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LUIZ CARLOS CAETANO

AVALIAÇÃO DAS CITOCINAS INFLAMATÓRIAS EM RATOS OBESOS-MSG SUPLEMENTADOS OU NÃO COM TAURINA

CASCAVEL-PR

(Novembro/2013)

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AVALIAÇÃO DAS CITOCINAS INFLAMATÓRIAS EM RATOS OBESOS-MSG SUPLEMENTADOS OU NÃO COM TAURINA

Dissertação apresentada ao Programa De Pós-Graduação *Stricto Sensu* em Biociências e Saúde – Nível Mestrado, do Centro de Ciências Biológicas e da Saúde, da Universidade Estadual do Oeste do Paraná, como requisito parcial para a obtenção do título de Mestre em Biociências e Saúde.

Área de concentração: Biologia, processo saúde-doença e políticas de saúde

ORIENTADOR: Prof[a]. Dra. Sandra Lucinei Balbo
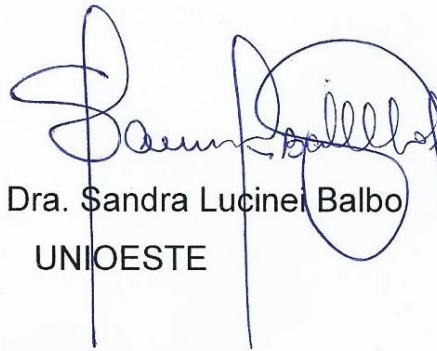CO-ORIENTADOR: Prof[a]. Dra. Maria Lúcia Bonfleur

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FOLHA DE APROVAÇÃO

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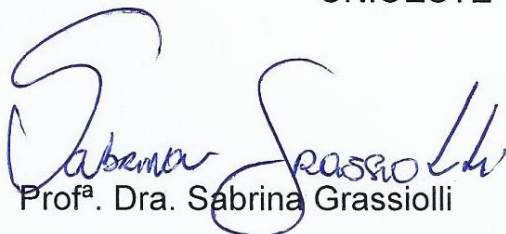# AVALIAÇÃO DAS CITOCINAS INFLAMATÓRIAS EM RATOS OBESOS-MSG SUPLEMENTADOS OU NÃO COM TAURINA

Esta dissertação foi julgada adequada para a obtenção do título de Mestre em Biociências e Saúde e aprovada em sua forma final pelo Orientador e pela Banca Examinadora.

Orientador: Profª. Dra. Sandra Lucinei Balbo

UNIOESTE

Profª. Dra. Célia Cristina Leme Beu

UNIOESTE

Profª. Dra. Sabrina Grassiolli

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Dedicado a Sidnei Bergamin, meu amado primo que, dia após dia, me motiva a nunca desistir, e a todos que lutam corajosamente em favor da vida.

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RESUMO GERAL

Dentre as várias alterações orgânicas decorrentes da obesidade, está o processo inflamatório crônico associado ao balanço das citocinas TNF-α, IL-1β, IL-6, IL-2, IFNγ, IL-4 e IL-10, e, há evidências de que o aminoácido taurina (TAU) possui efeito anti-inflamatório. Assim, neste trabalho investigamos o perfil inflamatório plasmático e do tecido adiposo retroperitoneal de ratos obesos-MSG, suplementados ou não, com o aminoácido TAU. Ratos *Wistar* receberam injeções subcutâneas de MSG (4mg/kg de peso corporal/dia) ou salina hiperosmótica, durante os primeiros 5 dias de vida e foram distribuídos nos grupos MSG e CON, respectivamente. Após os 21 dias de vida, metade de cada grupo recebeu 2,5% de TAU na água de beber, sendo separados nos grupos CON, CON + TAU (CTAU), MSG e MSG + TAU (MTAU). Aos 120 dias de vida os animais foram eutanasiados. Ratos MSG apresentaram obesidade acompanhada de hipertrigliceridemia e resistência à insulina (RI). Todavia, não afetou a expressão de IκBα e JNK. A suplementação com TAU aumentou 61% a expressão do IkBα no grupo CTAU em relação ao grupo CON e 107% nos animais MTAU em comparação com os obesos-MSG. As expressões de TNF-α, IL-1β e IL-6 no tecido adiposo retroperitoneal foram semelhantes nos 4 grupos de animais estudados, assim como as concentrações plasmáticas do TNF-α, IL-1β, IL-6, IL-2, IFNγ, IL-4 e IL-10. É possível concluir que o tratamento neonatal com MSG não influencia o perfil inflamatório dos animais. Concluímos também que a TAU aumentou a expressão proteica do IkBα nos animais controle e MSG, sem afetar as citocinas inflamatórias. Desta forma sugerimos que a TAU possa exercer seus efeitos anti-inflamatórios no tecido adiposo, via NF-κB.

Palavras-chaves: Obesidade; Glutamato Monossódico (MSG); Citocinas; Inflamação; NF-κB; JNK; Taurina.

GENERAL ABSTRACT

Among the several organic alterations arising from obesity, chronic inflammation is associated with the balance of cytokines TNF-α, IL-1β, IL-6, IL-2, IFNg, IL-4 and IL-10, and there is evidence the amino acid taurine (Tau) has anti-inflammatory effect. Therefore, this study investigated the inflammatory profile in plasma and retroperitoneal adipose tissue of MSG-obese rats, supplemented or not, with the TAU. Male *Wistar* rats received subcutaneous injections of MSG (4mg/kg body weight/day) or hyperosmotic saline during the first 5 days of life, composing the control (CON) and MSG groups. After 21 days, half of each group received TAU 2.5% in drinking water, and separated into 04 groups: CON, CON with TAU (CTAU), MSG and MSG with TAU (MTAU). At 120 days of age, the animals were euthanized. The MSG rats showed an increase in Lee Index, retroperitoneal and perigonadal fat pads deposition, insulin and triglycerides plasmatic concentrations and HOMA-IR, when compared to CON animals, showing that the treatment with MSG led to obesity. The TAU supplementation attenuated retroperitoneal fat deposition, as well as TG concentration. The MSG treatment did not alter the expression of JNK and IκBα. However, the supplementation with TAU increased 61% the expression of IkBα in CTAU group compared to the CON and 107% in the MTAU animals compared to the MSG. The expression of TNF-α, IL-1β and IL-6 in the retroperitoneal adipose tissue were similar in the four groups of animals, as well as plasma concentrations of TNF-α, IL-1β, IL-6, IL-2, IFNγ, IL-4 and IL-10. It is possible to conclude that neonatal treatment with MSG does not influence the inflammatory profile of the animals. We also conclude that the TAU increased 61% of IkBα protein expression in the control group and 107% in the MSG-obese animals, without affecting the inflammatory cytokines. Thus we suggest that TAU can exert their anti-inflammatory effects in adipose tissue, via NF-kB.

Keywords: Obesity; Monosodium Glutamate (MSG); Citokines; Inflammation; NF-κB; JNK; Taurine.

SUMÁRIO

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

ANGPTL2 – *Angiopoietinlike protein 2*

AP-1 – Ativador da proteina-1

CCL2 – *Chemokine (C-C motif) ligand 2*

COL – Colesterol

CXCL5 – *CXC-chemokine ligand 5*

DM2 – Diabetes *Mellitus* tipo 2

ER – Retículo endoplasmático

GLUT-4 – *Glucose transporter type 4*

IFN-γ – *Interferon - gamma*

IKKB – *I-Kappa-B Kinase*

IL-10 – Interleucina - 10

IL-12 – Interleucina - 12

IL-1β – Interleucina - 1 beta

IL-2 – Interleucina - 2

IL-4 – Interleucina - 4

IL-6 – Interleucina - 6

IR – Receptor da insulina

IRS – Substrato do receptor da insulina

IκB – Inibidores kappa B

IκBα – Inibidor kappa B alfa

JNK – c-*Jun N-terminal Kinase*

LPS – Lipopolissacarídeo

MSG – Glutamato monossódico

mTOR – *Mammalian target of rapamycin*

NAMPT – Nicotinamida fosforribosiltransferase

NF-κB – Fator nuclear – kappa B

NPY – Neuropeptídeo Y

PDX-1 – *Pancreatic and duodenal homeobox* 1

PI3K – *Phosphoinositide-3-kinase*

RBP4 – *Retinol-binding protein 4*

S6K – S6-*Kinase*

SERP – Resíduos inibidores da serina

SFRP5 – *Secreted frizzled-related protein 5*

SUS – Sistema Único de Saúde

TAU – Taurina

TauBr – Taurina Bromamina

TauCl – Taurina Cloramina

TG – Triglicerídeo

TLR – *Toll Like Receptor*

TNFα – Fator de necrose tumoral – alfa

INTRODUÇÃO GERAL

A obesidade acompanha a humanidade desde o início das civilizações, recebendo diferentes interpretações de acordo com cada cultura. No período Neolítico (aproximadamente 10.000 anos a.C.), as "deusas" eram admiradas e cultuadas por seus volumosos quadris, coxas e seios, sendo um símbolo de beleza e fertilidade. Já na Idade Média, o sobrepeso era visto pelos japoneses como um desvio moral e, a Igreja Católica europeia, o associava com o pecado da gula. A associação entre a obesidade e saúde pública já era relatada por Hipócrates em seus manuscritos. Galeno, discípulo de Hipócrates, classificou a obesidade em natural (moderada) e mórbida (exagerada), cuja origem estava associada com a falta de disciplina do indivíduo e o tratamento associava exercício físico e uma dieta pobre em calorias. A associação entre o excesso de peso e beleza deixou de existir com obras de Arte a partir do século XIII, que passaram a retratar corpos de damas magras e com formas delineadas (CUNHA, NETO e JÚNIOR, 2006).

Atualmente a obesidade é considerada um dos mais graves problemas de saúde pública do mundo (CINTRA, ROPELLE e PAULI, 2011), sendo considerada a segunda maior causa de morte passível de prevenção (BRASIL, 2005), e atinge mais de meio bilhão de pessoas. O risco de mortalidade associado à obesidade ultrapassa os riscos da subnutrição (WHO, 2010) por ser fator desencadeante de uma série de outras patologias, em especial as disfunções cardiovasculares e o Diabetes *Mellitus* tipo 2 (DM2). O desenvolvimento da obesidade deriva de um saldo positivo entre a ingesta e a utilização de energia, favorecendo a hipertrofia do tecido adiposo (ANDERSON, 1972). Esse desbalanço está associado à interação entre fatores ambientais, comportamentais e genéticos, mas os hábitos ocidentais contemporâneos, que incluem o sedentarismo e as dietas hipercalóricas, são os grandes responsáveis pela rápida disseminação dessa pandemia. As consequências da obesidade vão além de problemas físicos, comprometendo também questões psicológicas, comportamentais, trabalhistas, educacionais e sentimentais. Isso responsabiliza e compromete todos os segmentos da sociedade na busca pela reversão desse quadro mundial (BRASIL, 2012).

Atualmente o tecido adiposo é um dos principais focos das pesquisas em obesidade, devido a uma revolução no entendimento da função biológica deste

tecido. Com a descoberta das adipocinas, esse tecido passou a ser considerado um órgão endócrino, exercendo fortíssimas influências no balanço energético, na homeostase da glicose (WAJCHENBERG, 2000; DÂMASO, 2003; CINTRA, ROPELLE e PAULI, 2011; LEGGATE *et. al*, 2012) e também no sistema imunológico. Muitos estudos atuais estão focados no processo inflamatório crônico desencadeado pela obesidade, pois o aumento da secreção de citocinas como a leptina, resistina, fator de necrose tumoral – alfa (TNF-α) e interleucinas (ILs) pelos adipócitos levam à resposta inflamatória com efeitos em todo o organismo (DÂMASO, 2003; CINTRA, ROPELLE e PAULI, 2011; LEGGATE *et. al*, 2012; LIRA *et. al*, 2012; PARK *et. al*, 2012).

A transcrição dos genes das citocinas inflamatórias, no tecido adiposo, é ativada por duas vias principais: a do *I-Kappa-B Kinase* (IKKβ) e da c-*Jun N-terminal Kinase* (JNK). Fatores como as próprias citocinas inflamatórias, ativam a IKKβ que promove a ubiquitinação dos inibidores κB (IκB) que se desligam do fator nuclear kB (NF-κB) e são degradados. O NF-κB, migra para o interior do núcleo celular e ativa a transcrição de diversos genes associados ao processo inflamatório, dentre eles os genes do TNFα, IL-1β e IL-6 (SCHMID e BIRBACH, 2008). A JNK é outra quinase ativada por estímulos inflamatórios (DAVIS, 2000). Quando fosforilada, ela estimula a migração do ativador de proteína-1 (AP-1) para o núcleo, onde irá ativar a transcrição das citocinas pró-inflamatórias (DAVIS, 2000; MORSE *et. al*, 2003).

Com o objetivo de entender os mecanismos fisiopatológicos envolvidos com a obesidade, vários modelos experimentais de animais são utilizados. Dentre eles, encontram-se os submetidos a alterações genéticas (CHENTOUF *et. al*, 2011; HUANG *et. al*, 2012), dietas hipercalóricas (NASCIMENTO *et. al*, 2008; EL MESALLAMY *et. al*, 2010; CHANG *et. al*, 2011; GENTILE *et. al*, 2011; MADANI *et. al*, 2012) ou por administração de drogas (NASCIMENTO *et. al*, 2008; EL MESALLAMY *et. al*, 2010; CHANG *et. al*, 2011; GENTILE *et. al*, 2011), como por exemplo, a neurointoxicação neonatal por glutamato monossódico (MSG).

A aplicação subcutânea de MSG causa lesões em núcleos hipotalâmicos fundamentais para o balanço energético corpóreo, induzindo, assim, o desenvolvimento da obesidade, bem como de suas comorbidades (OLNEY, 1969; SIMONS *et. al*, 2007; MORRISON *et. al*, 2008; FERREIRA *et. al*, 2011; PATIL *et. al*, 2011; ROMAN-RAMOS *et. al*, 2011).

Na tentativa de prevenir ou elaborar estratégias terapêuticas para minimizar os danos corpóreos causados pela obesidade, muitos tratamentos vêm sendo

testados, como os processos cirúrgicos, atividades físicas, tratamentos farmacológicos, dentre outros. Um dos tratamentos utilizados na pesquisa cientifica atual é a suplementação com o aminoácido taurina (TAU), que atua na prevenção da deposição de gordura (CARNEIRO *et. al*, 2009; CHANG *et. al*, 2011; GENTILE *et. al*, 2011; NARDELLI *et. al*, 2011); melhora o metabolismo hepático; e reduz a disponibilidade de triglicerídeos (TG) e colesterol (COL) para os demais tecidos (EL MESALLAMY *et. al*, 2010; CHANG *et. al*, 2011)

Além de apresentar papel fundamental na modulação da homeostase da glicose, a Tau também aumenta a secreção e a sensibilidade à insulina (CARNEIRO *et. al*, 2009; RIBEIRO *et. al*, 2009). Recentemente, foi demonstrado que a suplementação com TAU diminuiu as citocinas pró-inflamatórias em ratos com diabetes por aloxana (DAS E SIL, 2012). Porém, esse efeito é contraditório na literatura (CHANG *et. al*, 2011).

Considerando que estudos publicados recentemente têm mostrado o aumento das citocinas e proteínas associadas à inflamação, tanto em pacientes obesos quanto em modelos experimentais de obesidade animal; considerando que a TAU vem sendo estudada como uma provável estratégia contra os efeitos da obesidade, além de estar sendo investigada como um possível agente anti-inflamatório; justifica-se a importância de estudar marcadores inflamatórios em ratos obesos-MSG, submetidos ou não, à suplementação com TAU. Assim, neste trabalho investigamos o perfil inflamatório plasmático e do tecido adiposo retroperitoneal de ratos obesos-MSG, suplementados ou não, com o aminoácido TAU.

REVISÃO GERAL DE LITERATURA

Obesidade

Atualmente a obesidade é considerada um dos maiores fenômenos clínico-epidemiológicos do mundo (CINTRA, ROPELLE e PAULI, 2011), atingindo cerca de 500 milhões de adultos no ano de 2008 e 43 milhões de crianças em 2010 (WHO, 2010). No Brasil, o aumento nas prevalências do sobrepeso e da obesidade são alarmantes, os casos de sobrepeso passaram de 43% da população acima de 18 anos em 2006, para 51% em 2012, atingindo cerca de 54% dos homens e 48% das mulheres (BRASIL, 2012). Além da variação relacionada ao sexo, tal prevalência também apresenta tendencial diferença entre as classes sociais (BRASIL, 2012) o que evidencia a influência de fatores como renda e escolaridade no comportamento alimentar. O relatório produzido pela Comissão Nacional de Determinantes Sociais da Saúde de 2008 aponta a necessidade da formulação e implantação de estratégias para a redução das morbi-mortalidades relacionadas à alimentação inadequada e ao sedentarismo (BRASIL, 2011). Essa é uma necessidade global, visto que cerca de 65% da população mundial vive em países onde o sobrepeso mata mais que a subnutrição, pois tal disfunção é fator de risco para anomalias cardiovasculares (principais causas das mortes mundiais) e doenças associadas como hipertensão arterial e arteriosclerose, além de DM2 e dislipidemias, doenças respiratórias, lesões músculo-esqueléticas, câncer e disfunções psicológicas (WHO, 2010). Em comum, grande parte dessas doenças tem sua origem associada à gênese do excesso de peso (DÂMASO, 2003), o qual se estabelece por um balanço positivo, em que há um aumento na razão entre ingesta e gasto energético, causando um excessivo acúmulo de energia depositada na forma de gordura no tecido adiposo (ANDERSON, 1972). Os gastos públicos com as doenças crônicas não transmissíveis causam um ônus de bilhões de reais aos cofres públicos do Brasil (BRASIL, 2011), totalizando cerca de 75% das despesas do Sistema Único de Saúde (SUS) com atenção à saúde (BRASIL, 2005).

A obesidade tornou-se assunto para os profissionais da área médica a partir do final da década de 1970 e vem sendo interpretada como uma doença epidêmica desde 1990, por isso obteve grande destaque nas políticas mundiais de saúde dos últimos anos. A definição do termo saúde pode ser incluída entre os conceitos que,

embora aplicados a categorias concretas e de relevância, não permitem sua definição com objetividade a partir de elementos aceitos universalmente (SABROZA, 2012). Christopher Boorse em 1977 conceitua saúde numa visão totalmente orgânica e restrita à ausência de doença (SCLIAR, 2007), um conceito baseado numa única ótica, uma forma de pensar fragmentada e monodisciplinar que conduz a um conhecimento limitado. Barros (2002) critica esse modelo biologicista por, atualmente, dominar a prática médica (BARROS, 2002). O conceito mais utilizado atualmente, deriva da Constituição da OMS de 1946 que afirma que a boa saúde é um estado de completo desenvolvimento físico, social e bem-estar mental, e não meramente a ausência de doença ou enfermidade (WHO, 2010). Essa definição permite uma visão interdisciplinar do processo saúde-doença, por ser um meio de superar o isolacionismo das disciplinas e discutir o mesmo objeto por diferentes pontos de vista, permitindo-se assim, uma maximização no entendimento causa e consequência de tal objeto (SCHERER e PIRES, 2011). Nesse contexto, diversos debates mundiais tentaram implementar fatores econômicos, sociais e ambientais, na atenção primaria à saúde. A carta de Ottawa de 1986 definiu a promoção à saúde: como o processo de capacitação da comunidade para atuar na melhoria da sua qualidade de vida e saúde, demonstrando a crescente busca por um entendimento mais amplo do papel dos diversos (BRASIL, 2002) segmentos da sociedade no processo saúde-doença, papel esse que só pode ser vislumbrado se usada uma visão interdisciplinar.

A obesidade é considerada uma doença multifatorial, pois diversos fatores intrínsecos (genéticos, fisiológicos e psicológicos) e também ambientais contribuem para o seu estabelecimento (LIDFELDT, SAMSIOE e AGARDH, 2006; WHO, 2010). Dentre as influências ambientais se destaca o estilo de vida da sociedade ocidental, caracterizado por reduzido gasto energético, derivado do sedentarismo e do alto consumo energético, proveniente de dietas alimentares hipercalóricas (LIDFELDT, SAMSIOE e AGARDH, 2006). Segundo Brasil (2012), apenas 22,7% da população brasileira consomem a porção alimentar diária recomendada pela OMS, 31,5% da população consomem regularmente alimentos gordurosos e 53,8% tomam leite integral regularmente (BRASIL, 2012). Por conclusão, pode-se notar que as altas prevalências do sobrepeso e da obesidade na sociedade contemporânea, dependem de uma complexa interação entre fatores endógenos e ambientais, com fortes influências comportamentais (CINTRA, ROPELLE e PAULI, 2011).

Tanto a obesidade humana quanto as formas de obesidade desenvolvidas em animais de laboratório, estão associadas ao desenvolvimento do DM2, resultante da diminuição na captação de glicose pelas células, especificamente no tecido muscular e adiposo. Com relação aos mecanismos que contribuem para a etiopatogenia do DM2, podemos destacar a alteração na secreção de insulina e/ou a resistência à ação deste hormônio. Acredita-se que na fase inicial há predomínio da resistência, com hiperinsulinemia compensatória, com o intuito de manter a normoglicemia. Numa fase posterior, pode ocorrer falência das células-β, reduzindo a secreção de insulina, levando à hiperglicemia (PRATLEY e WEYER, 2001). Somado às disfunções cardiovasculares, o DM2 é uma das mais preocupantes comorbidades associadas à obesidade.

A função endócrina do tecido adiposo

O elo entre a obesidade e suas comorbidades começou a ser elucidado na década de 1990. Com a descoberta de secreções adipocitárias, denominadas adipocinas, o tecido adiposo passou a ser considerado um órgão endócrino. Via adipocinas, o tecido adiposo promove efeitos autócrinos e parácrinos, exercendo fortíssimas influências na ingesta alimentar, na termogênese, no armazenamento calórico e na homeostase da glicose (WAJCHENBERG, 2000; DÂMASO, 2003; CINTRA, ROPELLE e PAULI, 2011; LEGGATE *et. al*, 2012). Dentre as adipocinas, destacam-se citocinas também secretadas pelo sistema imunológico, em especial as de ação pró-inflamatória (TNF-α, a IL-1β e a IL-6) e as de ação anti-inflamatória (IL-4 e IL-10).

O tecido adiposo apresenta, além de adipócitos, terminações nervosas, vasos sanguíneos e certa coleção de leucócitos, em especial macrófagos polarizados M1 e M2 (TANTI *et. al*, 2012; PATEL, BURAS e BALASUBRAMANYAM, 2013). Os macrófagos M1 apresentam atividade pró-inflamatória, secretando mediadores como TNF-α e IL-1β, enquanto que os macrófagos M2 apresentam efeito imunomodulatório via secreção das citocinas anti-inflamatórias Il-4 e IL-10 (TANTI *et. al*, 2012; PATEL, BURAS e BALASUBRAMANYAM, 2013). Estímulos quimiotáxicos, produzidos em resposta ao aumento de ácidos graxos livres e à hipóxia tecidual derivada da hipertrofia dos adipócitos, promovem um aumento na infiltração de macrófagos polarizados M1 no tecido adiposo (Figura 1) (OUCHI *et. al*, 2011; TANTI *et. al*, 2012; PATEL, BURAS e BALASUBRAMANYAM, 2013). Essa associação

entre a hipertrofia dos adipócitos e a infiltração de macrófagos M1, aumenta a razão macrófagos M1/M2, o que promove um desbalanço na secreção das citocinas (OUCHI *et. al*, 2011; TANTI *et. al*, 2012; PATEL, BURAS e BALASUBRAMANYAM, 2013), desencadeando o quadro inflamatório crônico de baixa intensidade, característico da obesidade (CINTRA, ROPELLE e PAULI, 2011; LIRA *et. al*, 2012; PARK *et. al*, 2012), que pode ser a conexão entre a doença e o DM 2 (TANTI *et. al*, 2012; PATEL, BURAS e BALASUBRAMANYAM, 2013).
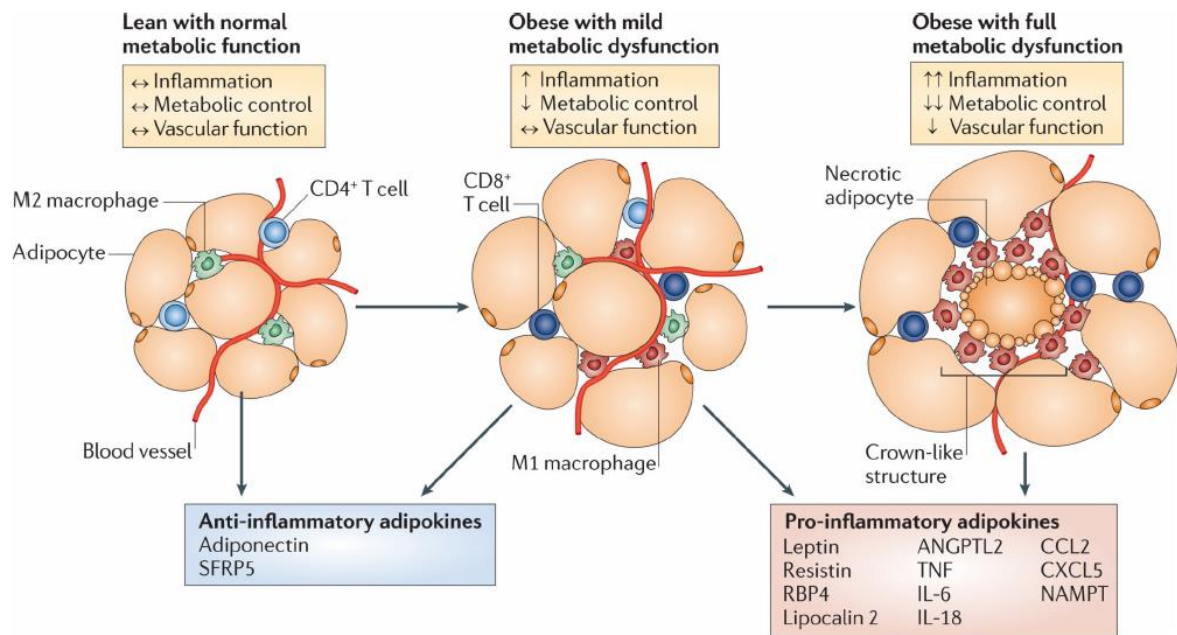
Figura 1: Modulação fenotípica do tecido adiposo. O tecido adiposo pode ser descrito em pelo menos três classificações estruturais e funcionais: magro com a função metabólica normal, obeso com disfunção metabólica leve e obeso com disfunção metabólica completa. Com o desenvolvimento da obesidade, os adipócitos sofrem hipertrofia devido ao aumento no armazenamento de triglicerídios. Em um quadro de obesidade limitada, é provável que o tecido mantenha a função metabólica relativamente normal, com baixos níveis de ativação de células imunitárias e função vascular suficiente. Entretanto, as mudanças qualitativas no tecido adiposo em expansão, podem promover a transição para um fenótipo disfuncional metabolicamente. No tecido adiposo normal, ocorre grande expressão de macrófagos M2, enquanto que a obesidade leva ao recrutamento e acúmulo de macrófagos M1, assim como as células T, no tecido adiposo. Adipocinas anti-inflamatórias, incluindo adiponectina e *secreted frizzled-related protein* 5 (SFRP5), são preferencialmente produzidas pelo tecido adiposo magro. Em estados de obesidade, o tecido adiposo gera grandes quantidades de fatores pró- inflamatórios, incluindo a leptina, resistina, *retinol-binding protein 4* (RBP4), lipocalina 2, *angiopoietinlike protein 2* (ANGPTL2), fator de necrose tumoral (TNF), IL -6, IL -18, *Ccchemokine ligand 2* (CCL2), *CXC-chemokine ligand 5* (CXCL5) e nicotinamida fosforribosiltransferase (NAMPT). Indivíduos obesos em um estado intermediário melhoraram os parâmetros metabólicos, diminuindo a expressão de marcadores inflamatórios e melhorando a função vascular em comparação com os

indivíduos que têm tecido adiposo metabolicamente disfuncional. O tecido adiposo metabolicamente disfuncional pode estar associado a níveis mais elevados de necrose dos adipócitos e os macrófagos M1 estão dispostos em torno destas células mortas em estruturas semelhantes a coroas (OUCHI *et. al*, 2011).

Durante a instalação e progressão do quadro inflamatório derivado da obesidade, as citocinas interagem com seus respectivos receptores, ativando vias metabólicas específicas. As duas vias de sinalização associadas a estímulos inflamatórios são as vias das quinases *I-Kappa-B-Kinase* (IKKβ) e c-Jun N-terminal *Kinase* (JNK), as mesmas quinases ativadas na resposta imune inata em resposta a agentes infecciosos (CINTRA, ROPELLE e PAULI, 2011; LIRA *et. al*, 2012; PARK *et. al*, 2012).

Tanto a IKKβ quanto a JNK apresentam efeito direto na via de sinalização da insulina, fosforilando o substrato do receptor de insulina (IRS) em resíduos de serina, acabam por reduzir a sua interação com o receptor da insulina (IR) e com a proteína *Phosphoinositide-3-kinase* (PI3K), promovendo, assim, um aumento na resistência à insulina (CINTRA, ROPELLE e PAULI, 2011; TANTI *et. al*, 2012; PATEL, BURAS e BALASUBRAMANYAM, 2013). Além desse efeito direto, IKKβ e JNK também promovem um aumento na transcrição dos genes de mediadores de ação inflamatória como o TNF-α, IL-1β e IL-6, proteína C-reativa e proteína sérica amiloide A (CINTRA, ROPELLE e PAULI, 2011; LIRA *et. al*, 2012; PARK *et. al*, 2012).

A ativação da quinase IKKβ promove a ubiquitinação dos inibidores kB (IkB) que, assim, se desligam do NF-kB e são, então, degradados. O NF-kB agora isolado, migra para o interior do núcleo celular e ativa a transcrição dos genes relacionados aos mediadores inflamatórios referidos (SCHMID e BIRBACH, 2008; CINTRA, ROPELLE e PAULI, 2011; TANTI *et. al*, 2012). A JNK, por sua vez, estimula a migração de um fator de transcrição gênica, conhecido como ativador de proteína-1 (AP-1), para o núcleo, onde também irá ativar a transcrição dos mediadores inflamatórios (Figura 2) (DAVIS, 2000; MORSE *et. al*, 2003; CINTRA, ROPELLE e PAULI, 2011; TANTI *et. al*, 2012). Esse aumento na expressão gênica derivado das vias IKKβ e JNK caracteriza o círculo vicioso promotor da inflamação crônica característica da obesidade (DAVIS, 2000; MORSE *et. al*, 2003; CINTRA, ROPELLE e PAULI, 2011).
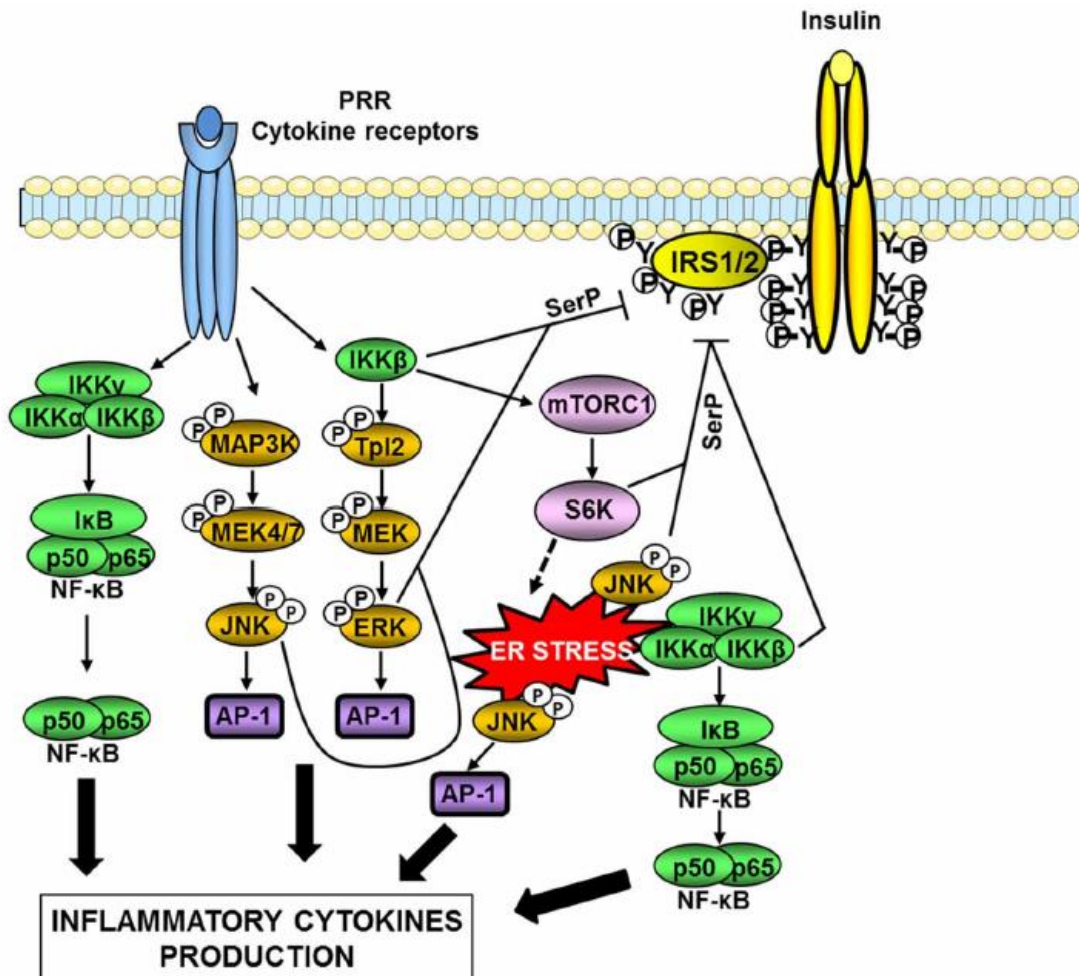
Figura 2: Quinases envolvidas na inflamação induzida pela obesidade. Na obesidade, uma rede de quinases de serina é ativada, incluindo JNK e IKKβ. JNK e IKKβ são ativadas via receptores como *Toll Like Receptors* (TLRs) ou via estresse de retículo endoplasmático (ER). Tais quinases participam da produção de citocinas inflamatórias através dos fatores de transcrição AP-1 e NF-kB. Muitas das citocinas inflamatórias produzidas são capazes de ativar essas quinases que levam a um circuito de amplificação via retroalimentação. JNK está envolvida na dessensibilização da sinalização da insulina através da fosforilação de IRS1/2 em resíduos inibidores da serina (SERP). Já a IKKβ pode fosforilar diretamente IRS1/2 em resíduos de serina, mas também pode agir diretamente através da ativação de mTORC1 e S6-*Kinase* (S6K). Tal ativação pode promover o estresse de ER levando a um novo ciclo de amplificação (TANTI *et. al*, 2012).

Modelos animais de obesidade

Na tentativa de compreender os mecanismos envolvidos com a progressão e os possíveis efeitos das disfunções crônicas como, por exemplo, a obesidade e suas comorbidades, diversos modelos animais experimentais são selecionados, desenvolvidos e utilizados ao redor do mundo. Dentre os modelos experimentais

utilizados no estudo da obesidade e suas co-morbidades destacam-se ratos com lesão no hipotálamo ventromedial (BRAY, 1991); linhagens com alterações genéticas recessivas, como os camundongos *ob/ob* (deficientes em leptina) (HUANG *et. al*, 2012) e os ratos *Zucker* (*fa/fa*) (incapazes de produzir os receptores para a leptina) (CHENTOUF *et. al*, 2011); e por adição de dietas hipercalóricas que simulam dietas da vida humana contemporânea (NASCIMENTO *et. al*, 2008; EL MESALLAMY *et. al*, 2010; CHANG *et. al*, 2011; GENTILE *et. al*, 2011; MADANI *et. al*, 2012). No geral, tais modelos animais apresentam uma série de características, como hiperfagia, hiperinsulinemia, reduzido gasto energético, aumento de peso corporal, intolerância à glicose e resistência à ação da insulina (NASCIMENTO *et. al*, 2008; EL MESALLAMY *et. al*, 2010; CHANG *et. al*, 2011; GENTILE *et. al*, 2011), que podem ser consequências de um desbalanço entre as atividades autonômicas. Esse desbalanço é proveniente da função exacerbada parassimpática e depleção simpática (BRAY, 1991).

Além dos modelos enfatizados, destaca-se um descrito inicialmente em 1969, conhecido como modelo MSG (OLNEY, ADAMO e RATNER, 1971). O MSG é um neurotransmissor excitatório (BHATTACHARYA, BHAKTA e GHOSH, 2011) que, quando administrado em animais neonatos, atinge o sistema nervoso central e provoca lesões hipotalâmicas, reduzindo aproximadamente 75% do número de neurônios do núcleo arqueado e eminência mediana (OLNEY, ADAMO e RATNER, 1971; ALARCON-AGUILAR *et. al*, 2008; PATIL *et. al*, 2011; ROMAN-RAMOS *et. al*, 2011) por meio da necrose e fagocitose circunvizinha (OLNEY, 1969; OLNEY, ADAMO E RATNER, 1971).

O animal submetido ao tratamento neonatal por MSG é caracterizado por apresentar obesidade; normo ou hipofagia (BALBO *et. al*, 2000; BALBO *et. al*, 2007); redução da termogênese no tecido adiposo marrom (OLNEY, ADAMO e RATNER, 1971; ROMAN-RAMOS *et. al*, 2011); aumento de NPY (STRICKER-KRONGRAD e BECK, 2004; ALARCON-AGUILAR *et. al*, 2008); redução na secreção do hormônio do crescimento e do hormônio luteinizante (SASAKI, KAWAI e OHTA, 1994) levando a um déficit no crescimento (MAITER *et. al*, 1991) e hipogonadismo, respectivamente (SASAKI, KAWAI e OHTA, 1994; PATIL *et. al*, 2011).

Ocorre, também, aumento na concentração lipídica plasmática (ALARCON-AGUILAR *et. al*, 2008; NARDELLI *et. al*, 2011; ROMAN-RAMOS *et. al*, 2011); redução na concentração de adrenalina no coração e intestino e aumento nas glândulas adrenais (MORRISON *et. al*, 2008); e ainda aumento na atividade

acetilcolinesterásica, sugerindo um desarranjo funcional autonômico (LUCINEI BALBO *et. al*, 2000).

Um dos maiores alvos nos estudos em animais MSG é a função pancreática e a secreção de insulina pelas células β, pois os mesmos se caracterizam por apresentarem hiperinsulinemia; intolerância à glicose e resistência à insulina (HIRATA *et. al*, 1997; BALBO *et. al*, 2007; ROMAN-RAMOS *et. al*, 2011). Além dessas características, está bem evidenciado que as ilhotas pancreáticas dos animais obesos-MSG secretam mais insulina em resposta à glicose quando comparado com animais magros (BALBO *et. al*, 2002; GRASSIOLLI *et. al*, 2006).

Ferreira e colaboradores (2009) mostraram que ratos MSG apresentam lesões nos glomérulos renais e alta excreção de albumina (FERREIRA *et. al*, 2009). No fígado ocorrem danos hepatocelulares moderados, acúmulo de células inflamatórias ao redor da veia central (BHATTACHARYA, BHAKTA e GHOSH, 2011), aumento na concentração de TG hepáticos (NARDELLI *et. al*, 2011), e redução das transaminases hepáticas, que pode estar associado às lesões (ROMAN-RAMOS *et. al*, 2011).

Com relação ao processo inflamatório e a obesidade-MSG, Alacorn-Aguilar e colaboradores (2008) mostraram um aumento na liberação de citocinas pró-inflamatórias no tecido adiposo de camundongos MSG (ALARCON-AGUILAR *et. al*, 2008), assim como Roman-Ramos e colaboradores (2011), que igualmente descreveram a elevação na quantidade de mRNAs de IL-6, TNFα, resistina no tecido adiposo deste modelo (ROMAN-RAMOS *et. al*, 2011). O aumento na liberação da leptina também promove um ciclo vicioso potencializador da inflamação, pois causa resistência à mesma e também aumenta ainda mais a liberação das citocinas (ALARCON-AGUILAR *et. al*, 2008; CHEN *et. al*, 2008; ROMAN-RAMOS *et. al*, 2011). Esse perfil inflamatório ativa PPARα/γ, como ficou demonstrado por Roman-Ramos e colaboradores (2011), que induz ao aumento na concentração de adiponectina, no tecido adiposo, como uma tentativa de reduzir o processo inflamatório (ROMAN-RAMOS *et. al*, 2011). Entretanto, a resistência a adiponectina também foi evidenciada em modelos MSG (ALARCON-AGUILAR *et. al*, 2008).

Taurina

Considerando os efeitos crônicos da obesidade e suas comorbidades, diferentes planos terapêuticos e estratégias preventivas são utilizadas na tentativa

de reverter ou prevenir essa síndrome. Os índices mundiais para o sobrepeso e a obesidade mostram que apenas a modulação alimentar e a prática de exercícios físicos não são suficientes para o controle dessa pandemia (CINTRA, ROPELLE e PAULI, 2011). Tratamentos cirúrgicos ou baseados em fármacos podem ser muito eficientes nesse combate. Uma das substâncias que parece estar envolvida na redução dos sintomas da obesidade é a TAU. O interesse científico sobre os efeitos favoráveis da TAU contra a obesidade pode ser explicada por um grande acervo de especulações sobre suas finalidades farmacológicas, uma vez que já foram destacadas suas propriedades de proteção contra perturbações metabólicas e sua ação antioxidante (CARNEIRO *et. al*, 2009; EL MESALLAMY *et. al*, 2010; CHANG *et. al*, 2011) ainda mais por se mostrar reduzida num quadro de obesidade, podendo gerar assim, um ciclo vicioso (CHANG *et. al*, 2011).

A TAU (ácido 2-aminoetanossulfônico) é o aminoácido livre mais abundante nos seres humanos (MARCINKIEWICZ e KONTNY, 2012). Trata-se de um aminoácido não essencial obtido na dieta ou convertido a partir da cisteína ou metionina (MARCINKIEWICZ e KONTNY, 2012; SOLON *et. al*, 2012). É comumente encontrado em altas taxas no plasma (RIBEIRO *et. al*, 2009), retina, miocárdio, musculatura esquelética, fígado e no cérebro (CHANG *et. al*, 2011), representando cerca de 0,1% do peso corporal na maioria dos mamíferos (KIM e CHA, 2013). Em condições normais a TAU auxilia na regulação da osmolaridade tecidual (RIBEIRO *et. al*, 2009; CHANG *et. al*, 2011), na integridade das membranas celulares (HUXTABLE, 1992) e na atividade de seus canais iônicos (RIBEIRO *et. al*, 2010).

Como demonstrado por vários autores (CARNEIRO *et al*, 2009; CHANG *et. al*, 2011; GENTILE *et. al*, 2011; NARDELLI *et. al*, 2011), a TAU previne a deposição de gordura, podendo levar à redução do peso corporal. Quanto ao tecido adiposo, a TAU é eficiente na redução do percentual e no tamanho máximo dos adipócitos (CHANG *et. al*, 2011). Seus efeitos no coração foram descritos por Das, Vasan e Sil (2012), onde a TAU aumentou a translocação de receptores GLUT-4 para a membrana das células do miocárdio por aumentar a fosforilação de IR e de IRS1, melhorando o transporte de glicose mediado por insulina, reduzindo o estresse oxidativo e a apoptose neste tecido (DAS, VASAN e SIL, 2012). Quando administrada diretamente no hipotálamo, a TAU potencializa o poder anorexígeno da insulina, reduzindo a liberação de neuropeptídeo Y (NPY) (SOLON *et. al*, 2012).

No fígado a TAU melhora o perfil lipídico, reduzindo a concentração hepática de TG em hamsters com dieta hiperlipídica e ratos MSG e (EL MESALLAMY *et. al*,

2010; CHANG *et. al*, 2011; NARDELLI *et. al*, 2011), auxiliando na redução da disponibilidade de TG e COL para os demais tecidos (YOKOGOSHI *et. al*, 1999; YAMAMOTO *et. al*, 2000). Ainda relacionado ao seu possível efeito hepato-protetor, a TAU ajuda a reduzir ou até prevenir a esteatose (CHANG *et. al*, 2011; GENTILE *et. al*, 2011) inflamação ou até mesmo lesões hepáticas (GENTILE *et. al*, 2011).

Nas células beta de animais magros, a TAU potencializa a permeabilidade à glicose, além de acelerar a resposta da liberação da insulina (CARNEIRO *et. al*, 2009; RIBEIRO *et. al*, 2009). Por aumentar *Pancreatic and duodenal homeobox* 1 (PDX-1), a TAU melhora a expressão de genes necessários para a produção da insulina (CARNEIRO *et. al*, 2009). Ela também aumenta a concentração dos íons Ca^{+2} citoplasmático, potencializando a liberação de vesículas contendo a insulina. Quanto ao glucagon, a TAU aumenta sua secreção em animais magros em estado de jejum, devido à elevação da concentração de íons Ca^{+2} no citoplasma células alfa (RIBEIRO *et. al*, 2010). Além dos efeitos nas ilhotas, a TAU também exerce efeitos em tecidos periféricos de camundongos magros, aumentando a captação e a oxidação da glicose (CARNEIRO *et. al*, 2009; RIBEIRO *et. al*, 2009). Porém, em ratos obesos-MSG a TAU não influenciou a homeostase da glicose, a secreção e a ação da insulina (NARDELLI *et. al*, 2011).

Além dos efeitos sobre disfunções metabólicas descritos acima, outra possível via de aplicação terapêutica da TAU que vem recebendo grande enfoque cientifico é o seu papel anti-inflamatório. Em um quadro de inflamação aguda, quimiotaxinas secretadas em resposta à presença de agentes infecciosos como, por exemplo, microorganismos, estimulam a infiltração de fagócitos (MARCINKIEWICZ e KONTNY, 2012; KIM e CHA, 2013), que se acumulam nas regiões de inflamação e tecidos infectados (KIM e CHA, 2013). Tais leucócitos atuam engolfando microorganismos invasores e eliminando-os com oxidantes microbicidas, como o peróxido de hidrogênio (H_2O_2), que é convertido em bactericidas ainda mais potentes, o ácido hipocloroso ($Cl^- + H_2O_2 + H^+ \rightarrow HOCl + H_2O$) e o ácido hipobromoso ($Br^- + H_2O_2 + H^+ \rightarrow HOBr + H_2O$) (KIM e CHA, 2013) pelo sistema de peroxidases das células fagocitárias (MARCINKIEWICZ e KONTNY, 2012; KIM e CHA, 2013). Os altos níveis de TAU em fagócitos e seu acúmulo em lesões inflamatórias sugerem o seu papel na imunidade inata (SCHULLER-LEVIS e PARK, 2004; MARCINKIEWICZ e KONTNY, 2012). A TAU pode reagir e desintoxicar o HOCl e o HOBr formando, assim, a taurina cloramina (Taurina + HOCl $\rightarrow$ TauCl + H_2O) e taurina bromamina (Taurina + HOBr $\rightarrow$ TauBr + H_2O), respectivamente

(MARCINKIEWICZ e KONTNY, 2012; KIM e CHA, 2013). Ambas exercem propriedades antimicrobianas e anti-inflamatórias, inibindo a produção de mediadores inflamatórios como, óxido nítrico, TNF-α, ILs e prostaglandinas (MARCINKIEWICZ e KONTNY, 2012; KIM e CHA, 2013).

Uma grande diversidade de estudos têm salientado o poder da TauCl, em especial, em reduzir a superprodução de mediadores inflamatórios em diferentes modelos celulares. Em macrófagos de camundongos, a TauCl atenuou as superproduções de TNF-α (MARCINKIEWICZ *et. al*, 1995; MARCINKIEWICZ *et. al*, 2000; MARCINKIEWICZ *et. al*, 2005) e IL-6 (MARCINKIEWICZ *et. al*, 1995; MARCINKIEWICZ *et. al*, 2005), derivadas de estímulos por lipopolissacarídeos (LPS) e interferon-γ (IFN-γ). Marcinkiewicz e colaboradores (1998) demonstraram uma redução na superprodução de TNF-α, IL-1β, IL-6 em células polimorfonucleares de camundongo estimuladas por LPS e IFN-γ (MARCINKIEWICZ *et. al*, 1998). Em células dendríticas de camundongos, também estimuladas por LPS e IFN-γ, a TauCl mitigou a superprodução de TNF-α, IL-2, IL-6, IL-10, IL-12 (MARCINKIEWICZ *et. al*, 1999). Em culturas de tecido adiposo humano, a TauCl também exerceu efeitos protetores, reduzindo as superproduções de TNF-α, IL-6, IL-8 estimuladas por LPS sem, entretanto, afetar a produção de IL-10 (MARCINKIEWICZ e KONTNY, 2012).

Em geral, a redução nos mediadores inflamatórios descrita pode estar associada ao efeito da TAU na via do Nf-κB. A TauCl inativa a atividade enzimática da quinase IKK e inibe a fosforilação do resíduo de serina 32 do IκB-α evitando, assim, a sua degradação e a consequente transcrição de genes de mediadores inflamatórios via NF-κB. Em macrófagos NR8383 estimulados por LPS e IFN- γ e tratados com TauCl, o Nf-κB permaneceu em associação com IκB no citoplasma e a sua migração nuclear foi impedida (BARUA, LIU e QUINN, 2001). Em extratos cardíacos de ratos diabéticos por aloxana, o tratamento com TAU levou à redução de NF-κB, TNF-α, IL-6 (DAS, VASAN e SIL, 2012) e ao aumento do PPAR-α no fígado de ratos obesos por dieta hiperlipídica (CHANG *et. al*, 2011). Como os efeitos da taurina são relativos em função do modelo de obesidade empregado, o estudo dos efeitos da suplementação de taurina no processo inflamatório, decorrente da obesidade MSG induzida em ratos, é fundamental para a elucidação das consequências dessa síndrome.

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ARTIGO CIENTÍFICO

TAURINE SUPPLEMENTATION ENHANCES IκBα PROTEIN EXPRESSION IN ADIPOSE TISSUE AND AMELIORATES SERUM ANTI-INFLAMMATORY CYTOKINE LEVELS IN MSG OBESITY

ARTIGO CIENTÍFICO

Taurine supplementation enhances IκBα protein expression in adipose tissue and ameliorates serum anti-inflammatory cytokine levels in MSG obesity

Luiz Carlos Caetano[1], *Maria Lúcia Bonfleur[1], Rosane Aparecida Ribeiro[2], Tarlliza Romanna Nardelli[3], Camila Lubaczeuski[1], Everardo Magalhães Carneiro[3], Sandra Lucinei Balbo[1]

1 Laboratório de Fisiologia Endócrina e Metabolismo (LAFEM), Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná (UNIOESTE), Cascavel, PR, Brazil.
2 Universidade Federal do Rio de Janeiro (UFRJ), Campus UFRJ-Macaé, Macaé, RJ, Brazil.
3 Laboratório de Pâncreas Endócrino e Metabolismo, Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil.

***Correspondence to:** Maria Lúcia Bonfleur
Laboratório de Fisiologia Endócrina e Metabolismo,
Cascavel, PR, Brazil CEP: 858119-110
E-mail: mlbonfleur@hotmail.com
Phone/Fax: +55 45 3220 3257

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Abstract

Obesity is associated with low-grade inflammation, which impairs insulin action and enhances body fat storage. The sulphated amino acid, taurine (TAU), regulates glucose homeostasis, lipid metabolism and presents anti-inflammatory actions. Here, we evaluated the inflammatory profiles of the serum and retroperitoneal adipose tissue from monosodium glutamate (MSG) obese rats, supplemented or not with TAU. Male *Wistar* rats received subcutaneous injections of MSG (4 mg/kg body weight/day, MSG group) or hypertonic saline (CTL) during the first 5 days of life. From 21 to 120 days of age, half of each MSG and CTL group received 2.5% TAU in their drinking water (CTAU and MTAU). At 120 days of age, MSG rats were obese and hyperinsulinemic. TAU supplementation reduced fat deposition without affecting insulinemia in MTAU rats. The MSG treatment did not change protein expression of IκBα and pJNK$_{1/2}$ in the retroperitoneal adipose tissue. In contrast, TAU supplementation increased IκBα protein expression in both the MTAU and CTAU groups. Furthermore, no alteration in TNF-α, IL-1β or IL-6 content was observed in adipose tissue following MSG obesity or supplementation. MSG rats presented lower serum TNF-α, IL-4 and IL-10 levels, which was prevented by TAU treatment. In conclusion, MSG rats did not present alterations in pro-inflammatory markers in retroperitoneal fat stores, but presented lower serum anti-inflammatory cytokines levels. TAU increased IκBα protein content in the adipose tissue and normalized serum TNF-α, IL-4 and IL-10 levels in MTAU rats; this effect may contribute to the preventive action of this amino acid upon adiposity.

Introduction

The chronic inflammation of adipose tissue that is associated with obesity contributes to the development of insulin resistance (IR) and type 2 diabetes (T2D)[1]. Adipose tissue has become a major topic of research in obesity since it is not only an energy store, but an endocrine organ that synthesizes and releases leptin, cytokines, adiponectin and resistin, which play a role in food intake, thermogenesis, energy expenditure and glucose homeostasis regulation [2; 3]. In different experimental models of obesity, as well as in obese and T2D subjects, pro-inflammatory cytokines are enhanced in the serum and adipose tissue, leading to a chronic low-grade inflammation that contributes to IR and increases body fat deposition [3-8].

Transcription of the pro-inflammatory cytokine genes is activated by two major pathways; the I-kappa-B kinase (IKKB) and c-Jun N-terminal kinase (JNK) pathways [9]. Inflammatory cytokines activate IKKB by ubiquitination of the inhibitors of kappa B (IκB), which releases the nuclear factor kappa B (NF-κB) for migration to the nucleus, where it in turn activates the transcription of tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 genes [9]. The TNF-α is a pro-inflammatory cytokine that is primarily involved in the genesis of the chronic inflammatory process in obesity [10; 11]. JNK is also activated by inflammatory stimuli [12] and translocates the activator protein (AP)-1 to the nucleus, increasing the transcription of pro-inflammatory cytokines [12; 13].

Taurine (TAU) is a non-essential amino acid obtained from the diet or synthesized from cysteine or methionine [14]. TAU has preventive effects upon obesity development [15-18], improving hepatic metabolism [17], reducing triglycerides in the plasma and liver [16], regulating glucose homeostasis and increasing insulin secretion and sensitivity [19; 20]. This amino acid also presents anti-inflammatory actions [21-23].

The administration of monosodium glutamate (MSG) to neonatal rodents causes lesions in the hypothalamic arcuate nucleus and median eminence, leading to neuroendocrine disturbances and obesity [6; 24-27]. These rodents present a pre-diabetic state, typical in overweight or obese humans, with normoglycemia, hyperinsulinemia, glucose intolerance, insulin resistance (IR)[16; 28; 29] and dyslipidemia [6; 16; 26]. Other studies, however, report controversial data regarding inflammatory cytokines in MSG obesity [4; 6; 30-33]. Here, we analyzed the inflammatory profile of the serum and retroperitoneal adipose tissue of MSG-obese rats that were supplemented, or not, with TAU. We provide evidence that TAU supplementation decreases adiposity, enhances IκBα protein in the adipose tissue and prevents alterations in the circulating levels of the anti-inflammatory cytokines, IL-4 and IL-10, in MSG-treated rats.

Materials and Methods

Obesity induction and TAU supplementation

All experiments were approved by the UNIOESTE's Committee on Ethics in Animal Experimentation (certificate n°: 00712). Pregnant *Wistar* rats were maintained in the sectorial animal house of the Endocrine Physiology and Metabolism Laboratory under a 12h light/dark cycle (lights on 7:00-19:00h), with controlled temperature (21 ± 2°C). At birth, newborn male rats received subcutaneous injections of MSG (4g/kg body weight (BW) per day, MSG group) or hypertonic saline (1.25g/kg BW/day, CTL group) during the first five days of life. From weaning (21th day) to 120 days of age, half of the MSG and CTL groups received 2.5% TAU in their drinking water (MTAU and CTAU groups). Rats had free access to standard rodent chow (Nuvital[®], Colombo, Brazil) and water during the entire experimental period.

Obesity evaluation and general serum biochemical and cytokine parameters

At the end of the experimental period, 8h-fasted rats were weighed and the nasoanal length was measured to calculate the Lee index [from the ratio of BW $(g)^{1/3}$/nasoanal length (cm) x 1000] [(34)]. Blood was collected from the tip of the tail for glycemia measurement using a glucose analyzer (Accu-Chek Advantage[®], Roche Diagnostics, Switzerland). Subsequently, all rat groups were euthanized by decapitation and total blood was collected; the serum obtained was used for insulin quantification by radioimmunoassay. Serum levels of the inflammatory markers, IL-1β, IL-2, IL-4, IL-6, IL-10, IFNγ and TNF-α, were measured by the RECYTMAG-65K multiplex ELISA kit (MILLIPLEX[®], Millipore Corporate Headquarters, Billerica, MA, USA) by Genesis Institute for Scientific Analysis (São Paulo, SP, BRA). In addition, the perigonadal and retroperitoneal fat pads were removed and weighed.

Western Blotting

For determination of protein expression of the inflammatory pathways and cytokines in the adipose tissue; retroperitoneal fat pads of all rat groups were homogenized in anti-protease buffer containing, 7 mol/l urea, 2 mol/l thiourea, 5 mmol/l EDTA, 1 mmol/l sodium fluoride, 1 mmol/l orthovanadate, 1 mmol/l pyrophosphate, 1 mmol/l phenylmethanesulfonyl fluoride and 2 mmol/l aprotinin. The homogenate was centrifuged at 12,600 g for 30 min. The supernatant was collected and the protein concentration was measured by Bradford assay. Subsequently, samples were incubated at 100°C for 5 min with Laemmli buffer. Proteins were separated by electrophoresis on biphasic polyacrylamide gel (SDS-PAGE). Afterwards, samples were transferred to nitrocellulose membranes (BioRad[®], Hercules, CA, USA). The

membranes were treated with a blocking buffer (5% non-fat dried milk, 10 mM Tris, 150 mM NaCl, and 0.02% Tween 20) and were subsequently incubated overnight with primary antibodies against IL-1β, TNFα (Biolegend®, CA, USA), IL-6, IκBα (Santa Cruz®, TX, USA) and phospho (p) JNK$_{1/2}$ proteins (Cell Signaling®, MA, USA). Detection was performed after a 2h incubation period using a horseradish peroxidase-conjugated secondary antibody (Thermo Scientific®, CA, USA) followed by exposure to an ImageQuant LAS 4000 Mini (GE® Healthcare Bio-Sciences, Uppsala, Sweden), which detects the chemiluminescence in the nitrocellulose membranes. The band intensities were quantified with the free software Image J® (National Institute of Mental Health, USA). After assaying the target proteins, Western blotting was repeated using a primary antibody to the α-tubulin protein (Sigma Aldrich®, MO, USA) as an internal control.

Statistical analysis

Results are presented as means ± SEM for the number of determinations (n) indicated. The statistical analyses were performed using two-way analysis of variance (ANOVA) followed by the Duncan's post-test (P < 0.05) with the Statistica 5.0 software (StatSoft, Tulsa, OK, USA).

Results

Obesity development, glycemia and insulin level evaluation

As observed in Table 1, BW and nasoanal length were 20% and 12%, respectively, lower in MSG than in CTL rats (P < 0.0001 and P < 0.0001, respectively). MSG treatment efficiently induced obesity, since MSG rats presented a higher Lee index and an increase of 49% and 90% in retroperitoneal and perigonadal fat pads, respectively, in comparison with the CTL group (P < 0.002 and P < 0.0001; Tab. 1). TAU supplementation prevented fat deposition in MTAU rats, with a 17% and 13% reduction in retroperitoneal and perigonadal fat stores, respectively, when compared with the MSG group (P < 0.003 and P < 0.02; Tab. 1). TAU also promoted a decrease of 22% in the retroperitoneal fat pads of the CTAU group, compared with the CTL group (P < 0.02; Tab. 1). These effects were not accompanied by alterations in BW, body length or mass in any of the supplemented groups. In addition, although no alterations in fasting glycemia were observed in the groups (Tab. 1), MSG rats maintained normal glucose circulating levels with 3-fold higher serum insulin concentrations than those of CTL rats (P < 0.002). TAU did not modify this parameter in either of the supplemented groups (Tab. 1).

Protein expression of inflammatory markers in the retroperitoneal adipose tissue

As demonstrated in Figure 1A, the expression of IκBα protein was similar in MSG and CTL retroperitoneal adipose tissues. TAU increased IκBα protein expression in both supplemented groups, with a 104% and 61% higher IκBα content in the retroperitoneal fat pads of MTAU and CTAU rats, respectively, when compared with their corresponding controls ($P < 0.002$ and $P < 0.005$; Fig. 1A). The expression of the pJNK1/2 protein was similar in all groups (Fig. 1B). No alteration in the expression of the pro-inflammatory proteins, TNF-α, IL-1β and IL-6, was observed in the retroperitoneal fat stores of any of the rat groups (Fig. 2A, B and C).

Serum cytokine levels

The serum concentrations of the pro-inflammatory cytokines, TNF-α, IL-1β, IL-6, IL-2 and IFNγ, are shown in Figure 3. MSG rats presented a decrease of 42% in serum TNF-α levels, compared to the CTL group ($P < 0.03$; Fig. 3A). TAU supplementation prevented this alteration in the MTAU rats (Fig. 3A). The serum IL-1β, IL-6, IL-2 and IFNγ concentrations were similar in all groups (Fig. 3B-E).

Figure 4 shows the serum anti-inflammatory cytokine levels in MSG rats supplemented, or not, with TAU. Serum IL-4 and IL-10 levels were 39% and 49%, respectively, lower in MSG rats compared with CTL ($P < 0.04$; Fig. 4A and 4B). TAU supplementation normalized IL-4 and partially enhanced IL-10 circulating levels in MTAU rats.

Discussion

MSG-treated rodents are characterized by massive body fat stores, IR [16; 28; 29] and dyslipidemia [6; 16; 26]. The present study demonstrated these characteristics in MSG rats and also demonstrated that TAU supplementation decreased fat accumulation in MTAU rats without altering serum insulin levels (Tab. 1), a finding that is in accordance with a previous observation from our research group [16].

Adipose tissue is currently considered as an endocrine organ responsible for the production of adipokines, including pro-inflammatory cytokines [35]. Studies have demonstrated that chronic inflammation of the adipose tissue contributes to metabolic disorders in obesity [30; 36; 37]. The synthesis of pro-inflammatory mediators is regulated by gene transcription through the activity of several transcription factors, including NF-κB and AP-1 [12; 13; 38]. NF-κB is found in the cytoplasm, forming a complex with a member of the IkB family. Phosphorylation of the IκBα is rapid and contributes to the classic inflammatory

response [39]. When phosphorylated, IκBα is ubiquitinated and degraded by proteasomes releasing NF-κB [40]. The free NF-κB migrates to the nucleus and binds to promoter regions of pro-inflammatory genes [12; 41-43]. In the present study, although MSG obesity did not alter the IκBα content in the retroperitoneal adipose tissue, TAU increased IkB-α protein in all of the supplemented groups (Fig. 1A), which indirectly demonstrates that the activity of NF-κB is lowered in MTAU rats. Barua et al. [38] showed that TAU chloramine (TauCl) reduces migration of NF-κB to the nucleus of cells cloned from rat alveolar macrophages and stabilizes the cytosolic content of IκBα. In addition, we demonstrated that $pJNK_{1/2}$ protein expression was not affected by MSG treatment or TAU supplementation. Therefore, our data indicate that TAU may modulate the inflammatory processes in the retroperitoneal adipose tissue of the MTAU group via NF-κB inhibition.

Our results also demonstrate that MSG obesity, associated or not with TAU supplementation, does not alter the protein expression of pro-inflammatory cytokines in retroperitoneal fat depots (Fig. 2). Hotamisligil et al. [30] reported increased gene expression of TNF-α in the perigonadal adipose tissue of four different genetic models of obesity and T2D. Increased gene expression of TNF-α, IL-6 and IL-1β was reported in the visceral adipose tissue of obese humans [44; 45] and in the perigonadal adipose tissue of high-fat diet obese mice [46]. Although the consensus in the literature indicates an increase in inflammatory cytokines in different experimental models of obesity and T2D, data for MSG obesity are controversial. Studies have reported increased gene [6; 31; 32] and protein expressions [31] of TNF-α and IL-6 in the white adipose tissue of MSG mice. Similar results were also reported in the brains of mice at 8, 10 and 14 days after MSG treatment [47]. However, other studies found no differences in TNF-α gene expression in the perigonadal adipose tissue of *Swiss* MSG mice [30], in splenic macrophages from *C57Bl/6* MSG mice [4] or in the periodontal tissue of MSG rats [33].

In vitro and in vivo studies and clinical trials consider that TAU and its derivatives constitute potential therapeutic agents against inflammatory diseases [22; 23; 48; 49]. Lin et al. [50] reported that TAU supplementation reduced infiltration of M1-polarized macrophages and increased M2 macrophages, leading to a reduced production of inflammatory cytokines in the adipose tissue of high-fat diet mice. According to Das et al. [51], the antioxidant activity of TAU reduced the effects of a potent inflammatory mediator known as hypochlorous acid (HClO), forming the stable compound, TauCl [52], which reduces NF-κB, TNF-α and IL-6 in the heart of alloxan diabetic rats. TAU supplementation reduced plasma levels of TNF-α and IL-6 in alcohol-fed rats [53]. In contrast, no modification in liver TNF-α and IL-1β levels of high-fat diet obese hamsters supplemented with TAU was reported [17]. In the current study, we observed that TAU did not alter pro-inflammatory protein expression in the retroperitoneal

fat stores; however changes in IκBα protein content were observed as well as modified serum cytokine levels.

Studies report that higher plasma concentrations of inflammatory markers are detected in obese individuals [54; 55] and in *ob/ob* and *db/db* mice [4]. Our study demonstrated that serum TNF-α, IL-4 and IL-10 levels were lower in MSG rats, and that no modifications were observed in serum IL-1β, IL-6, IL-2 and IFNγ concentrations (Fig. 3). In contrast, previous studies showed no alteration in circulating levels of TNF-α [4; 6; 31; 56] and IL-10 [56] in MSG obese rodents. A previous study from our laboratory presented lower TNF-α mRNA in the periodontal tissue of MSG rats [33]. In addition, lower serum IL-4 and IL-10 levels may contribute to obesity development and metabolic disruption in MSG rats, as IL-4 has been found to inhibit adipogenesis and improve lipolysis in 3T3-L1 adipocytes by enhancing the activity of hormone-sensitive lipase via protein kinase A activation [57]. In mice with a transient overexpression of IL-4, a better glucose homeostasis and reduced lipid accumulation in fat tissues has been reported [58]. IL-4 also showed cytoprotective effects in pancreatic beta-cells against pro-inflammatory cytokines such as INF-γ and IL-1β [59]. Furthermore, IL-10 has been shown to decrease weight gain, prevent insulin desensitization and suppress macrophage infiltration in adipose tissue from high-fat mice [60]. *Otsuka Long-Evans Tokushima Fatty* rats present lower IL-10 secretion from the adipose tissue and when submitted to physical exercise, associated or not with metformin, demonstrate enhanced IL-10 release, which correlates with a reduction in adipose tissue [61]. These data indicate that the preventive effect of TAU upon fat deposition in MSG obesity may be associated with the preservation of normal serum levels of IL-4 and IL-10 in MTAU rats.

In conclusion, our results demonstrate that, in contrast to other experimental models of obesity, MSG obese-rats do not exhibit chronic inflammation in their adipose tissue nor any increase in pro-inflammatory cytokines in the serum. Some authors have suggested that this effect may due to the low severity of MSG obesity [4; 30]. Conversely, we believe that this differential feature is probably linked to the hypercorticosteronemia that is present in the MSG model [56; 62-65]. Furthermore, for the first time, the present study demonstrated lower IL-4 and IL-10 levels in the serum of MSG rats, which may contribute to disruption of glucose control and fat deposition. TAU supplementation prevented fat accumulation in the MTAU rats, an action that may be associated with enhanced IκBα content in the adipose tissue and normal circulating IL-4 and IL-10 levels.

Acknowledgments

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Conflict of Interest

All contributing authors report no conflicts of interest.

Authorship

LCC, TRN, CL: execution of experiments: SLB and MLB; conception and experimental design, data interpretation and manuscript writing; RAR: data interpretation and manuscript writing; EMC: intellectual contribution and provision of materials and reagents.

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Table 1: Obesity parameters, and fasting glycemia and insulinemia in 120-day old CTL, CTAU, MSG and MTAU rats.

	CTL	CTAU	MSG	MTAU
BW (g)	433 ± 9^a	433 ± 5^a	339 ± 10^b	346 ± 6^b
Nasoanal length (cm)	23.7 ± 0.4^a	23.6 ± 0.3^a	20.6 ± 0.4^b	20.9 ± 0.3^b
Lee Index	321 ± 3^a	321 ± 3^a	339 ± 4^b	337 ± 4^b
Retroperitoneal fat pad (g/100g BW)	1.3 ± 0.06^a	1.0 ± 0.08^b	1.9 ± 0.09^c	1.6 ± 0.05^d
Perigonadal fat pad (g/100g BW)	1.3 ± 0.06^a	1.1 ± 0.08^a	2.5 ± 0.12^b	2.2 ± 0.09^c
Glucose (mg/dL)	68 ± 1	66 ± 3	63 ± 4	67 ± 3
Insulin (ng/mL)	0.61 ± 0.06^a	1.0 ± 0.16^{ab}	1.9 ± 0.30^b	1.8 ± 0.28^b

Data are means $\pm$ SEM (n=6-10). Different letters indicate significant difference. Two-way ANOVA followed by the Duncan's post-test, $P < 0.05$.

Figure legends

Figure 1: *TAU supplementation enhances IκBα protein expression in the retroperitoneal tissue of MSG rats*. Total IκBα (A) and phospho (p)-JNK$_{1/2}$ (B) protein expressions in retroperitoneal adipose tissue of 120-day-old CTL, CTAU, MSG and MTAU rats. Data are means $\pm$ SEM of the optical densitometric values (n = 4-6). Different letters over the bars indicate significant difference. Two-way ANOVA followed by Duncan's post-test, $P < 0.05$.

Figure 2: *MSG obesity or TAU supplementation does not alter pro-inflammatory cytokine levels in the retroperitoneal adipose tissue*. TNF-α (A), IL-1β (B) and IL-6 (C) protein expressions in retroperitoneal adipose tissue of 120-day-old CTL, CTAU, MSG and MTAU rats. Bars represent the means $\pm$ SEM of the values, determined by optical densitometry (n = 4-6). Different letters indicate significant difference. Two-way ANOVA followed by the Duncan's post-test, $P < 0.05$.

Figure 3: *MSG obesity rats present lower serum TNF-α levels that can be normalized by TAU.* Serum levels of the pro-inflammatory cytokines; TNF-α (A), IL-1β (B), IL-6 (C) IL-2(D) and IFNγ (E) in CTL, CTAU, MSG and MTAU rats. Bars represent the means ± SEM of the values (n=5-10). Different letters indicate significant difference. Two-way ANOVA followed by the Duncan's post-test, P < 0.05.

Figure 4: *Anti-inflammatory cytokines were reduced in MSG obesity and TAU prevented this alteration.* Serum levels of the anti-inflammatory cytokines: IL-4 (A) and IL-10 (B) in CTL, CTAU, MSG and MTAU rats. Bars represent the means ± SEM of the values (n=5-10). Different letters indicate significant difference. Two-way ANOVA followed by the Duncan's post-test, P < 0.05.
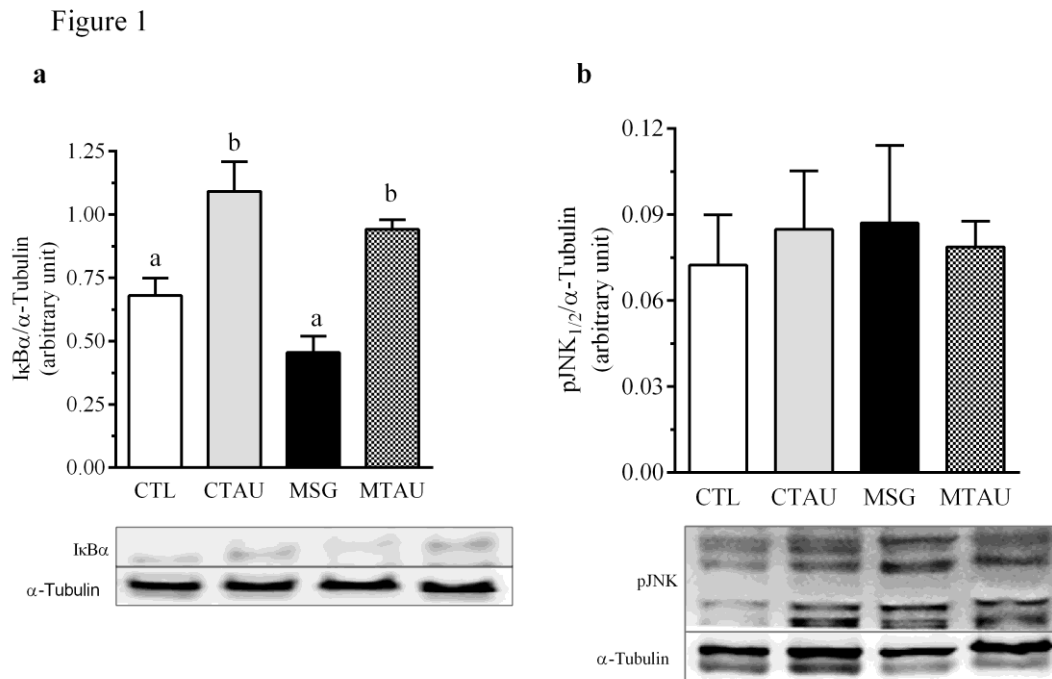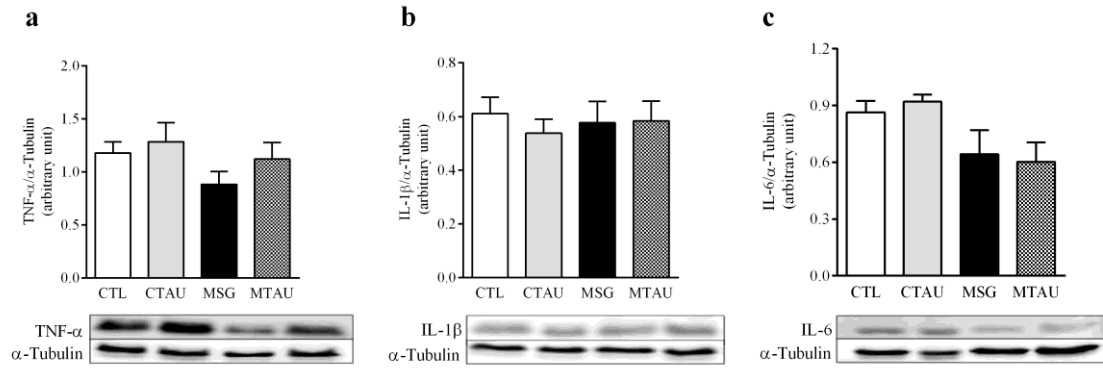
Figure 1
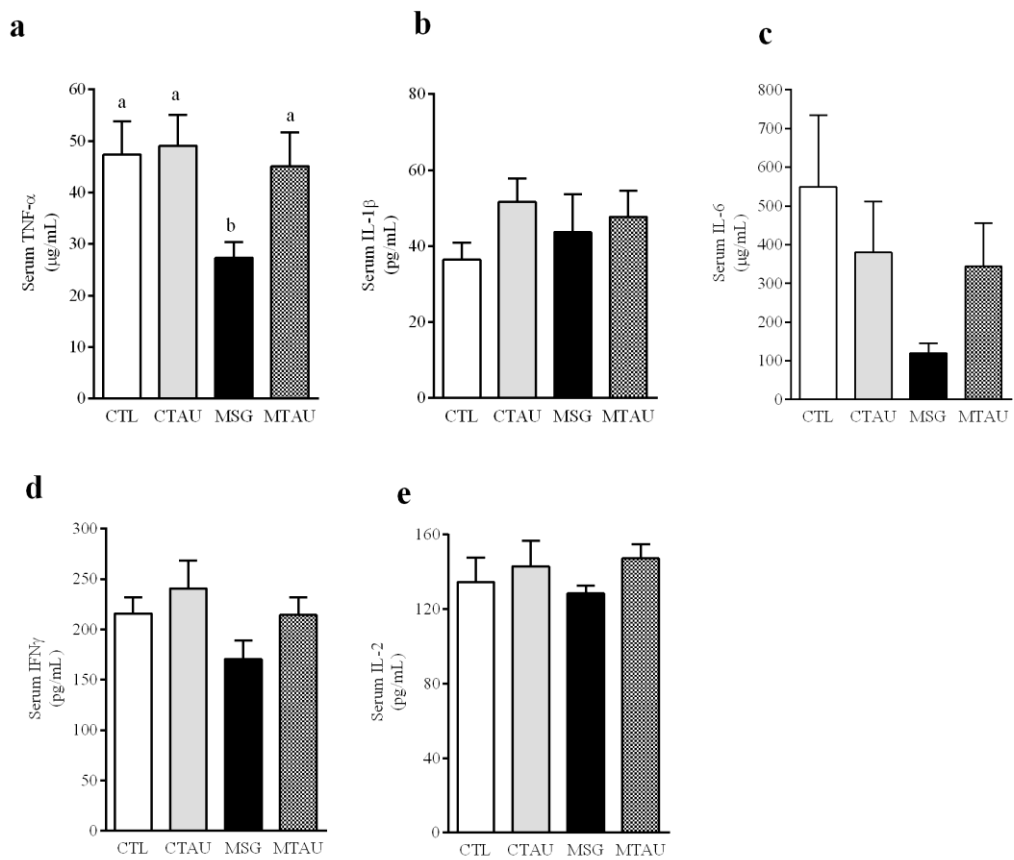
Figure 2

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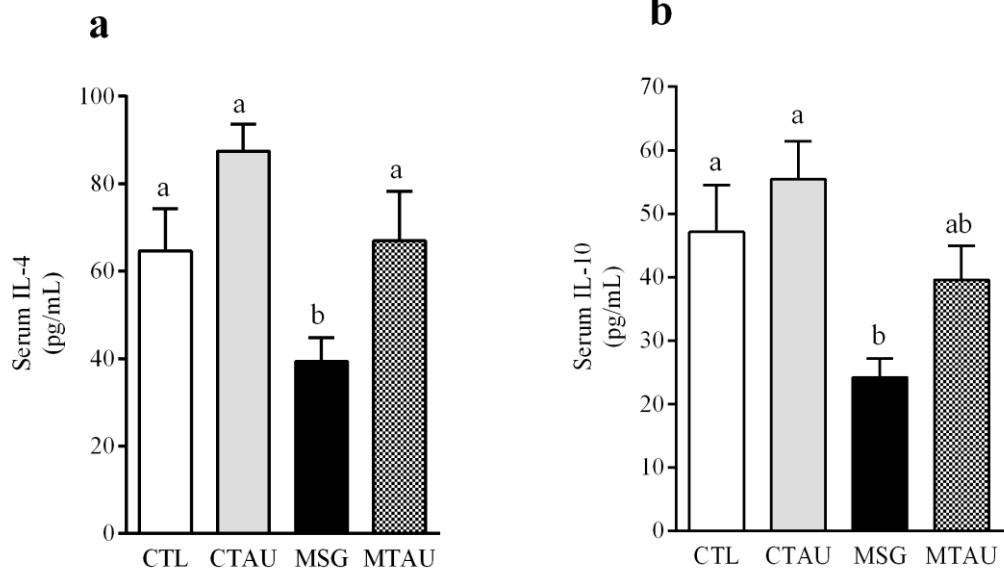
ANEXO A:

Certificado do Comitê de Ética em Experimentação Animal e Aulas Práticas - Universidade Estadual do Oeste do Paraná.

unioeste
Universidade Estadual do Oeste do Paraná

Comitê de Ética na Experimentação Animal e Aulas Práticas – CEEAAP/UNIOESTE

PARECER DE APROVAÇÃO DE PROJETO

Protocolo de Experimentação Animal n°: **00712**

Título do Projeto: **Efeito da obesidade-MSG e do aminoácido taurina associados ou não sobre as citocinas inflamatórias.**

Solicitante: **Sandra Lucinei Balbo (CCBS) Cascavel.**

O projeto acima foi **aprovado,** conforme Ata 03-2012 em reunião ocorrida em 15-05-2012 realizada pelo Comitê de Ética em Experimentação Animal e Aulas Práticas da Unioeste, desde que seguido o protocolo proposto e avaliado por este Comitê.

Lembramos que, de acordo com as atribuições, o CEEAAP se resguarda do direito de realizar visitas aos locais onde os projetos serão executados com finalidade de acompanhamento.

Ao término da vigência do projeto, após o envio do relatório final, o coordenador receberá um Certificado de que o protocolo realizado seguiu os princípios da experimentação animal, de acordo com sua respectiva ata de aprovação em data especificada.

Cascavel, 15 de Maio de 2012.

Profª Drª Luciana Oliveira de Fariña
Coordenadora do CEEAAP/Unioeste
Portaria 3244/2011 - GRE

ANEXO B:

Normas do periódico
BRITISH JOURNAL OF NUTRITION (ISSN: 0007-1145)

Directions to Contributors
British Journal of Nutrition
(Revised November 2013)

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The publication remit of the journal. The *British Journal of Nutrition* 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 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; papers on pharmaceutical agents or substances that are considered primarily as medicinal agents; studies in which a nutrient or extract is administered by a route other than orally (unless the specific aim of the study is to investigate parenteral nutrition) nor studies using supra-physiological amounts of nutrients (unless the specific aim of the study is to investigate toxic effects).

Guidelines on studies reporting *in vivo* or *in vitro* models. Studies involving animal models of human nutrition and health or disease will be considered for publication provided that the amount of a nutrient or combination of nutrients used could reasonably be expected to be achieved in humans.

Studies involving *in vitro* models will be considered for publication provided that the amount of a nutrient or combination of nutrients is 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 what the cell type used in the model would encounter *in vivo*.

Guidelines on studies reporting the effects of extracts. Studies involving extracts will be considered for publication provided that 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 humans (or in animals if they are the specific target of an intervention).

Studies involving extracts in *in vitro* models will be considered for publication provided that 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*.

Ethical standards for studies involving humans or other vertebrate animals

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). A paper describing any experimental work on human subjects must include the following statement in the materials/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]."

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 at www.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 using inhumane procedures, or experiments that have not been reviewed and approved by an animal experimentation ethics committee. When reporting on experiments involving the use of vertebrate animals, authors must state 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].

General guidelines

As a contributor you must follow the guidelines set out below. Prospective authors may contact the Publications Office directly on +44 (0)1223 347954 (telephone) or bjn.edoffice@cambridge.org (email).

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Papers should be accompanied by a statement of acceptance of the conditions laid down in the Directions to Contributors. The statement must affirm that the submission represents original work that has not been published previously, that it is not currently being considered by another journal, and that if accepted for the *British Journal of Nutrition* it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Nutrition Society. It should also confirm that each author has seen and approved the contents of the submitted manuscript. **At the time of acceptance the authors should provide a completed copy of the 'Licence to Publish' (in lieu of copyright transfer), which is available on the Nutrition Society's web pages** (http://www.nutritionsociety.org/publications/nutrition-society-journals/british-journal-of-nutrition); the Society no longer requires copyright of the material published in the journal, only a 'Licence to Publish.' **The authors or their institutions retain the copyright.**

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Full Papers, Reviews, Systematic Reviews, Horizons Articles and Workshop Reports should be submitted to: http://bjn.msubmit.net/ Please contact the Publications Office on edoffice@nutsoc.org.uk regarding any other types of article. Articles reporting randomised trials must conform to the standards set by the Consolidated Standards of Reporting Trials consortium http://www.consort-statement.org/ . Studies reporting the findings of microarray analyses must comply with the "Minimum Information about a Microarray Experiment" (MIAME) guidelines (http://www.mged.org/Workgroups/MIAME/miame.html) and the data must be deposited in a publically accessible repository.

Review Articles The 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 Publications 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 the 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 which 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 which are likely to produce substantial advances in nutritional science. These articles should be thought-provoking and possibly controversial.

Systematic Reviews and meta-analyses. The journal endorses the Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA) Statement, a guideline to help authors report a systematic review and meta-analysis http:/prisma-statement.org (see *British Medical Journal* (2009) **339**, b2535). A systematic review or meta-analysis of randomised trials and other evaluation studies should follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (http:/prisma-statement.org).

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Revision of manuscripts following review. When substantial revisions are required to manuscripts, 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.

Form of full papers submitted for publication. The onus of preparing a paper in a form suitable for sending to press lies with the author. Authors are advised to consult a current issue in order to make themselves familiar with the *British Journal of Nutrition* 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 the *British Journal of Nutrition*. The requirements of *British Journal of Nutrition* are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors (ICMJE), and authors are encouraged to consult the latest guidelines, which contain a lot of useful generic information about preparing scientific papers http://www.icmje.org/ .

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editorial process. If a concern is raised about possible plagiarism in an article published in the *British Journal of Nutrition*, this will be investigated fully and dealt with in accordance with the Committee on Publication Ethics guidelines (www. http://publicationethics.org/).

Authors are invited to nominate up to four potential referees who may then be asked by the Editorial Board to help review the work.

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 is required.

Spelling should generally be that of the *Concise Oxford Dictionary* (1995), 9th ed. Oxford: Clarendon Press. Papers should normally be divided into the following parts:

(a) *Title page:* authors' names should be given without titles or degrees and one forename may be given in full. The name and address of the institution where the work was performed should be given, as well as the main address for each author. The name and address of the author to whom correspondence should be sent should be clearly stated, together with telephone and fax numbers and email address. Other authors should be linked to their address using superscript Arabic numerals. Any necessary descriptive material about the authors, e.g. Beit Memorial Fellow, should appear at the end of the paper in the Acknowledgments.

Authorship. Although discretion is allowed, authors must fulfill the criteria set out by the International Committee of Medical Journal Editors (http://www.icmje.org) and described by as follows by Calder (*Br J Nutr* (2009) **101**, 775). Authors should meet each of the following requirements by substantial contributions to '(a) the conception and design, acquisition of data, or analysis and interpretation of data; (b) the drafting the article or revising it critically for important intellectual content; (c) 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 acknowledged in the Acknowledgments section.

If the paper is one of a series of papers that have a common main title followed by a subtitle specific to the individual paper, numbering should not be used to indicate the sequence of papers. The format should be 'common title: specific subtitle', with a short common title, e.g. Partitioning of limiting protein and energy in the growing pig: testing quantitative rules against experimental data.

The title page should also contain a shortened version of the paper's title, not exceeding forty-five letters and spaces in length, suitable for use as a running title in the published paper.

Authors are asked to supply three or four key words or phrases (each containing up to three words) on the title page of the typescript.

(b) *Abstract*: each paper must open with an abstract of **not more than 250 words**. The abstract should be a single paragraph of continuous text outlining the aims of the work, the experimental approach taken, the principal results and the conclusions and their relevance to nutritional science.

(c) *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 pages**.

(d) *Experimental methods*: methods should appear after the introduction. The methods section must include a subsection that describes the methods used for statistical analysis (see the section on statistical analysis below) and the sample size must be justified by the results of appropriate calculations and related to the study outcomes.

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

(e) *Results*: these should be given as concisely as possible, using figures or tables as appropriate. Data must not be duplicated in tables and figures.

(f) *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 pages**.

(g) *Acknowledgments*: here you may acknowledge individuals or organizations that provide advice and/or support (non-financial). Formal financial support and funding should be listed in the following section.

(h) *Financial Support:* please provide details of the sources of financial support for all authors, including grant numbers. This 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. 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)". 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."

(i) *Conflict of Interest:* Conflict of interest exists when an author has interests that might inappropriately influence his or her judgement, even if that judgement is not influenced; for further detail, see http://www.icmje.org/ethical_4conflicts.html. Because of this, authors must disclose potentially conflicting interests so that others can make judgements about such effects. At the time of submission authors should disclose any financial arrangements or connections they may have that are pertinent to the submitted manuscript and that may be perceived as potentially biasing their paper. Non-financial interests that could be relevant in this context should also be disclosed. If there are no interests to declare, the title should be inserted followed by "None".

(j) *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.

(k) *References*: these should be given in the text using the Vancouver system. They 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[1,2–4]'. If a reference is cited more than once the same number should be used each time. References cited only in tables and figure legends and not in the text 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. At the end of the paper, on a page(s) separate from the text, references should be listed in numerical order. 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. Names and initials of authors of unpublished work should be given in the text as 'unpublished results' and not included in the References. Titles of journals should appear in their abbreviated form using the NCBI LinkOut page http://www.ncbi.nlm.nih.gov/projects/linkout/journals/jourlists.fcgi?typeid=1&type=journals&operation=Show References to books and monographs should include the town of publication and the number of the edition to which reference is made. Thus:

1. Setchell KD, Faughnan MS, Avades T *et al.* (2003) Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women. *Am J Clin Nutr* **77**, 411–419.
2. Barker DJ, Winter PD, Osmond C *et al.* (1989) Weight in infancy and death from ischaemic heart disease. *Lancet* **ii**, 577–580.
3. Forchielli ML & Walker WA (2005) The role of gut-associated lymphoid tissues and mucosal defence. *Br J Nutr* **93**, Suppl. 1, S41–S48.
4. Bradbury J, Thomason JM, Jepson NJA *et al.* (2003) A nutrition education intervention to increase the fruit and vegetable intake of denture wearers. *Proc Nutr Soc* **62**, 86A.
5. Frühbeck G, Gómez-Ambrosi J, Muruzabal FJ *et al.* (2001) The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab* **280**, E827–E847.
6. Han KK, Soares JM Jr, Haidar MA *et al.* (2002) Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. *Obst Gynecol* **99**, 389–394.
7. Uhl M, Kassie F, Rabot S *et al.* (2004) Effect of common Brassica vegetables (Brussels sprouts and red cabbage) on the development of preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in liver and colon of Fischer 344 rats. *J Chromatogr* **802**B, 225–230.
8. Hall WL, Vafeiadou K, Hallund J *et al.* (2005) Soy isoflavone enriched foods and inflammatory biomarkers of cardiovascular risk in postmenopausal women: interactions with genotype and equol production. *Am J Clin Nutr* (In the Press).
9. Skurk T, Herder C, Kraft I *et al.* (2004) Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* (Epublication ahead of print version).
10. Skurk T, Herder C, Kraft I *et al.* (2005) Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* **146**, 1006–1011; Epublication 2 December 2004.
11. Bradbury J (2002) Dietary intervention in edentulous patients. PhD Thesis, University of Newcastle.
12. 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.
13. Bruinsma J (editor) (2003) *World Agriculture towards 2015/2030: An FAO Perspective*. London: Earthscan Publications.
14. Griinari JM & Bauman DE (1999) Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In *Advances in Conjugated Linoleic Acid Research*, vol. 1, pp. 180–200 [MP Yurawecz, MM Mossoba, JKG Kramer, MW Pariza and GJ Nelson, editors]. Champaign, IL: AOCS Press.
15. Henderson L, Gregory J, Irving K *et al.* (2004) *National Diet and Nutrition Survey: Adults Aged 19 to 64 Years*. vol. 2: *Energy, Protein, Fat and Carbohydrate Intake*. London: The Stationery Office.
16. International Agency for Research on Cancer (2004) *Cruciferous Vegetables, Isothiocyanates and Indoles. IARC Handbooks of Cancer Prevention* no. 9 [H Vainio and F Bianchini, editors]. Lyon, France: IARC Press.
17. Linder MC (1996) Copper. In *Present Knowledge in Nutrition*, 7th ed., pp. 307–319 [EE Zeigler and LJ Filer Jr, editors]. Washington, DC: ILSI Press.
18. 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.
19. 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.

References to material available on websites should include the full Internet address, and the date of the version cited. Thus:

20. Department of Health (1997) Committee on Toxicity of Chemicals in Food Consumer Products and the Environment. Statement on vitamin B$_6$ (pyridoxine) toxicity. http://www.open.gov.uk/doh/hef/B6.htm
21. Kramer MS & Kakuma R (2002) *The Optimal Duration of Exclusive Breastfeeding: A Systematic Review*. Rome: WHO; available at http://www.who.int/nut/documents/optimal_duration_of_exc_bfeeding_review_eng.pd
22. Hooper L, Thompson RL, Harrison RA *et al*. (2004) Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database of Systematic Reviews*, issue 4, CD003177.
http://www.mrw.interscience.wiley.com/cochrane/clsysrev/articles/CD003177/frame.html
23. Nationmaster (2005) HIV AIDS – Adult prevalence rate. http://www.nationmaster.com/graph-T/hea_hiv_aid_adu_pre_rat (accessed June 2005).

(l) *Supplementary data:* Additional data (e.g. data files, large tables) relevant to the paper can be submitted for publication online only, where they are made available via a link from the abstract and the paper. The paper should stand alone without these data. Supplementary data should be supplied as a PDF for the review process and must be cited in a relevant place in the text of the paper.

Mathematical modelling of nutritional processes. Papers in which mathematical modelling of nutritional processes forms the principal element will be considered for publication provided: (a) they are based on sound biological and mathematical principles; (b) they advance nutritional concepts or identify new avenues likely to lead to such advances; (c) assumptions used in their construction are fully described and supported by appropriate argument; (d) they are described in such a way that the nutritional purpose is clearly apparent; (e) the contribution of the model to the design of future experimentation is clearly defined.

Units. Results should be presented in metric units according to the International System of Units (see Quantities, Units and Symbols in Physical Chemistry, 3rd ed. (2007) Cambridge: RSC Publishing), and Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences (1972) London: The Royal Society – as reproduced in *Proceedings of the Nutrition Society* (1972) **31**, 239–247). SI units should be used throughout the paper. The author will be asked to convert any values that are given in any other form. The only exception is where there is a unique way of expressing a particular variable that is in widespread use. Energy values must be given in Joules (MJ or kJ) using the conversion factor 1 kcal = 4·184 kJ. If required by the author, the value in kcal can be given afterwards in parentheses. Temperature is given in degrees Celsius (ºC). Vitamins should be given as mg or μg, not as IU.

For substances of known molecular mass (Da) or relative molecular mass, e.g. glucose, urea, Ca, Na, Fe, K, P, values should be expressed as mol/l; for substances of indeterminate molecular mass (Da) or relative molecular mass, e.g. phospholipids, proteins, and for trace elements, e.g. Cu, Zn, then g/l should be used.

Time. The 24 h clock should be used, e.g. 15.00 hours.

Units are: year, month, week, d, h, min, s, kg, g, mg, μg, litre, ml, μl, fl. To avoid misunderstandings, the word litre should be used in full, except in terms like g/l. Radioactivity should be given in becquerels (Bq or GBq) not in Ci. 1 MBq = 27·03 μCi (1Bq = 1 disintegration/s).

Statistical treatment of results. Data from individual replicates should not be given for large experiments, but may be given for small studies. The methods of statistical analysis used should be described, and references to statistical analysis packages included in the text, for example: Statistical Analysis Systems statistical software package version 6.11 (SAS Institute, Cary, NC, USA). The description should provide enough information for a statistician with access to the data to reproduce the results presented. Information such as analysis of variance tables should be given in the paper only if they are relevant to the discussion. A statement of the number of replicates, their average value and some appropriate measure of variability is usually sufficient. Authors must state whether their data follow a Gaussian distribution or not, and the choice of statistical tests must be consistent with the distribution of the data.

Justification for the sample size must be given. If the study is based on a power calculation, details of this should be provided including the desired effect size and power as well as the estimate of variability that was used.

Comparisons between means can be made by using either confidence intervals (CI) or significance tests. The most appropriate of such measures is usually the standard error of a difference between means (SED) or the standard errors of the means (SEM). The SEM represents the uncertainty associated with the estimation of a given mean and is not directly related to the SED or comparisons among means in mixed models as it is in fixed effects models. The SED estimates the uncertainty associated with the difference between two means; because it is used in various mean comparisons tests, SED can be implied within the tests *per se*. The standard deviation (SD) is more useful only when there is specific interest in the variability of individual values and no treatment means are being compared. The sample size (n per treatment) should also be stated in text or in the table. Standard analysis of variance assumes homogeneous variance. Unless there is heterogeneous variance, as tested by an appropriate statistic, or there is unequal n, a pooled SEM or SED simplifies tables and is preferred. The number of decimal places quoted should be sufficient but not excessive. If data transformations are being used, text should clearly state which variables have been transformed in which way and how that was decision was reached (e.g., tests for normality, diagnostic plots).

Authors should consider whether their study is rather of explorative (hypothesis-generating) or confirmative (hypothesis-testing) nature. This is particularly important when results from multiple tests are being presented, which can be the case when various treatments are being compared, multiple endpoints are considered, or different subgroups are being analysed. Such multiple testing issues occur often in exploratory studies, and authors should take care not to overstate findings in these situations. At least the number of significant results should be compared to the number of tests compared, where 1 in 20 findings would be expected by chance alone. Methods that control certain error rates (experiment-wise error rate, false discovery rate, etc…) such as post-hoc tests can be used in this context, but are not obligatory, as long as the exploratory nature of the results is made clear. In confirmative studies, pre-planned comparisons or primary endpoints should be stated upfront and analysed by appropriate tools such as contrast testing for pre-planned comparisons. Unnecessary multiple testing corrections with respect to secondary comparisons or endpoints should be avoided to not compromise the power of the study.

Measurements on the same experimental unit over time or in different sections of tissue generally are not independent. If the repeated measures are taken from the same animal or human subject, which are expected to be randomly chosen to represent a population, an appropriate mixed model should be fitted while investigating the best covariance of error structures. All major statistical software packages offer a wide variety of structures; the one chosen should be stated.

If comparisons between means are made using CI, the format for presentation is, e.g. 'difference between means 0·73 (95 % CI 0·314, 1·36) g'. If significance tests are used, a statement that the difference between the means for two groups of values is (or is not) statistically significant should include the level of significance attained, preferably as an explicit P value (e.g. $P=0.016$ or $P=0.32$) rather than as a range (e.g. $P<0.05$ or $P>0.05$). It should be stated whether the significance levels quoted are one-sided or two-sided (when relevant). Where a multiple comparison procedure is used, a description or explicit reference should be given. Where appropriate, a superscript notation may be used in tables to denote levels of significance; similar superscripts should denote lack of a significant difference.

When the method of analysis is unusual, or if the experimental design is at all complex, further details (e.g., experimental plan, raw data, confirmation of assumptions, analysis of variance tables, etc.) should be included. Adequate detail should be provided for a subsequent reader to interpret and potentially repeat the approach used. For example, the statistical model should be provided or described in adequate detail, and all blocking factors and criteria should be provided. Regressions should provide appropriate estimates of parameter uncertainty (not necessarily provided by graphing software).

Figures. Figures should not be incorporated into the article file and should be supplied as separate electronic files. Figure legends should be grouped in a section at the end of the 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.

In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, ○, ●, Δ, ▲, □, ■, ×, +. 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 is reduced to fit the printed page.

Figures and diagrams can be prepared using most applications but please do not use the following: cdx, chm, jnb or PDF. All figures should be numbered and legends should be provided. Each figure, with its legend, should be comprehensible without reference to the text and should include definitions of abbreviations. Latin names for unusual species should be included unless they have already been specified in the text. Each figure will be positioned near the point in the text at which it is first introduced unless instructed otherwise.

Note that authors will be charged 350 GBP for the publication of colour figures. Authors from countries entitled to free journal access through Research4Life will be exempt from these charges: http://www.research4life.org/

Refer to a recent copy of the journal for examples of figures.

Image integrity. Images submitted with a manuscript should be minimally processed (e.g. the addition of labelling). Authors should retain their original data, as Editors may request them for comparison during manuscript review. If such data are unavailable the manuscript may be withdrawn from the review process. Submitted manuscripts in which there appear to be manipulation of images will be withdrawn from the editorial process.

Some image processing is acceptable (and may be unavoidable), but the final image must accurately represent the original data. 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. Grouping or cropping of images must be identified in the legend and indicated by clear demarcation. Adjustment of brightness, contrast or colour balance is acceptable if applied to the whole image and to controls and if data do not disappear as the result of the manipulation. If a concern is raised about possible image manipulation in an article published in the *British Journal of Nutrition*, this will be investigated fully and dealt with in accordance with the Committee on Publication Ethics guidelines (www.http://publicationethics.org/).

Plates. The *British Journal of Nutrition* will also consider the inclusion of illustrations and photomicrographs. The size of photomicrographs may have to be altered in printing; in order to avoid mistakes the magnification should be shown by scale on the photograph itself. The scale with the appropriate unit together with any lettering should be drawn by the author, preferably using appropriate software.

Tables. 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 appear in the footnote in the order that they appear in the table (reading from left to right across the table, then down each column). Abbreviations in tables must be defined in footnotes. 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.

Tables should be placed at the end of the text. Each table will be positioned near the point in the text at which it is first introduced unless instructed otherwise.

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Chemical formulas. These should be written as far as possible on a single horizontal line. With inorganic substances, formulas may be used from first mention. With salts, it must be stated whether or not the anhydrous material is used, e.g. anhydrous $CuSO_4$, or which of the different crystalline forms is meant, e.g. $CuSO_4.5H_2O$, $CuSO_4.H_2O$.

Descriptions of solutions, compositions and concentrations. Solutions of common acids, bases and salts should be defined in terms of molarity (M), e.g. 0.1 M-NaH_2PO_4. Compositions expressed as mass per unit mass (w/w) should have values expressed as ng, µg, mg or g per kg; similarly for concentrations expressed as mass per unit volume (w/v), the denominator being the litre. If concentrations or compositions are expressed as a percentage, the basis for the composition should be specified (e.g. % (w/w) or % (w/v) etc.). The common measurements used in nutritional studies, e.g. digestibility, biological value and net protein utilization, should be expressed as decimals rather than as percentages, so that amounts of available nutrients can be obtained from analytical results by direct multiplication. See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences*. London: The Royal Society, 1972 (para. 8).

Cell lines. The Journal expects authors to deposit cell lines (including microbial strains) used in any study to be published in publicly accessible culture collections, for example, the European Collection of Cell Cultures (ECACC) or the American Type Culture Collection (ATCC) and to refer to the collection and line or strain numbers in the text (e.g. ATCC 53103). Since the authenticity of subcultures of culture collection specimens that are distributed by individuals cannot be ensured, authors should indicate laboratory line or strain designations and donor sources as well as original culture collection identification numbers.

Gene nomenclature and symbols. The use of symbols and nomenclature recommended by the HUGO Gene Nomenclature Committee (http://www.genenames.org/) is encouraged. Information on human genes is also available from Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene), on mouse genes from the Mouse Genome Database (http://www.informatics.jax.org/) and on rat genes from the Rat Genome Database (http://rgd.mcw.edu/).

Nomenclature of vitamins. Most of the names for vitamins and related compounds that are accepted by the Editors are those recommended by the IUNS Committee on Nomenclature. See *Nutrition Abstracts and Reviews* (1978) **48**A, 831–835.

Acceptable name Other names*

Vitamin A
Retinol Vitamin A_1
Retinaldehyde, retinal Retinene
Retinoic acid (all-*trans* or 13-*cis*) Vitamin A_1 acid
3-Dehydroretinol Vitamin A_2

Vitamin D
Ergocalciferol, ercalciol Vitamin D_2 calciferol
Cholecalciferol, calciol Vitamin D_3

Vitamin E
α-, β- and γ-tocopherols plus
tocotrienols

Vitamin K
Phylloquinone Vitamin K_1
Menaquinone-n (MK-n)† Vitamin K_2
Menadione Vitamin K_3,
menaquinone,
menaphthone

Vitamin B_1
Thiamin Aneurin(e), thiamine

Vitamin B_2
Riboflavin Vitamin G, riboflavine,
lactoflavin

Niacin
Nicotinamide Vitamin PP
Nicotinic acid

Folic Acid
Pteroyl(mono)glutamic acid Folacin, vitamin B_c or M

Vitamin B_6
Pyridoxine Pyridoxol
Pyridoxal
Pyridoxamine

Vitamin B_{12}
Cyanocobalamin
Hydroxocobalamin Vitamin B_{12a} or B_{12b}
Aquocobalamin
Methylcobalamin
Adenosylcobalamin

Inositol

Myo-inositol *Meso*-inositol
Choline
Pantothenic acid
Biotin Vitamin H
Vitamin C
Ascorbic acid
Dehydroascorbic acid

*Including some names that are still in use elsewhere, but are not used by the *British Journal of Nutrition*.

†Details of the nomenclature for these and other naturally-occurring quinones should follow the Tentative Rules of the IUPAC-IUB Commission on Biochemical Nomenclature (see *European Journal of Biochemistry* (1975) **53**, 15–18)

Generic descriptors. The terms **vitamin A**, **vitamin C** and **vitamin D** may still be used where appropriate, for example in phrases such as 'vitamin A deficiency', 'vitamin D activity'.

Vitamin E. The term **vitamin E** should be used as the descriptor for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of α-tocopherol. The term **tocopherols** should be used as the generic descriptor for all methyl tocols. Thus, the term **tocopherol** is not synonymous with the term **vitamin E**.

Vitamin K. The term **vitamin K** should be used as the generic descriptor for 2-methyl-1,4-naphthoquinone (menaphthone) and all derivatives exhibiting qualitatively the biological activity of phylloquinone (phytylmenaquinone).

Niacin. The term **niacin** should be used as the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.

Vitamin B_6. The term **vitamin B_6** should be used as the generic descriptor for all 2-methylpyridine derivatives exhibiting qualitatively the biological activity of pyridoxine.

Folate. Due to the wide range of C-substituted, unsubstituted, oxidized, reduced and mono- or polyglutamyl side-chain derivatives of pteroylmonoglutamic acid that exist in nature, it is not possible to provide a complete list. Authors are encouraged to use either the generic name or the correct scientific name(s) of the derivative(s), as appropriate for each circumstance.

Vitamin B_{12}. The term **vitamin B_{12}** should be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. The term **corrinoids** should be used as the generic descriptor for all compounds containing the corrin nucleus and thus chemically related to cyanocobalamin. The term **corrinoid** is not synonymous with the term **vitamin B_{12}**.

Vitamin C. The terms **ascorbic acid** and **dehydroascorbic acid** will normally be taken as referring to the naturally-occurring L-forms. If the subject matter includes other optical isomers, authors are encouraged to include the L- or D- prefixes, as appropriate. The same is true for all those vitamins which can exist in both natural and alternative isomeric forms.

Amounts of vitamins and summation. Weight units are acceptable for the amounts of vitamins in foods and diets. For concentrations in biological tissues, SI units should be used; however, the authors may, if they wish, also include other units, such as weights or international units, in parentheses.

See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences* (1972) paras 8 and 14–20. London: The Royal Society.

Nomenclature of fatty acids and lipids. In the description of results obtained for the analysis of fatty acids by conventional GLC, the shorthand designation proposed by Farquhar JW, Insull W, Rosen P, Stoffel W & Ahrens EH (*Nutrition Reviews* (1959), **17**, Suppl.) for individual fatty acids should be used in the text, tables and figures. Thus, 18 : 1 should be used to represent a fatty acid with eighteen carbon atoms and one double bond; if the position and configuration of the double bond is unknown. The shorthand designation should also be used in the abstract. If the positions and configurations of the double bonds are known, and these are important to the discussion, then a fatty acid such as linoleic acid may be referred to as *cis*-9,*cis*-12-18 : 2 (positions of double bonds related to the carboxyl carbon atom 1). However, to illustrate the metabolic relationship between different unsaturated fatty acid families, it is sometimes more helpful to number the double bonds in relation to the terminal methyl carbon atom, *n*. The preferred nomenclature is then: 18 : 3*n*-3 and 18 : 3*n*-6 for α-linolenic and γ-linolenic acids respectively; 18 : 2*n*-6 and 20 : 4*n*-6 for linoleic and arachidonic acids respectively and 18 : 1*n*-9 for oleic acid. Positional isomers such as α- and γ-linolenic acid should always be clearly distinguished. It is assumed that the double bonds are methylene-interrupted and are of the *cis*-configuration (see Holman RT in *Progress in the Chemistry of Fats and Other Lipids* (1966) vol. 9, part 1, p. 3. Oxford: Pergamon Press). Groups of fatty acids that have a common chain length but vary in their double bond content or double bond position should be referred to, for example, as C_{20} fatty acids or C_{20} PUFA. The modern nomenclature for glycerol esters should be used, i.e. triacylglycerol, diacylglycerol, monoacylglycerol *not* triglyceride, diglyceride, monoglyceride. The form of fatty acids used in diets should be clearly stated, i.e. whether ethyl esters, natural or refined fats or oils. The composition of the fatty acids in the dietary fat and tissue fats should be stated clearly, expressed as mol/100 mol or g/100 g total fatty acids.

Nomenclature of micro-organisms. The correct name of the organism, conforming with international rules of nomenclature, should be used: if desired, synonyms may be added in parentheses when the name is first mentioned. Names of bacteria should conform to the current Bacteriological Code and the opinions issued by the International Committee on Systematic Bacteriology. Names of algae and fungi must conform to the current International Code of Botanical Nomenclature. Names of protozoa should conform to the current International Code of Zoological Nomenclature.

Nomenclature of plants. For plant species where a common name is used that may not be universally intelligible, the Latin name in italics should follow the first mention of the common name. The cultivar should be given where appropriate.

Other nomenclature, symbols and abbreviations. Authors should consult recent issues of the *British Journal of Nutrition* for guidance. The IUPAC rules on chemical nomenclature should be followed, and the recommendations of the Nomenclature Committee of IUBMB and the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature and

Nomenclature Commission of IUBMB in *Biochemical Nomenclature and Related Documents* (1992), 2nd ed., London: Portland Press (http://www.chem.qmul.ac.uk/iupac/bibliog/white.html). The symbols and abbreviations, other than units, are essentially those listed in *British Standard* 5775 (1979–1982), *Specifications for Quantities, Units and Symbols*, parts 0–13. Day should be abbreviated to d, for example 7 d, except for 'each day', '7th day' and 'day 1'.

Elements and simple chemicals (e.g. Fe and CO_2) can be referred to by their chemical symbol (with the exception of arsenic and iodine, which should be written in full) or formula from the first mention in the text; the title, text and table headings, and figure legends can be taken as exceptions,. Well-known abbreviations for chemical substances may be used without explanation, thus: RNA for ribonucleic acid and DNA for deoxyribonucleic acid. Other substances that are mentioned frequently (five or more times) may also be abbreviated, the abbreviation being placed in parentheses at the first mention, thus: lipoprotein lipase (LPL), after that, LPL, and an alphabetical list of abbreviations used should be included. Only accepted abbreviations may be used in the title and text headings. If an author's initials are mentioned in the text, they should be distinguished from other abbreviations by the use of stops, e.g. 'one of us (P. J. H.)…'. For UK counties the official names given in the *Concise Oxford Dictionary* (1995) should be used and for states of the USA two-letter abbreviations should be used, e.g. MA (not Mass.) and IL (not Ill.). Terms such as 'bioavailability' or 'available' may be used providing that the use of the term is adequately defined.

Spectrophotometric terms and symbols are those proposed in *IUPAC Manual of Symbols and Terminology for Physicochemical Quantities and Units* (1979) London: Butterworths. The attention of authors is particularly drawn to the following symbols: m (milli, $10 \cdot 3$), μ (micro, $10 \cdot 6$), n (nano, $10 \cdot 9$) and p (pico, $10 \cdot 12$). Note also that ml (millilitre) should be used instead of cc, μm (micrometre) instead of μ (micron) and μg (microgram) instead of γ.

Numbers. Numerals should be used with units, for example, 10 g, 7 d, 4 years (except when beginning a sentence, thus: 'Four years ago...'); otherwise, words (except when 100 or more), thus: one man, ten ewes, ninety-nine flasks, three times (but with decimal, 2·5 times), 100 patients, 120 cows, 136 samples.

Abbreviations. The following abbreviations are accepted without definition by the *British Journal of Nutrition:*

ADP (GDP) adenosine (guanosine) 5'-disphosphate

AIDS acquired immune deficiency syndrome

AMP (GMP) adenosine (guanosine) 5'-monophosphate

ANCOVA analysis of covariance

ANOVA analysis of variance

apo apolipoprotein

ATP (GTP) adenosine (guanosine) 5'-triphosphate

AUC area under the curve

BMI body mass index

BMR basal metabolic rate

bp base pair

BSE bovine spongiform encephalopathy

CHD coronary heart disease

CI confidence interval

CJD Creutzfeldt-Jacob disease

CoA and acyl-CoA co-enzyme A and its acyl derivatives

CV coefficient of variation

CVD cardiovascular disease

Df degrees of freedom

DHA docosahexaenoic acid

DM dry matter

DNA deoxyribonucleic acid

Dpm disintegrations per minute

EDTA ethylenediaminetetra-acetic acid

ELISA enzyme-linked immunosorbent assay

EPA eicosapentaenoic acid

Expt experiment (for specified experiment, e.g. Expt 1)

FAD flavin-adenine dinucleotide

FAO Food and Agriculture Organization (except when used as an author)

FFQ food-frequency questionnaire

FMN flavin mononucleotide

GC gas chromatography

GLC gas–liquid chromatography

GLUT glucose transporter

GM genetically modified

Hb haemoglobin

HDL high-density lipoprotein

HEPES 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid

HIV human immunodeficiency virus

HPLC high-performance liquid chromatography

Ig immunoglobulin

IHD ischaemic heart disease

IL interleukin
IR infra red
kb kilobases
K_m Michaelis constant
LDL low-density lipoprotein
MHC major histocompatibility complex
MRI magnetic resonance imaging
MS mass spectrometry
MUFA monounsaturated fatty acids
NAD+, NADH oxidized and reduced nicotinamide-adenine dinucleotide
NADP+, NADPH oxidized and reduced nicotinamide-adenine dinucleotide phosphate
NEFA non-esterified fatty acids
NF-κB nuclear factor kappa B
NMR nuclear magnetic resonance
NS not significant
NSP non-starch polysaccharide
OR odds ratio
PAGE polyacrylamide gel electrophoresis
PBS phosphate-buffered saline
PCR polymerase chain reaction
PG prostaglandin
PPAR peroxisome proliferator-activated receptor
PUFA polyunsaturated fatty acids
RDA recommended dietary allowance
RER respiratory exchange ratio
RIA radioimmunoassay
RMR resting metabolic rate
RNA, mRNA etc. ribonucleic acid, messenger RNA etc.
rpm revolutions per minute
RT reverse transcriptase
SCFA short-chain fatty acids
SDS sodium dodecyl sulphate
SED standard error of the difference between means
SFA saturated fatty acids
SNP single nucleotide polymorphism
TAG triacylglycerol
TCA trichloroacetic acid
TLC thin-layer chromatography
TNF tumour necrosis factor
UN United Nations (except when used as an author)
UNICEF United Nations International Children's Emergency Fund
UV ultra violet
VLDL very-low-density lipoprotein
V_{O2} O_2 consumption
V_{O2max} maximum O_2 consumption
WHO World Health Organization (except when used as an author)
Use of three-letter versions of amino acids in tables: Leu, His, etc.
CTP, UTP, GTP, ITP, as we already use ATP, AMP etc.

Disallowed words and phrases. The following are disallowed by the *British Journal of Nutrition*:
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c.a. or around (use approximately or about)
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