheumatology**, v. 34, n. 3, p. 357–72, 2016.

CALDER, P. C. Fatty acids and inflammation: The cutting edge between food and pharma. **European Journal of Pharmacology**, v. 668, p. S50–S58, 2011.

CALDER, P. C. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. **Biochimica et Biophysica Acta**, v. 1851, n. 4, p. 469–84, abr. 2015.

CHEN, X. et al. Adiponectin: A biomarker for rheumatoid arthritis? **Cytokine & Growth Factor Reviews**, v. 24, n. 1, p. 83–89, 2013.

CHUNG, C. P. et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. **Atherosclerosis**, v. 196, n. 2, p. 756–763, fev. 2008.

CLELAND, L. G. et al. Reduction of cardiovascular risk factors with longterm fish oil treatment in early rheumatoid arthriti. **The Journal of Rheumatology**, v. 33, n. 10, 2006.

COSTA, J. V.; DUARTE, J. S. Tecido Adiposo E Adipocinas. **Acta Med Port**, v. 19, p. 251–256, 2006.

CROWSON, C. S. et al. Contribution of obesity to the rise in incidence of rheumatoid arthritis. **Arthritis Care and Research**, v. 65, n. 1, p. 71–77, 2013.

DAS28 - Home of the Disease activity score and DAS28. Disponível em: <http://www.das-score.nl/das28/en/>.

DEL PRETE, A.; SALVI, V.; SOZZANI, S. Adipokines as potential biomarkers in rheumatoid arthritis. **Mediators of Inflammation**, v. 2014, p. 1–12, 2014.

DESSÌ, A.; ATZEI, A.; FANOS, V. Cranberry in children: prevention of recurrent urinary tract infections and review of the literature. **Revista Brasileira de Farmacognosia**, v. 21, n. 5, p. 807–813, out. 2011.

DUFFEY, K. J.; SUTHERLAND, L. A. Adult consumers of cranberry juice cocktail have lower C-reactive protein levels compared with nonconsumers. **Nutrition Research**, v. 35, n. 2, p. 118–126, 2015.

DUTHIE, G. G. et al. Increased salicylate concentrations in urine of human volunteers after consumption of cranberry juice. **Journal of Agricultural and Food Chemistry**, v. 53, n. 8, p. 2897–900, 20 abr. 2005.

ELLABBAN, A. et al. Impact of obesity on functional and laboratory parameters in patients with rheumatoid arthritis. **Egyptian Rheumatology and Rehabilitation**, v. 43, n. 1, p. 21, 2016.

FANTUZZI, G. Adipose tissue, adipokines, and inflammation. **Journal of Allergy and Clinical Immunology**, v. 115, n. 5, p. 911–920, 2005.

FANTUZZI, G. Adiponectin in inflammatory and immune-mediated diseases. **Cytokine**, v. 64, n. 1, p. 1–10, out. 2013.

FATIMA, S. S. et al. New roles of the multidimensional adipokine: Chemerin. **Peptides**, v. 62, p. 15–20, dez. 2014.

FELSON, D. T. et al. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. **Arthritis and rheumatism**, v. 36, n. 6, p. 729–40, jun. 1993.

FERRACCIOLI, G.; GREMESE, E. Adiposity, joint and systemic inflammation: the additional risk of having a metabolic syndrome in rheumatoid arthritis. **Swiss Medical Weekly**, v. 141, n. May, p. 1–10, 2011.

FOCHESATTO ,F, L; BARROS, E. **Medicina Interna na Prática Clínica**. Porto Alegre: Artmed; 2013

FOXMAN, B. et al. Cranberry juice capsules and urinary tract infection after surgery: results of a randomized trial. **American Journal of Obstetrics and Gynecology**, v. 213, n. 2, p. 194.e1-8, ago. 2015.

FRANSEN J, WELSING P M, DE KEIJZER R M, VAN R. P. L. Disease activity scores using C-reactive protein: CRP may replace ESR in the assessment of RA disease activity [abstract]. **Ann Rheum Dis**, v. 62 (Suppl, p. 151, 2004.

GARAULET, M. et al. Adiponectin, the controversial hormone. **Public Health Nutrition**, v. 10, n. 10A, p. 1145–50, out. 2007.

GILES, J. T. et al. Adiponectin is a mediator of the inverse association of adiposity with radiographic damage in rheumatoid arthritis. **Arthritis Care and Research**, v. 61, n. 9, p. 1248–1256, 2009.

GILES, J. T.; VAN DER HEIJDE, D. M.; BATHON, J. M. Association of circulating adiponectin levels with progression of radiographic joint destruction in rheumatoid arthritis. **Annals of the Rheumatic Diseases**, v. 70, n. 9, p. 1562–8, set. 2011.

GOELDNER, I. et al. Association of anticyclic citrullinated peptide antibodies with extra-articular manifestations, gender, and tabagism in rheumatoid arthritis patients from southern Brazil. **Clinical Rheumatology**, v. 30, n. 7, p. 975–980, 22 jul. 2011.

GOLDSMITH, C. H. et al. Criteria for clinically important changes in outcomes: development, scoring and evaluation of rheumatoid arthritis patient and trial profiles. OMERACT Committee. **The Journal of Rheumatology**, v. 20, n. 3, p. 561–5, mar. 1993.

GUAY, D. R. P. Cranberry and Urinary Tract Infections. **Drugs**, v. 69, n. 7, p. 775–807, maio 2009.

GUIMARÃES, D. E. D. et al. Adipocitocinas: uma nova visão do tecido adiposo. **Revista de Nutrição**, v. 20, n. 5, p. 549–559, out. 2007.

HAFFNER, S. M.; MIETTINEN, H.; STERN, M. P. The homeostasis model in the San Antonio Heart Study. **Diabetes Care**, v. 20, n. 7, p. 1087–92, jul. 1997.

HARRIS, W. S. Nonpharmacologic treatment of hypertriglyceridemia: focus on fish oils. **Clinical Cardiology**, v. 22, n. 6 Suppl, p. II40-3, jun. 1999.

HUTCHESON, J. Adipokines influence the inflammatory balance in autoimmunity. **Cytokine**, v. 75, n. 2, p. 272–279, 2015.

IWASAKI, Y.; YAMAMOTO, K. [Basic research overview in rheumatoid arthritis]. **Nihon rinsho. Japanese Journal of Clinical Medicine**, v. 74, n. 6, p. 889–96, jun. 2016.

JAMES, M. J.; GIBSON, R. A.; CLELAND, L. G. Dietary polyunsaturated fatty acids and inflammatory mediator production. **The American Journal of Clinical Nutrition**, v. 71, n. 1 Suppl, p. 343S–8S, jan. 2000.

JAMES, M.; PROUDMAN, S.; CLELAND, L. Fish oil and rheumatoid arthritis: past, present and future. **The Proceedings of the Nutrition Society**, v. 69, n. 3, p. 316–23, ago. 2010.

KANEKO, S. et al. Rheumatoid arthritis and cytokines. **Nihon rinsho. Japanese Journal of Clinical Medicine**, v. 74, n. 6, p. 913–8, jun. 2016.

KEREKES, G. et al. Rheumatoid arthritis and metabolic syndrome. **Nature reviews. Rheumatology**, v. 10, n. 11, p. 691–6, nov. 2014.

KINSELLA, J. E. Alpha-linolenic acid: functions and effects on linoleic acid metabolism and eicosanoid-mediated reactions. **Advances in Food and Nutrition Research**, v. 35, p. 1–184, 1991.

KINSELLA, J. E.; LOKESH, B.; STONE, R. A. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. **The American Journal of Clinical Nutrition**, v. 52, n. 1, p. 1–28, jul. 1990.

KOLAHI, S. et al. Fish oil supplementation decreases serum soluble receptor activator of nuclear factor-kappa B ligand/osteoprotegerin ratio in female patients with rheumatoid arthritis. **Clinical biochemistry**, v. 43, n. 6, p. 576–80, abr. 2010.

KUMAR, L. D. et al. Advancement in contemporary diagnostic and therapeutic approaches for rheumatoid arthritis. **Biomedicine & Pharmacotherapy**, v. 79, p. 52–61, 2016.

LAWRENCE, R. C. et al. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. **Arthritis & Rheumatism**, v. 41, n. 5, p. 778–

799, maio 1998.

LIPSKY, P. E. Artrite Reumatoide. In: **Medicina Interna de Harrison**. 14. ed. Rio de Janeiro: [s.n.]. p. 1996–1997.

LOUZADA-JUNIOR, P. et al. Análise descritiva das características demográficas e clínicas de pacientes com artrite reumatóide no estado de São Paulo, Brasil. **Revista Brasileira de Reumatologia**, v. 47, n. 2, p. 84–90, abr. 2007.

MAKI, K. C. et al. Consumption of a cranberry juice beverage lowered the number of clinical urinary tract infection episodes in women with a recent history of urinary tract infection. **The American Journal of Clinical Nutrition**, v. 103, n. 6, p. 1434–42, jun. 2016.

MARCHAND, S. et al. Nutrient signature of Quebec (Canada) cranberry (Vaccinium macrocarpon Ait.). **Revista Brasileira de Fruticultura**, v. 35, n. 1, p. 292–304, mar. 2013.

MARQUES NETO, J. F. et al. Estudo multicêntrico da prevalência da artrite reumatóide do adulto em amostras da populaçao brasileira. **Revista brasileira de reumatologia**, p. 169–73, 1993.

MAYSER, P. et al. Omega-3 fatty acid-based lipid infusion in patients with chronic plaque psoriasis: results of a double-blind, randomized, placebo-controlled, multicenter trial. **Journal of the American Academy of Dermatology**, v. 38, n. 4, p. 539–47, abr. 1998.

MedicinaNET. Disponível em: <http://www.medicinanet.com.br/>. (“MedicinaNET”, [s.d.])

MCINNES, I. B.; SCHETT, G. The Pathogenesis of Rheumatoid Arthritis. **New England Journal of Medicine**, v. 365, n. 23, p. 2205–2219, 8 dez. 2011.

MILES, E. A.; CALDER, P. C. Influence of marine n-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis. **The British Journal of Nutrition**, v. 107, n. S2, p. S171-84, jun. 2012.

MOTA, L. M. H. DA et al. Consenso da Sociedade Brasileira de Reumatologia 2011 para o diagnóstico e avaliação inicial da artrite reumatoide. **Revista Brasileira de Reumatologia**, v. 51, n. 3, p. 207–219, jun. 2011.

MYASOEDOVA, E. et al. Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007. **Arthritis and rheumatism**, v. 62, n. 6, p. 1576–82, jun. 2010.

NAKAJIMA, A. Cardiovascular diseases in rheumatoid arthritis. **Nihon rinsho. Japanese Journal of Clinical Medicine**, v. 74, n. 6, p. 1012–6, jun. 2016.

NEUMANN, E. et al. Rheumatoid arthritis progression mediated by activated synovial fibroblasts. **Trends in Molecular Medicine**, v. 16, n. 10, p. 458–468, out. 2010.

NEUMANN, E. et al. Adipocytokines as driving forces in rheumatoid arthritis and related inflammatory diseases? **Arthritis and Rheumatism**, v. 63, n. 5, p. 1159–1169, 2011.

NORLING, L. V; PERRETTI, M. The role of omega-3 derived resolvins in arthritis. **Current Opinion in pharmacology**, v. 13, n. 3, p. 476–81, jun. 2013.

NOVOTNY, J. A. et al. Cranberry juice consumption lowers markers of cardiometabolic risk, including blood pressure and circulating C-reactive protein, triglyceride, and glucose concentrations in adults. **The Journal of Nutrition**, v. 145, n. 6, p. 1185–93, jun. 2015.

NURMOHAMED, M. T.; HESLINGA, M.; KITAS, G. D. Cardiovascular comorbidity in rheumatic diseases. **Nature reviews. Rheumatology**, v. 11, n. 12, p. 693–704, dez. 2015.

O. BURR, G. The essential fatty acids fifty years ago. **Progress in Lipid Research**, v. 20, p. xxvii–xxix, 1981.

OHASHI, K.; OUCHI, N.; MATSUZAWA, Y. Anti-inflammatory and anti-atherogenic properties of adiponectin. **Biochimie**, v. 94, n. 10, p. 2137–2142, 2012.

OLIVEIRA, B. M. G. B. DE et al. Síndrome metabólica em pacientes com diagnóstico de artrite reumatoide acompanhados em um Hospital Universitário do Nordeste brasileiro. **Revista Brasileira de Reumatologia**, v. 56, n. 2, p. 117–125, 2016.

PARK, Y. et al. Effect of n-3 polyunsaturated fatty acid supplementation in patients with rheumatoid arthritis: a 16-week randomized, double-blind, placebo-controlled, parallel-design multicenter study in Korea. **The Journal of Nutritional Biochemistry**, v. 24, n. 7, p. 1367–72, jul. 2013.

PICERNO, V. et al. One year in review: the pathogenesis of rheumatoid arthritis. **Clinical and Experimental Rheumatology**, v. 33, n. 4, p. 551–558, 2015.

PINA, A. et al. Arando na profilaxia das infecções urinárias recorrentes: revisão baseada na evidência. **Revista Portuguesa de Clínica Geral**, v. 27, n. 5, p. 452–457, 2011.

PREVOO, M. L. et al. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. **Arthritis and rheumatism**, v. 38, n. 1, p. 44–8, jan. 1995.

PROUDMAN, S. M.; CLELAND, L. G.; JAMES, M. J. Dietary omega-3 fats for treatment of inflammatory joint disease: efficacy and utility. **Rheumatic Diseases Clinics of North America**, v. 34, n. 2, p. 469–79, maio 2008.

QIN, B. et al. Body mass index and the risk of rheumatoid arthritis: a systematic review and dose-response meta-analysis. **Arthritis Research & Therapy**, v. 17, n. 1, p. 86, 2015.

RAJAEI, E. et al. The Effect of Omega-3 Fatty Acids in Patients With Active Rheumatoid Arthritis Receiving DMARDs Therapy: Double-Blind Randomized Controlled Trial. **Global Journal of Health Science**, v. 8, n. 7, p. 18–25, 2016.

ROBINSON, K.; PRINS, J.; VENKATESH, B. Clinical review: adiponectin biology and its role in inflammation and critical illness. **Critical Care (London, England)**, v. 15, n. 2, p. 221, 2011.

RODRIGUEZ-MATEOS, A. et al. Cranberry (poly)phenol metabolites correlate with improvements in vascular function: A double-blind, randomized, controlled, dose-response, crossover study. **Molecular Nutrition & Food Research**, v. 60, n. 10, p. 2130–2140, 31 maio 2016.

RONTOYANNI, V. G. et al. Marine n-3 fatty acids for cardiovascular risk reduction and disease control in rheumatoid arthritis: "kill two birds with one stone"? **Current Pharmaceutical Design**, v. 18, n. 11, p. 1531–42, 2012.

SALES, C.; OLIVIERO, F.; SPINELLA, P. Role of omega-3 polyunsaturated fatty acids in diet of patients with rheumatic diseases. **Reumatismo**, v. 60, n. 2, p. 95–101, 2008a.

SALES, C.; OLIVIERO, F.; SPINELLA, P. Fish oil supplementation in rheumatoid arthritis. **Reumatismo**, v. 60, n. 3, p. 174–9, 2008b.

SCHELLEKENS, G. A. et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. **Arthritis & Rheumatism**, v.43, n.1, p.155-163, 2000.

SCHERER, P. E. et al. A novel serum protein similar to C1q, produced exclusively in

adipocytes. **The Journal of Biological Chemistry**, v. 270, n. 45, p. 26746–9, 10 nov. 1995.

SCOTECE, M. et al. Role of adipokines in atherosclerosis: Interferences with cardiovascular complications in rheumatic diseases. **Mediators of Inflammation**, v. 2012, p. 1–14, 2012.

SCOTECE, M. et al. Adiponectin and leptin: New targets in inflammation. **Basic and Clinical Pharmacology and Toxicology**, v. 114, n. 1, p. 97–102, 2014.

SCOTT, D. L. et al. Disease activity in rheumatoid arthritis: preliminary report of the Consensus Study Group of the European Workshop for Rheumatology Research. **Clinical and Experimental Rheumatology**, v. 10, n. 5, p. 521–5, 1991.

SCRIVO, R. et al. Rheumatic diseases and obesity: Adipocytokines as potential comorbidity biomarkers for cardiovascular diseases. **Mediators of Inflammation**, v. 2013, p. 1–14, 2013.

SEMERANO, L. et al. Novel Immunotherapeutic Avenues for Rheumatoid Arthritis. **Trends in Molecular Medicine**, v. 22, n. 3, p. 214–29, mar. 2016.

SIEGEL, J. N.; ZHEN, B.-G. Use of the American College of Rheumatology N (ACR-N) index of improvement in rheumatoid arthritis: Argument in favor. **Arthritis & Rheumatism**, v. 52, n. 6, p. 1637–1641, jun. 2005.

SIMÃO, T. N. C. **Efeito do consumo de suco de cranberry de baixa caloria sobre fatores de risco cardiovascular em indivíduos com síndrome metabólica**. [s.l.] Universidade Estadual de Londrina, 2012.

SIMÃO, T. N. C. et al. Reduced-energy cranberry juice increases folic acid and adiponectin and reduces homocysteine and oxidative stress in patients with the metabolic syndrome. **British Journal of Nutrition**, v. 110, n. 10, p. 1885–1894, 11 nov. 2013.

SMITH, W.; MUKHOPADHYAY, R. Essential fatty acids: the work of George and Mildred Burr. **The Journal of Biological Chemistry**, v. 287, n. 42, p. 35439–41, 12 out. 2012.

SIPPEL, C. et al. PROCESSOS INFLAMATÓRIOS DA OBESIDADE. **Revista de Atenção à Saúde**, v. 12, p. 48–56, 2014.

SKALSKA, U.; KONTNY, E. Adiponectin Isoforms and Leptin Impact on Rheumatoid Adipose Mesenchymal Stem Cells Function. **Stem Cells International**, v. 2016, p. 1–7, 2016.

SKEOCH, S.; BRUCE, I. N. Atherosclerosis in rheumatoid arthritis: is it all about

inflammation? **Nature Reviews. Rheumatology**, v. 11, n. 7, p. 390–400, jul. 2015.

SMOLEN, J. S. et al. A simplified disease activity index for rheumatoid arthritis for use in clinical practice. **Rheumatology (Oxford, England)**, v. 42, n. 2, p. 244–57, fev. 2003.

SMOLEN, J. S. et al. Rheumatoid arthritis. **The Lancet**, v. 0, n. 0, p. 1580–1588, maio 2016.

SUÁREZ-MAHECHA, H. et al. IMPORTÂNCIA DE ÁCIDOS GRAXOS POLIINSATURADOS PRESENTES EM PEIXES DE CULTIVO E DE AMBIENTE NATURAL PARA A NUTRIÇÃO HUMANA. **Boletim do Instituto de Pesca**, v. 28(1), p. 101–110, 2002.

TIPTON, D. A.; CHRISTIAN, J.; BLUMER, A. Effects of cranberry components on IL-1β-stimulated production of IL-6, IL-8 and VEGF by human TMJ synovial fibroblasts. **Archives of Oral Biology**, v. 68, p. 88–96, ago. 2016.

TOUSSIROT, É. et al. The role of leptin in the pathophysiology of rheumatoid arthritis. **Life Sciences**, v. 140, p. 29–36, nov. 2015.

TRAYHURN, P.; WOOD, I. S. Adipokines: inflammation and the pleiotropic role of white adipose tissue. **The British Journal of Nutrition**, v. 92, n. 3, p. 347–355, 2004.

TURESSON, C. et al. Incidence and predictors of severe extra-articular disease manifestations in an early rheumatoid arthritis inception cohort. **Annals of the Rheumatic Diseases**, v. 66, n. 11, p. 1543–4, nov. 2007.

VAN DER HEIJDE, D. M. et al. Validity of single variables and composite indices for measuring disease activity in rheumatoid arthritis. **Annals of the Rheumatic Diseases**, v. 51, n. 2, p. 177–81, fev. 1992.

VAN DER HEIJDE, D. M. et al. Development of a disease activity score based on judgment in clinical practice by rheumatologists. **The Journal of Rheumatology**, v. 20, n. 3, p. 579–81, mar. 1993.

VAN GESTEL, A. M.; HAAGSMA, C. J.; VAN RIEL, P. L. C. M. Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. **Arthritis & Rheumatism**, v. 41, n. 10, p. 1845–1850, out. 1998.

VINSON, J. A. et al. Phenol antioxidant quantity and quality in foods: fruits. **Journal of Agricultural and Food Chemistry**, v. 49, n. 11, p. 5315–21, nov. 2001.

WAJCHENBERG, B. L. Tecido adiposo como glândula endócrina. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 44, n. 1, p. 1–32, 2000.

WOOLF, A. D. How to assess musculoskeletal conditions. History and physical examination. **Best Practice & Research. Clinical rheumatology**, v. 17, n. 3, p. 381–402, jun. 2003.

ZHANG, Y. et al. Positional cloning of the mouse obese gene and its human homologue. **Nature**, v. 372, n. 6505, p. 425–32, 1 dez. 1994.

APÊNDICES

APÊNDICE A - FICHA DE AVALIAÇÃO

FICHA DE AVALIAÇÃO DOS PACIENTES COM ARTRITE REUMATÓIDE

NOME: _____FONE:_____
Endereço:_____
RG: _____
IDADE ou DN: _____
SEXO: feminino ( ) masculino ( )

AVALIAÇÃO TEMPO (____)

TEMPO DE DIAGNÓSTICO: _____

DAS 28: _____

COMPROMETIMENTO SISTEMICO EXTRA-ARTICULAR:
pulmonar ( ), vasculite ( ), ocular ( ), nodulos reumatoides ( ), cardíaco ( ), SNC ( )

OUTRAS DOENÇAS:
HAS ( ), DM ( ), dislipidemia ( ), IAM ( ), AVC ( ), outros: _____
outra colagenose ( ): _____

MEDICAÇÕES
( ) Prednisona dose: _____
( ) Metotrexate dose: _____
( ) Hidroxicloroquina/Cloroquina dose: _____
( ) Sulfassalazina dose: _____
( ) Leflunomide dose: _____
( ) Etanercepte dose: _____
( ) Adalimumabe dose: _____
( ) Infliximabe dose: _____
( ) Tocilizumabe dose: _____
( ) Abatacepte dose: _____
( ) Rituximabe dose: _____
( ) Ciclofosfamida dose: _____
( ) outros: _____

TABAGISMO: sim ( ) não ( )

ATIVIDADE FISICA: sim ( ) não ( )
tipo: _____ frequência: _____ há quanto tempo: _____

DADOS ANTROPOMÉTRICOS

Altura (cm)	Peso (kg)	IMC (kg/m2)	Circunferência Abdominal (cm)	Pressão Arterial (mm/Hg)

APÊNDICE B - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Termo de Consentimento Livre e Esclarecido

Título da pesquisa: “Influência da ingestão de óleo de peixe e suco de Cranberry de baixa caloria sobre o metabolismo, processo inflamatório e o estresse oxidativo em pacientes com artrite reumatoide”

Prezado (a) Senhor (a):

Gostaríamos de convidá-lo (a) a participar da pesquisa “Influência da ingestão de óleo de peixe e suco de Cranberry de baixa caloria sobre o processo inflamatório e o estresse oxidativo em pacientes com artrite reumatoide”, realizada no Hospital Universitário de Londrina. O objetivo dessa pesquisa é avaliar se o consumo de óleo de peixe e suco de Cranberry, isoladamente ou associados, podem melhorar a inflamação e o estresse oxidativo decorrentes da artrite reumatoide, bem como reduzir a atividade da doença. A sua participação é muito importante e ela se daria da seguinte forma: em um primeiro momento, como de rotina, você passará por uma avaliação clínica ambulatorial pelo médico reumatologista e uma coleta de sangue para análises laboratoriais. A partir desse momento, de acordo com um sorteio, você será incluído em um de 3 grupos: (1) grupo do óleo de peixe (consumo diário de 10 cápsulas de óleo de peixe por 90 dias (2) grupo óleo de peixe + suco de Cranberry (90 dias) ou (3) grupo controle (dieta habitual por 90 dias). Após esse período você retornará ao ambulatório para que as avaliações descritas acima sejam repetidas e possamos verificar os efeitos destas intervenções. Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você: recusar-se a participar, ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos ainda que as informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.

O consumo de suco de Cranberry e/ou de óleo de peixe não causam riscos à saúde das pessoas. Os benefícios esperados são redução da inflamação, do estresse oxidativo e de

fatores que possam favorecer o desenvolvimento de doenças do coração. Além disso, espera-se também que ocorra uma melhora dos sintomas da doença como dores, inchaço, rigidez nas articulações e na sua qualidade de vida.

Informamos que o (a) senhor (a) não pagará nem será remunerado por sua participação.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos pode nos contatar Elis Fatel (Telefone: 43-91617713; e-mail: elis.fatel@hotmail.com) e Flávia Troncon Rosa (Telefone: 43-96990769; e-mail: flaviatrosa@yahoo.com.br) ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Kock, nº 60, ou no telefone 33712490. Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas, devidamente preenchida e assinada entregue a você.

Londrina, ___ de _____de 201_____.

Pesquisador Responsável: Prof. Dr Isaias Dichi. RG: 9.922.731-1

_____, tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar voluntariamente da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):_____

Data:_____

Obs: Caso o participante da pesquisa seja menor de idade, deve ser incluído o campo para assinatura do menor e do responsável.

ANEXOS

ANEXO A – PARECER CONSUBSTANCIADO DO COMITÊ DE ÉTICA EM PESQUISA EM SERES HUMANOS DA UNIVERSIDADE ESTADUAL DE LONDRINA

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL/ HOSPITAL REGIONAL DO NORTE DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Influência da ingestão de óleo de peixe e suco de cranberry de baixa caloria sobre o processo inflamatório e o estresse oxidativo em pacientes com artrite reumatoide

Pesquisador: Isaias Dichi
Área Temática:
Versão: 4
CAAE: 13426014.6.0000.5231
Instituição Proponente: CCS - Departamento de Clínica Médica
Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 617.289
Data da Relatoria: 14/04/2014

Apresentação do Projeto:
Trata-se da resposta a uma pendência indicada no parecer 575.753.

Objetivo da Pesquisa:
Idem ao parecer 575.753.

Avaliação dos Riscos e Benefícios:
Idem ao parecer 575.753.

Comentários e Considerações sobre a Pesquisa:
Idem ao parecer 575.753.

Considerações sobre os Termos de apresentação obrigatória:
Idem ao parecer 575.753.

Recomendações:
Não há.

Conclusões ou Pendências e Lista de Inadequações:
Não há.

Endereço: AVENIDA ROBERT KOCH, 60
Bairro: VILA OPERÁRIA **CEP:** 86.038-440
UF: PR **Município:** LONDRINA
Telefone: (43)3371-2490 **E-mail:** cep268@uel.br

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL/ HOSPITAL REGIONAL DO NORTE DO

Continuação do Parecer: 617.289

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Parecer referendado.

LONDRINA, 15 de Abril de 2014

Assinador por:
Paula Mariza Zedu Alliprandini
(Coordenador)

Endereço: AVENIDA ROBERT KOCH, 60
Bairro: VILA OPERÁRIA **CEP:** 86.038-440
UF: PR **Município:** LONDRINA
Telefone: (43)3371-2490 **E-mail:** cep268@uel.br

ANEXO B – Normas para publicação da Revista Brasileira de Reumatologia e do *British Journal of Nutrition*

REVISTA BRASILEIRA DE REUMATOLOGIA - INSTRUÇÕES AOS AUTORES

Escopo e política

A Revista Brasileira de Reumatologia (RBR) publica artigos sobre temas reumatológicos e correlatos, após análise por seu Conselho Editorial. A RBR é catalogada no Web of Science, PUBMED/MEDLINE, Lilacs (Literatura Latino-Americana e do Caribe em Ciências da Saúde), Scopus, Index Copernicus e Scielo (Scientific Electronic Library Online).

Ao submeter o manuscrito, os autores assumem a responsabilidade de o trabalho não ter sido previamente publicado nem estar sendo analisado por outra revista. Os manuscritos devem ser inéditos, ter sido objeto de análise e com anuência de todos os autores.

Artigos aceitos para publicação passam a ser propriedade da revista.

A abreviatura de seu título é Rev Bras Reumatol, que deve ser usada em bibliografias, notas de rodapé e em referências bibliográficas.

Não há taxas para submissão e avaliação de artigos.

A Revista adota o sistema iThenticate para todos os manuscritos submetidos; na identificação de alto índice de pareamento os arquivos são submetidos a avaliação pelos editores.

Os manuscritos serão avaliados inicialmente por editor-chefe e/ou editor associado, e se considerado em conformidade com formato e escopo da Revista, seguirão para avaliação por no mínimo dois revisores com conhecimento científico na área de pesquisa, de procedência nacional ou estrangeira. Após as

devidas correções e possíveis sugestões, o manuscrito receberá os pareceres favoráveis ou desfavoráveis dos revisores, que subsidiarão a subsequente reavaliação e recomendação de aceite ou rejeição pelo editor associado. As decisões finais serão de responsabilidade do editor-chefe".

FORMA E PREPARAÇÃO DE MANUSCRITOS

APRESENTAÇÃO DO MANUSCRITO

O manuscrito pode ser submetido em português ou inglês, em espaço duplo, com margens de 2,5 cm. No texto não devem ser empregadas abreviaturas não convencionais, gírias (jargões) médicas ou redação tipo telegráfica. A citação de medicamentos e produtos farmacêuticos deve ser feita utilizando-se apenas a nomenclatura farmacológica, sem menção do nome comercial.

ESTRUTURA DO MANUSCRITO

*Manuscript**, *Title Page**, *Cover Letter* e *Author Agreement** devem ser enviados em arquivos individuais. Tabelas e figuras devem ser numeradas conforme citadas no texto e enviadas em arquivos separados, com títulos e legendas correspondentes. (*arquivos obrigatórios)

Página do título

Deve conter: a) título do artigo; b) nome completo dos autores e sua titulação mais importante; c) departamento(s) e instituição(ões) onde se originou o trabalho; d) nome, endereço completo e e-mail válido do autor responsável para correspondência; e) conflito de interesse e agências financiadoras relevantes; f) título resumido com no máximo 60 caracteres.

Author Agreement

É o documento no qual os autores declaram a originalidade do manuscrito, além de aprovarem o artigo objeto da submissão, a autoria e a ordem da lista de autores. Deve ser assinado por todos os autores. A seguir é apresentado um modelo.

Caro Editor,

Os autores, abaixo assinados, declaram que este manuscrito é original, não foi publicado antes e não se encontra submetido para qualquer outra publicação.

Gostaríamos de pedir a atenção do Editor para a presente publicação de nós autores, referente a aspectos do presente manuscrito submetido. Confirmamos que o manuscrito foi lido e aprovado por todos os autores signatários e que não há nenhum outro

autor a fazer parte senão os listados. Confirmamos também que a ordem dos autores listada no manuscrito foi aprovada por todos.

Entendemos que o Autor para Correspondência será o único contato para o processo editorial. Ele será o único responsável pela comunicação com os demais autores acerca do progresso da submissão, da revisão do manuscrito e de sua aprovação final.

(Assinatura de todos os autores)

Artigo Original

Deve conter: página do título, página de resumo com palavras-chave, introdução, material e métodos ou pacientes e métodos, resultados e discussão, agradecimentos, referências, tabelas, figuras e legendas das figuras. Não deve exceder 5.000 palavras, incluindo-se as referências e excluindo-se a página do título, resumo, tabelas e legendas. Pode exibir até seis figuras ou tabelas e até 50 referências.

Página de resumo

Deve conter: a) objetivo, métodos, resultados e conclusões, não excedendo 250 palavras; b) três a cinco palavras-chave.

Introdução

A finalidade dessa seção é definir o propósito e as razões para a realização do trabalho. Não se recomenda extensa revisão da literatura.

Pacientes e métodos ou Material e métodos

Deve incluir informações suficientes que permitam a reprodução do trabalho e, quando pertinente, a aprovação pelo Comitê de Ética institucional. Os métodos empregados na análise estatística devem sempre ser citados.

Resultados

Devem ser claros e concisos. Tabelas e gráficos não devem duplicar informações.

Discussão

Deve ser concisa, interpretando os resultados no contexto da literatura atual. É conveniente não ultrapassar a metade do número de páginas do trabalho completo.

Agradecimentos

Apenas às pessoas que contribuíram, por exemplo, com técnicas, discussão e envio de pacientes. Auxílio financeiro deve ser referido na página do título.

Referências

Devem ser citadas no texto em algarismos arábicos, sobrescritos e depois da pontuação, sem parênteses ou colchetes. A numeração deve ser sequencial, de acordo com a ordem de citação no texto. Nas referências com mais de seis autores, devem ser citados os seis primeiros, seguidos pela expressão *et al*. Sugere-se a utilização dos

programas Reference Manager ou Endnote, seguindo-se o estilo Vancouver. Exemplos de referência para diferentes formatos são apresentados a seguir. Os autores devem consultar o NLM's Citing Medicine para mais informações sobre os formatos das referências.

Artigo de revista

1. Rivero MG, Salvatore AJ, Gomez-Puerta JA, Mascaro JM, Jr., Canete JD, Munoz-Gomez J *et al*. Accelerated nodulosis during methotrexate therapy in a patient with systemic lupus erythematosus and Jaccoud's arthropathy. Rheumatology (Oxford) 2004; 43(12):1587-8.

Artigo extraído de endereço eletrônico

2. Cardozo JB, Andrade DMS, Santiago MB. The use of bisphosphonate in the treatment of avascular necrosis: a systematic review. Clin Rheumatol 2008. Available from: http://www.springerlink.com.w10069.dotlib.com.br/content/l05j4j3332041225/fulltext.pdf. [Accessed in February 24, 2008].

Livro

3. Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Tabelas e Figuras

Cada tabela ou figura deverá ser numerada em algarismo arábico e enviada em arquivo separado (.jpg, .tif, .png, .xls, .doc) com 300 dpi no mínimo. Título e legenda devem estar no mesmo arquivo da figura ou tabela a que se referem. Tabelas e ilustrações devem ser autoexplicativas, com informações suficientes para sua compreensão sem que se tenha de recorrer ao trabalho. Fotomicrografias devem incluir a escala apropriada.

Artigo de Revisão

Revisões, preferencialmente sistemáticas, podem ser submetidas à RBR, devendo abordar com profundidade um tema de interesse para o reumatologista. Não apresentam estruturação padronizada, prescindindo de introdução ou discussão. Devem apresentar resumo sem subdivisões, com três a cinco palavras-chave, e não devem exceder 6.000 palavras, incluindo-se as referências e excluindo-se a página do título, resumo, tabelas e legendas. Podem exibir até cinco figuras ou tabelas e até 70 referências.

Relato de Caso

Deve incluir resumo e palavras-chave, sem necessidade de subdivisões. O texto, porém, apresenta as seguintes seções: introdução, que deve ser concisa; relato de caso, contendo a descrição e a evolução do quadro clínico, exames laboratoriais, ilustrações e tabelas (que substituem as seções material e métodos e resultados); e discussão. Deve

conter no máximo seis autores, e não deve exceder 1.500 palavras, incluindo-se as referências e excluindo-se a página do título, resumo, tabelas e legendas. Pode exibir até duas figuras ou tabelas e até 15 referências.

Comunicação breve

Aborda um ponto ou detalhe específico de um tema. Deve incluir resumo com no máximo 250 palavras, e três a cinco palavras-chave. O texto não necessita subdivisões, deve ter até 2.500 palavras incluindo-se as referências e excluindo-se a página do título, resumo, tabelas e legendas. Pode exibir até três figuras ou tabelas e até 25 referências.

Regras para aplicar tempos verbais apropriados de acordo com o contexto ou seção

Contexto ou seção	**Tempo verbal apropriado**
Resumo	**Passado**
Introdução	**Presente, quando se referir a fatos estabelecidos e conhecimento prévio**
Métodos, materiais e resultados	**Passado**
Discussão/Conclusão	**Combinado de passado (quando se referir a resultados obtidos no trabalho) e presente (quando se referir a fatos estabelecidos e conhecimento prévio); às vezes pode ser utilizado o futuro (especialmente quando se referir a perspectivas de trabalhos a serem realizados)**
Atribuições	**Passado Ex.: Andrade *et al.* relataram...**
Descrição de Tabelas e Figuras	**Presente**
Conhecimento estabelecido e resultados prévios	**Presente**

Regras gerais para se obter uma boa escrita em um artigo científico:

Prefira a voz ativa

As sentenças devem ser curtas, claras e objetivas

A unidade de medida deve ser abreviada quando empregada com valores numéricos (p. ex., 1 mg), mas escrita por extenso quando separada de valor numérico. Utilize o Sistema Internacional de Unidades (*SI units*) para definir as unidades de medida. Lembre-se de deixar um espaço entre o número e a unidade (p. ex., 10 mg/dL), exceto quando for porcentagem, que deve estar junto (p. ex., 70%). O plural das unidades de medida é a mesma forma do singular (p. ex., 1 mL, 10 mL; 1 h, 10 h). Quando iniciarem a frase, os números devem estar por extenso, e não em algarismo arábico

Defina a abreviação na primeira vez que aparecer no texto principal. Após a definição, use sempre a abreviação em vez da forma por extenso. Evite o uso de abreviações no título e no resumo

Ao escrever em inglês, não utilize contrações (p. ex., prefira *does not* em vez de *doesn't*)

Livro recomendado: Rogers SM. Mastering scientific and medical writing: a self-help guide. Berlin: Springer; 2007.

Considerações éticas e legais

A RBR segue as normas do *Uniform Requirements for Manuscripts* (URM) *Submitted to Biomedical Journals* desenvolvidas pelo *The International Committee of Medical Journal Editors* (ICMJE) - fevereiro de 2006.

Conflito de interesse

A confiança pública no processo de revisão por pares e a credibilidade dos artigos publicados dependem, em parte, de como o conflito de interesse é administrado durante a redação, a revisão por pares e a decisão editorial. O conflito de interesse existe quando um autor (ou instituição do autor), revisor ou editor tem relações financeiras ou pessoais que influenciem de forma inadequada (viés) suas ações (tais relações são também conhecidas como duplo compromisso, interesses conflitantes ou fidelidades conflitantes). Essas relações variam entre aquelas com potencial insignificante até as com grande potencial para influenciar o julgamento, e nem todas as relações representam verdadeiro conflito de interesse. O potencial conflito de interesse pode existir dependendo se o indivíduo acredita ou não que a relação afete seu julgamento científico. Relações financeiras (tais como emprego, consultorias, posse de ações, testemunho de especialista pago) são os conflitos de interesse mais facilmente identificáveis e os mais suscetíveis de minar a credibilidade da revista, dos autores e da própria ciência. No entanto, podem ocorrer conflitos por outras razões, tais como relações pessoais, competição acadêmica e

paixão intelectual.

Consentimento informado

Os pacientes têm o direito à privacidade, que não deve ser infringida sem o consentimento informado. A identificação de informações, incluindo os nomes dos pacientes, iniciais ou números no hospital, não devem ser publicadas em descrições, fotografias e genealogias, a menos que a informação seja essencial para os propósitos científicos e o paciente (ou responsável) dê o consentimento livre e esclarecido para a publicação. O consentimento informado para este propósito requer que o manuscrito a ser publicado seja mostrado ao paciente. Os autores devem identificar os indivíduos que prestam assistência a escrever e divulgar a fonte de financiamento para essa assistência. Detalhes identificadores devem ser omitidos se não são essenciais. O anonimato completo é difícil de se conseguir; no entanto, no caso de qualquer dúvida, o consentimento deve ser obtido. Por exemplo, mascarar a região ocular em fotografias de pacientes é uma proteção de anonimato inadequada. Se as características de identificação são alteradas para proteger o anonimato, como na linhagem genética, os autores devem garantir que as alterações não distorçam o significado científico. Quando o consentimento informado foi obtido, ele deve ser indicado no artigo publicado.

Princípios éticos

Ao relatar experimentos em seres humanos, os autores devem indicar se os procedimentos seguidos estiveram de acordo com os padrões éticos do comitê responsável por experimentação humana (institucional e nacional) e com a Declaração de Helsinki de 1975, revisado em 2000. Se houver dúvida se a pesquisa foi realizada em conformidade com a Declaração de Helsinki, os autores devem explicar a razão para sua abordagem e demonstrar que o corpo de revisão institucional aprovou explicitamente os aspectos duvidosos do estudo. Ao relatar experimentos com animais, os autores devem indicar se as orientações institucionais e nacionais para o cuidado e a utilização de animais de laboratório foram seguidas.

Registro de ensaios clínicos

Os ensaios clínicos devem ser registrados segundo recomendação da OMS em www.who.int/ictrp/en/. A definição de ensaios clínicos incluem ensaios preliminares (fase I): um estudo prospectivo com o recrutamento de indivíduos submetidos a qualquer intervenção relacionada à saúde (medicamentos, procedimentos cirúrgicos, aparelhos, terapias comportamentais, regime alimentar, mudanças nos cuidados de saúde) para avaliar os efeitos em desfechos clínicos (qualquer parâmetro biomédico e de saúde, inclusive medidas farmacocinéticas e reações adversas). A RBR tem o direito de não

publicar trabalhos que não cumpram estas e outras normas legais e éticas explicitadas nas diretrizes internacionais.

Financiamento e apoio

Os autores devem, também, informar se receberam financiamento ou apoio de instituições como CNPq, CAPES, Fundos Remanescentes da SBR, instituições universitárias, laboratórios etc.

Secretaria Editorial RBR
Revista Brasileira de Reumatologia
Av. Brigadeiro Luiz Antonio, 2.466 - conjs. 93-94
CEP: 01402-000 - São Paulo - SP - Brasil
Tel./fax: (11) 3289-7165
e-mail: rbreumatol@terra.com.br

Envio de manuscritos:

O manuscrito deve ser submetido *online* através do *site:* http://ees.elsevier.com/bjr

BRITISH JOURNAL OF NUTRITION (BJN) - INSTRUCTIONS FOR CONTRIBUTORS

British Journal of Nutrition (BJN) is an international peer-reviewed journal that publishes original papers and review articles in all branches of nutritional science. The

underlying aim of all work should be to develop nutritional concepts.

SUBMISSION

This journal uses ScholarOne Manuscripts for online submission and peer review.

Complete guidelines for preparing and submitting your manuscript to this journal are provided below.

SCOPE

BJN encompasses the full spectrum of nutritional science and reports of studies in the following areas will be considered for publication: Epidemiology, dietary surveys, nutritional requirements and behaviour, metabolic studies, body composition, energetics, appetite, obesity, ageing, endocrinology, immunology, neuroscience, microbiology, genetics, and molecular and cell biology. The focus of all manuscripts submitted to the journal must be to increase knowledge in nutritional science.

The journal does NOT publish papers on the following topics: Case studies; papers on food technology, food science or food chemistry; studies of primarily local interest; studies on herbs, spices or other flavouring agents, pharmaceutical agents or that compare the effects of nutrients to those of medicines, complementary medicines or other substances that are considered to be primarily medicinal agents; studies in which a nutrient or extract is not administered by the oral route (unless the specific aim of the study is to investigate parenteral nutrition); studies using non-physiological amounts of nutrients (unless the specific aim of the study is to investigate toxic effects); food contaminants.

In vivo and in vitro models

Studies involving animal models of human nutrition and health or disease will only be considered for publication if the amount of a nutrient or combination of nutrients used could reasonably be expected to be achieved in the human population.

Studies involving in vitro models will only be considered for publication if the amount of a nutrient or combination of nutrients is demonstrated to be within the range that could reasonably be expected to be encountered in vivo, and that the molecular form of the nutrient or nutrients is the same as that which the cell type used in the model would encounter in vivo.

Extracts

Studies involving extracts will only be considered for publication if the source of starting material is readily accessible to other researchers and that there are appropriate measures for quality control, that the method of extraction is described in sufficient detail with appropriate quality control measures, that the nutrient composition of the extract is characterised in detail and that there are measures to control the quality of the composition of the extract between preparations, and that the amount of extract used could reasonably be

expected to be achieved in in the human population (or in animals if they are the specific target of an intervention).

Studies involving extracts in in vitro models will only be considered for publication if the above guidelines for studies involving extracts are followed, and that the amount and molecular form of the extract is the same as that which would be encountered by the cell type used in the model in vivo.

Probiotics

Studies involving probiotics may be considered provided that the primary focus of the study/review is the effects on nutrient absorption and/or metabolism. Studies/reviews that focus primarily on probiotics per se will not be considered.

Manuscripts submitted to BJN that are outside of the journal's scope or do not meet the above requirements will be rejected immediately.

REVIEW PROCESS

BJN uses a single blind review process.

As part of the online submission process, authors are asked to affirm that the submission represents original work that has not been published previously, and that it is not currently being considered by another journal. Authors must also confirm that each author has seen and approved the contents of the submitted manuscript. Finally, authors should confirm that permission for all appropriate uses has been obtained from the copyright holder for any figures or other material not in his/her copyright, and that the appropriate acknowledgement has been made to the original source.

At submission, authors are asked to nominate at least four potential referees who may then be asked by the Editorial Board to help review the work. Manuscripts are normally reviewed by two external peer reviewers and a member of the Editorial Board.

When substantial revisions are required to manuscripts after review, authors are normally given the opportunity to do this once only; the need for any further changes should at most reflect only minor issues. If a paper requiring revision is not resubmitted within 2 months, it may, on resubmission, be deemed a new paper and the date of receipt altered accordingly.

PUBLISHING ETHICS

BJN considers all manuscripts on the strict condition that:

The manuscript is your own original work, and does not duplicate any other previously published work;

The manuscript has been submitted only to the journal - it is not under consideration or peer review or accepted for publication or in press or published elsewhere;

All listed authors know of and agree to the manuscript being submitted to the journal;

and

The manuscript contains nothing that is abusive, defamatory, fraudulent, illegal, libellous, or obscene.

The Journal adheres to the Committee on Publication Ethics (COPE) guidelines on research and publications ethics.

Text taken directly or closely paraphrased from earlier published work that has not been acknowledged or referenced will be considered plagiarism. Submitted manuscripts in which such text is identified will be withdrawn from the editorial process. If a concern is raised about possible plagiarism in an article submitted to or published in BJN, this will be investigated fully and dealt with in accordance with the COPE guidelines.

ARTICLE TYPES

BJN publishes the following: Research Articles, Review Articles, Systematic Reviews, Horizons in Nutritional Science, Workshop Reports, Invited Commentaries, Letters to the Editor, Obituaries, and Editorials.

Research Articles, Reviews, Systematic Reviews, Horizons Articles, Letters to the Editor and Workshop Reports should be submitted to http://mc.manuscriptcentral.com/bjn. Please contact the Editorial Office onbjn.edoffice@cambridge.orgregarding any other types of article.

Review Articles

BJN is willing to accept critical reviews that are designed to advance knowledge, policy and practice in nutritional science. Current knowledge should be appropriately contextualised and presented such that knowledge gaps and research needs can be characterised and prioritised, or so that changes in policy and practice can be proposed along with suggestions as to how any changes can be monitored. The purpose or objective of a review should be clearly expressed, perhaps as question in the Introduction, and the review's conclusions should be congruent with the initial objective or question. Reviews will be handled by specialist Reviews Editors. Please contact the Editorial Office with any queries regarding the submission of potential review articles. All reviews, including systematic reviews and meta-analyses, should present the uncertainties and variabilities associated with the papers and data being reviewed; in particular BJN cautions against uncritical acceptance of definitions and non-specific global terminology, the advice of advisory bodies, and reference ranges for example.

Reviews: These articles are written in a narrative style, and aim to critically evaluate a specific topic in nutritional science.

Horizons in Nutritional Science: These are shorter than Review articles and aim to

critically evaluate recent novel developments that are likely to produce substantial advances in nutritional science. These articles should be thought-provoking and possibly controversial.

Systematic Reviews and meta-analyses: A systematic review or meta-analysis of randomised trials and other evaluation studies must be accompanied by a completed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement checklist, a guideline to help authors report a systematic review and meta-analysis (see British Medical Journal (2009) 339, b2535). Meta-analysis of observational studies must be accompanied by a completed Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting checklist, indicating the page where each item is included (see JAMA (2000) 283, 2008-2012). Manuscripts in these areas of review will not be sent for peer review unless accompanied by the relevant completed checklist.

Letters to the Editor

Letters are invited that discuss, criticise or develop themes put forward in papers published in BJN. They should not, however, be used as a means of publishing new work. Acceptance will be at the discretion of the Editorial Board, and editorial changes may be required. Wherever possible, letters from responding authors will be included in the same issue as the original article.

DETAILED MANUSCRIPT PREPARATION INSTRUCTIONS

Language

Papers submitted for publication must be written in English and should be as concise as possible. We recommend that authors have their manuscript checked by someone whose first language is English before submission, to ensure that submissions are judged at peer review exclusively on academic merit. Please see the Author Language Services section below for more information.

Spelling should generally be that of the *Concise Oxford Dictionary* (1995), 9th ed. Oxford: Clarendon Press. Authors are advised to consult a current issue in order to make themselves familiar with BJN as to typographical and other conventions, layout of tables etc. Sufficient information should be given to permit repetition of the published work by any competent reader of BJN.

Published examples of BJN article types can be found below:

Research Article

Review Article

Horizons Article

Letter to the Editor

Authorship

The Journal conforms to the International Committee of Medical Journal Editors (ICMJE) definition of authorship, as described by P.C. Calder (*Br J Nutr* (2009) 101, 775). Authorship credit should be based on:

Substantial contributions to conception and design, data acquisition, analysis and/or interpretation;

Drafting the article or revising it critically for important intellectual content; and

Final approval of the version to be published.

The contribution of individuals who were involved in the study but do not meet these criteria should be described in the Acknowledgments section.

Ethical standards

The required standards for reporting studies involving humans and experimental animals are detailed in an Editorial by G.C. Burdge (*Br J Nutr* (2014) 112).

Experiments involving human subjects

The notice of contributors is drawn to the guidelines in the World Medical Association (2000) Declaration of Helsinki: ethical principles for medical research involving human subjects, with notes of clarification of 2002 and 2004 (http://www.wma.net/en/30publications/10policies/b3/), the *Guidelines on the Practice of Ethics Committees Involved in Medical Research Involving Human Subjects* (3rd ed., 1996; London: The Royal College of Physicians) and the Guidelines for the ethical conduct of medical research involving children, revised in 2000 by the Royal College of Paediatrics and Child Health: Ethics Advisory Committee (*Arch Dis Child* (2000) 82, 177–182). Articles reporting randomised trials must conform to the standards set by the Consolidated Standards of Reporting Trials (CONSORT) consortium. A completed CONSORT Checklist (Consolidated Standards of Reporting Trials (CONSORT) consortium) must accompany manuscripts reporting randomised controlled trials. Submissions that do not include this information will not be considered for review until a completed CONSORT Checklist has been submitted and approved.

Required disclosures: A paper describing any experimental work on human subjects must include the following statement in the Experimental Methods section: "This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the [insert name of the ethics committee; a specific ethics number may be inserted if you wish]. Written [or Verbal] informed consent was obtained from all subjects/patients. [Where verbal consent was obtained this must be followed by a statement such as: Verbal consent was witnessed and formally recorded]." For clinical trials, the trial registry name, registration identification number, and the URL for the

registry should be included.

PLEASE NOTE: From 1 October 2014, as a condition for publication, all randomised controlled trials that involve human subjects submitted to BJN for review must be registered in a public trials registry. A clinical trial is defined by the ICMJE (in accordance with the definition of the World Health Organisation) as any research project that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes. Registration information must be provided at the time of submission, including the trial registry name, registration identification number, and the URL for the registry.

Experiments involving the use of other vertebrate animals

Papers that report studies involving vertebrate animals must conform to the 'ARRIVE Guidelines for Reporting Animal Research' detailed in Kilkenny et al. (*J Pharmacol Pharmacother* (2010) 1, 94-99) and summarised atwww.nc3rs.org.uk. Authors must ensure that their manuscript conforms to the checklist that is available from the nc3Rs website. The attention of authors is drawn particularly to the ARRIVE guidelines point 3b ('Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology', point 9c ('Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment') and point 17a ('Give details of all important adverse events in each experimental group'). The Editors will not accept papers reporting work carried out involving procedures that cause or are considered likely to cause distress or suffering which would confound the outcomes of the experiments, or experiments that have not been reviewed and approved by an animal experimentation ethics committee or regulatory organisation.

Required disclosures: Where a paper reports studies involving vertebrate animals, authors must state in the Experimental Methods section the institutional and national guidelines for the care and use of animals that were followed and that all experimental procedures involving animals were approved by the [insert name of the ethics committee or other approving body; wherever possible authors should also insert a specific ethics/approval number].

Manuscript Format

The requirements of BJN are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the ICMJE.

Typescripts should be prepared with 1.5 line spacing and wide margins (2 cm), the preferred font being Times New Roman size 12. At the ends of lines, words should not be hyphenated unless hyphens are to be printed. Line numbering and page numbering are

required.

Manuscripts should be organised as follows:

Cover letter

Papers should be accompanied by a cover letter including a brief summary of the work and a short explanation of how it advances nutritional science. The text for the cover letter should be entered in the appropriate box as part of the online submission process.

Title Page

The title page should include:

The title of the article;

Authors' names;

Name and address of department(s) and institution(s) to which the work should be attributed for each author;

Name, mailing address, email address, telephone and fax numbers of the author responsible for correspondence about the manuscript;

A shortened version of the title, not exceeding 45 characters (including letters and spaces) in length;

At least four keywords or phrases (each containing up to three words).

Authors' names should be given without titles or degrees and one forename may be given in full. Identify each author's institution by a superscript number (e.g. A.B. Smith[1]) and list the institutions underneath and after the final author.

Abstract

Each paper must open with an unstructured abstract of not more than 250 words. The abstract should be a single paragraph of continuous text without subheadings outlining the aims of the work, the experimental approach taken, the principal results (including effect size and the results of statistical analysis) and the conclusions and their relevance to nutritional science.

Introduction

It is not necessary to introduce a paper with a full account of the relevant literature, but the introduction should indicate briefly the nature of the question asked and the reasons for asking it. It should be no longer than two manuscript pages.

Experimental methods

The methods section must include a subsection that describes the methods used for statistical analysis (see the section on statistical analysis in the Appendix) and the sample size must be justified by the results of appropriate calculations and related to the study outcomes.

Justification of sample size: All manuscripts that report primary research must contain

a statistical justification of sample size that is stated explicitly in the Statistics sub-section of the Methods. Manuscripts that do not contain this information will be rejected automatically and returned to the authors for correction. The revised versions will be treated as new submissions. The information required must include, but not be restricted to, the following:-

Hypothesised effect size with appropriate justification.

A statement regarding statistical power (typically 80%) and the two-sided significance level (typically 0.05).

An explanation of how the statistical power was calculated.

If sample size is determined by the feasibility of recruitment minimally detectable effect sizes should be provided instead of power analysis.

The only exceptions are:-

Meta-analyses.

Exploratory or secondary analysis of observational studies based on large sample sizes

For studies involving humans subjects or experimental animals, the Methods section must include a subsection that reports the appropriate ethical approvals for the study (see Ethical Standards above).

All analytical procedures must be accompanied by a statement of within and between assay precision.

Diets: The nutrient composition of diets used in studies published in BJN must be described in detail, preferably in a table(s). Experimentally relevant differences in composition between diets are essential. For instance, studies of fat nutrition should always include fatty acid compositions of all diets.

PCR analysis: Where experiments involve measurement of mRNA including microarray analysis, for analysis of individual genes, mRNA should be measured by quantitative RTPCR. A statement about the quality and integrity of the RNA must be provided together with the results of eletrophoretic analysis of the purity of the PCR products. Unless published elsewhere, full details of the oligonuceoltide primers and of the PCR protocol must be stated either in the text or in Supplementary Material. The stability of reference genes used for normalisation of PCR data must be reported for the experimental conditions described. Where possible, analysis of mRNA levels should be accompanied by assessment of either protein levels or activities.

Microarray analysis: Studies involving microarray analysis of mRNA must conform to the "Minimum Information about a Microarray Experiment" (MIAME) guidelines including deposition of the raw data in an appropriate repository (the Access Code must be state din the

Methods). All microarray experiments must be accompanied by appropriate validation by quantitative RTPCR.

Results

These should be given as concisely as possible, using figures or tables as appropriate. Data must not be duplicated in tables and figures.

Discussion

While it is generally desirable that the presentation of the results and the discussion of their significance should be presented separately, there may be occasions when combining these sections may be beneficial. Authors may also find that additional or alternative sections such as 'conclusions' may be useful. The discussion should be no longer than five manuscript pages.

Acknowledgments

Here you may acknowledge individuals or organizations that provided advice and/or support (non-financial). Formal financial support and funding should be listed in the following section.

Financial Support

Please provide details of the sources of financial support for all authors, including grant numbers. For example, "This work was supported by the Medical research Council (grant number XXXXXXX)". Multiple grant numbers should be separated by a comma and space, and where research was funded by more than one agency the different agencies should be separated by a semi-colon, with "and" before the final funder. Grants held by different authors should be identified as belonging to individual authors by the authors' initials. For example, "This work was supported by the Wellcome Trust (A.B., grant numbers XXXX, YYYY), (C.D., grant number ZZZZ); the Natural Environment Research Council (E.F., grant number FFFF); and the National Institutes of Health (A.B., grant number GGGG), (E.F., grant number HHHH)".

This disclosure is particularly important in the case of research that is supported by industry. Support from industry not only includes direct financial support for the study but also support in kind such as provision of medications, equipment, kits or reagents without charge or at reduced cost and provision of services such as statistical analysis; all such support must be disclosed here and if no such support was received this must be stated. Where no specific funding has been provided for research, please provide the following statement: "This research received no specific grant from any funding agency, commercial or not-for-profit sectors."

In addition to the source of financial support, please state whether the funder contributed to the study design, conduct of the study, analysis of samples or data, interpretation of findings or the preparation of the manuscript. If the funder made no such contribution, please

provide the following statement: "[Funder's name] had no role in the design, analysis or writing of this article."

Conflict of Interest

Please provide details of all known financial, professional and personal relationships with the potential to bias the work. Where no known conflicts of interest exist, please include the following statement: "None."

For more information on what constitutes a conflict of interest, please see the International Committee of Medical Journal Editors (ICMJE) guidelines.

Authorship

Please provide a very brief description of the contribution of each author to the research. Their roles in formulating the research question(s), designing the study, carrying it out, analysing the data and writing the article should be made plain.

References

References should be numbered consecutively in the order in which they first appear in the text using superscript Arabic numerals in parentheses, e.g. 'The conceptual difficulty of this approach has recently been highlighted[1,2]'. If a reference is cited more than once, the same number should be used each time. References cited only in tables and figure legends should be numbered in sequence from the last number used in the text and in the order of mention of the individual tables and figures in the text.

Names and initials of authors of unpublished work should be given in the text as 'unpublished results' and not included in the References. References that have been published online only but not yet in an issue should include the online publication date and the Digital Object Identifier (doi) reference, as per the example below.

At the end of the paper, on a page(s) separate from the text, references should be listed in numerical order using the Vancouver system. When an article has more than three authors only the names of the first three authors should be given followed by '*et al.*' The issue number should be omitted if there is continuous pagination throughout a volume. Titles of journals should appear in their abbreviated form using the NCBI LinkOut page. References to books and monographs should include the town of publication and the number of the edition to which reference is made. References to material available on websites should follow a similar style, with the full URL included at the end of the reference, as well as the date of the version cited and the date of access.

Examples of correct forms of references are given below.

Journal articles

Rebello SA, Koh H, Chen C *et al.* (2014) Amount, type, and sources of carbohydrates

in relation to ischemic heart disease mortality in a Chinese population: a prospective cohort study. *Am J Clin Nutr* 100, 53-64.

Villar J, Ismail LC, Victora CG *et al.* (2014) International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project.*Lancet* 384, 857-868.

Alonso VR & Guarner F (2013) Linking the gut microbiota to human health. *Br J Nutr* 109, Suppl. 2, S21–S26.

Bauserman M, Lokangaka A, Gado J et al. A cluster-randomized trial determining the efficacy of caterpillar cereal as a locally available and sustainable complementary food to prevent stunting and anaemia. *Public Health Nutr*. Published online: 29 January 2015. doi: 10.1017/S1368980014003334.

Books and monographs

Bradbury J (2002) Dietary intervention in edentulous patients. PhD Thesis, University of Newcastle.

Ailhaud G & Hauner H (2004) Development of white adipose tissue. In *Handbook of Obesity. Etiology and Pathophysiology*, 2nd ed., pp. 481–514 [GA Bray and C Bouchard, editors]. New York: Marcel Dekker.

Bruinsma J (editor) (2003) *World Agriculture towards 2015/2030: An FAO Perspective*. London: Earthscan Publications.

World Health Organization (2003) *Diet, Nutrition and the Prevention of Chronic Diseases*. Joint WHO/FAO Expert Consultation. WHO Technical Report Series no. 916. Geneva: WHO.

Keiding L (1997) *Astma, Allergi og Anden Overfølsomhed i Danmark – Og Udviklingen 1987–199I (Asthma, Allergy and Other Hypersensitivities in Denmark, 1987–1991)*. Copenhagen, Denmark: Dansk Institut for Klinisk Epidemiologi.

Sources from the internet

Nationmaster (2005) HIV AIDS – Adult prevalence rate. http://www.nationmaster.com/graph-T/hea_hiv_aid_ad... (accessed June 2013).

Figures

Figures should be supplied as separate electronic files. Figure legends should be grouped in a section at the end of the manuscript text. Each figure should be clearly marked with its number and separate panels within figures should be clearly marked (a), (b), (c) etc. so that they are easily identifiable when the article and figure files are merged for review. Each figure, with its legend, should be comprehensible without reference to the text and should include definitions of abbreviations. The nature of the information displayed in the figures (e.g.

mean (SEM)) and the statistical test used must be stated.

We recommend that only TIFF, EPS or PDF formats are used for electronic artwork. Other non-preferred but usable formats are JPG, PPT and GIF files and images created in Microsoft Word. Note that these non-preferred formats are generally NOT suitable for conversion to print reproduction. For further information about how to prepare your figures, including sizing and resolution requirements, please see our artwork guide.

In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, ○, ●, △, ▲, □, ■, ×, +. Curves and symbols should not extend beyond the experimental points. Scale-marks on the axes should be on the inner side of each axis and should extend beyond the last experimental point. Ensure that lines and symbols used in graphs and shading used in histograms are large enough to be easily identified when the figure size is reduced to fit the printed page. Statistically significant effects should be indicated with symbols or letters.

Colour figures will be published online free of charge, and there is a fee of £350 per figure for colour figures in the printed version. If you request colour figures in the printed version, you will be contacted by CCC-Rightslink who are acting on our behalf to collect colour charges. Please follow their instructions in order to avoid any delay in the publication of your article.

Images submitted with a manuscript should be minimally processed; some image processing is acceptable (and may be unavoidable), but the final image must accurately represent the original data. Grouping or cropping of images must be identified in the legend and indicated by clear demarcation. Please refer to the Office of Research Integrity guidelines on image processing in scientific publication. Authors should provide sufficient detail of image-gathering procedures and process manipulation in the Methods sections to enable the accuracy of image presentation to be assessed. Authors should retain their original data, as Editors may request them for comparison during manuscript review.

Tables

Tables should be placed in the main manuscript file at the end of the document, not within the main text. Please donot supply tables as images (e.g. in TIFF or JPG format). Be sure that each table is cited in the text. Tables should carry headings describing their content and should be comprehensible without reference to the text. Tables should not be subdivided by ruled lines.

The dimensions of the values, e.g. mg/kg, should be given at the top of each column. Separate columns should be used for measures of variance (SD, SE etc.), the ± sign should not be used. The number of decimal places used should be standardized; for whole numbers

1.0, 2.0 etc. should be used. Shortened forms of the words weight (wt) height (ht) and experiment (Expt) may be used to save space in tables, but only Expt (when referring to a specified experiment, e.g. Expt 1) is acceptable in the heading.

Footnotes are given in the following order: (1) abbreviations, (2) superscript letters, (3) symbols. Abbreviations are given in the format: RS, resistant starch. Abbreviations in tables must be defined in footnotes in the order that they appear in the table (reading from left to right across the table, then down each column). Symbols for footnotes should be used in the sequence: *†‡§||¶, then ** etc. (omit * or †, or both, from the sequence if they are used to indicate levels of significance).

For indicating statistical significance, superscript letters or symbols may be used. Superscript letters are useful where comparisons are within a row or column and the level of significance is uniform, e.g. '^{a,b,c}Mean values within a column with unlike superscript letters were significantly different (*P*<0•05)'. Symbols are useful for indicating significant differences between rows or columns, especially where different levels of significance are found, e.g. 'Mean values were significantly different from those of the control group: **P*<0•05, ***P*<0•01, ****P*<0•001'. The symbols used for *P* values in the tables must be consistent.

Supplementary material

Additional data (e.g. data sets, large tables) relevant to the paper can be submitted for publication online only, where they are made available via a link from the paper. The paper should stand alone without these data. Supplementary Material must be cited in a relevant place in the text of the paper.

Although Supplementary Material is peer reviewed, it is not checked, copyedited or typeset after acceptance and it is loaded onto the journal's website exactly as supplied. You should check your Supplementary Material carefully to ensure that it adheres to journal styles. Corrections cannot be made to the Supplementary Material after acceptance of the manuscript. Please bear this in mind when deciding what content to include as Supplementary Material.

COPYRIGHT

Authors or their institutions retain copyright of papers published in BJN. The corresponding author should complete aLicense to Publish form on behalf of all authors, and upload this with the manuscript files at the time of submission. If the manuscript is not accepted, the form will be destroyed.

OPEN ACCESS

Authors in BJN have the option to publish their paper under a fully Open Access agreement, upon payment of a one-off Article Processing Charge. In this case, the final

published Version of Record will be made freely available to all in perpetuity under a creative commons license, enabling its re-use and re-distribution. This Open Access option is only offered to authors upon acceptance of an article for publication.

Authors choosing the Open Access option are required to complete the Open Access License to Publish form. More information about Open Access in BJN, including the current Article Processing Charge, can be found on our website.

AuthorAID

AuthorAID is a global network that provides free support, mentoring, resources and training to help researchers in low- and middle-income countries to write, publish and otherwise communicate their work.

Key features of AuthorAID are:

a community space for discussion and questions where researchers can benefit from advice and insights from members across the globe

access to a range of documents and presentations on best practice in writing and publication

world-wide training workshops and MOOCs on scientific writing

a chance to network with other researchers

personal mentoring by highly published researchers and professional editors

For any authors new to publishing research articles, we encourage you to make use of the AuthorAID resources before submitting your paper to BJN. Through the AuthorAID network, guidance can be found to help researchers through the process of writing and submitting scientific papers, advice about responding to reviewer comments, as well as research design and grant applications.

Please note that seeking support through AuthorAID will not guarantee acceptance for publication in BJN, or affect the editorial process in any way.

AUTHOR LANGUAGE SERVICES

BJN recommends that authors have their manuscripts checked by an English language native speaker before submission; this will ensure that submissions are judged at peer review exclusively on academic merit. We list a number of third-party servicesspecialising in language editing and/or translation, and suggest that authors contact as appropriate. Use of any of these services is voluntary, and at the author's own expense.

PROOFS

PDF proofs are sent to authors in order that they make sure that the paper has been correctly set up in type. Only changes to errors induced by typesetting/copy-editing or typographical errors will be accepted.

Corrected proofs should be returned within 2 days by email to:

Emma Pearce

Production Editor

Cambridge University Press

Telephone: +44 1223 325032

Fax: +44 1223 325802

Email: bjnproduction@cambridge.org

If corrected proofs are not received from authors within 7 days the paper may be published as it stands.

OFFPRINTS

A PDF file of the paper will be suppltied free of charge to the corresponding author of each paper, and offprints may be ordered on the order form sent with the proofs.

DIGITAL PRESERVATION POLICY

Cambridge University Press publications are deposited in the following digital archives to guarantee long-term digital preservation:

CLOCKSS (journals)

Portico (journals and books)

Further information can be found here.

CONTACT

Prospective authors may contact the Editorial Office directly on +44 (0) 1223 325977 (telephone) or bjn.edoffice@cambridge.org.

APPENDIX: MATHEMATICAL MODELLING, STATISTICS AND NOMENCLATURE