NAYARA RAMPAZZO MORELLI

ENVOLVIMENTO DO PESO CORPORAL E DOS COMPONENTES DA SÍNDROME METABÓLICA NO ESTRESSE OXIDATIVO E NITROSATIVO EM INDIVÍDUOS COM SOBREPESO E OBESOS

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ciências da Saúde, Centro de Ciências da Saúde, Universidade Estadual de Londrina, como requisito parcial para obtenção do título de mestre.

Orientador: Prof. Dr. Isaias Dichi.

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MORELLI, Nayara Rampazzo. **Envolvimento do peso corporal e dos componentes da síndrome metabólica no estresse oxidativo e nitrosativo em indivíduos com sobrepeso e obesos**. 2015. 70 f. Dissertação (Mestrado em Ciências da Saúde) – Universidade Estadual de Londrina, Londrina, 2015.

RESUMO

Em um estudo realizado anteriormente pelo nosso grupo, foi verificado um aumento no estresse oxidativo em indivíduos com sobrepeso com Síndrome Metabólica (SM), mas não em indivíduos com sobrepeso sem SM. Com a intenção de ampliar esses dados, o objetivo do presente estudo foi avaliar o estresse oxidativo e nitrosativo em indivíduos com sobrepeso e obesos. Os indivíduos selecionados foram divididos em três grupos: grupo controle (G1, n = 131) com íncice de massa corpórea (IMC) entre 20 e 24,9 Kg/m^2; grupo com sobrepeso (G2, n = 120) com IMC entre 25 e 29,9 Kg/m^2; e grupo com obesidade (G3, n = 79) com IMC ≥ 30 Kg/m^2. A SM foi definida seguindo os critérios de classificação estabelecidos pelo *Adult Treatment Panel III* (ATP III) e, posteriormente, os indivíduos foram redivididos de acordo com a presença ou não de cada um dos componentes da SM. Foi avaliada a capacidade antioxidante total do plasma pelo TRAP (*Total Radical-Trapping Antioxidant Parameter*), quimiluminescência iniciada por t-butil hidroperóxido (CL-LOOH), produtos avançados de oxidação proteica (AOPP) e os metabólitos do óxido nítrico (NOx). O grupo G3 apresentou níveis de AOPP mais altos em relação à G1 e G2 (p=0.001 e p=0.011, respectivamente), enquanto os níveis de NO foram mais baixos nos grupos G2 e G3 em comparação com G1 (p=0.009 e p=0.048, respectivamente). A seguir, foi realizado um ajuste para verificar a possível inflência da presença de SM sobre os resultados. AOPP não diferiu entre os grupos, enquanto níveis mais baixos de NO mantiveram-se significativos. Os dados ajustados pelo IMC mostraram que indivíduos com níveis elevados de triglicerídeos tiveram níveis mais altos de AOPP (p=0.001) e redução no TRAP/ácido úrico (p=0.036). Indivíduos com níveis mais baixos de HDL colesterol e com pressão arterial elevada mostraram aumento nos níveis de AOPP (p=0.001 e p=0.034, respectivamente) e redução nos níveis de NO (p=0.017 e p=0.043, respectivamente). Os indivíduos que apresentaram resistência à insulina tiveram níveis mais altos de AOPP (p=0.024). Em conclusão, apenas o estresse nitrosativo foi relacionado ao IMC, enquanto a oxidação de proteínas foi relacionada a cada componente da SM. Além disso, tanto o NO como a AOPP foram relacionados com a hipertensão. Os componentes da SM tiveram uma participação fundamental em indivíduos com sobrepeso e obesos, sendo que a hipertrigliceridemia foi o parâmetro que mostrou o maior grau de desequilíbrio redox.

Palavras-chave: Sobrepeso. Obesidade. Síndrome Metabólica. Estresse oxidativo. Estresse nitrosativo.

MORELLI, Nayara Rampazzo. **Involvement of body weight and the metabolic syndrome components on oxidative and nitrosative stress in overweight and obese subjects**. 2015. 70 p. Dissertation (Master's Degree in Health Sciences) – University of Londrina, Londrina, 2015.

ABSTRACT

In a previous study our group verified an increase in oxidative stress in overweight subjects with Metabolic Syndrome (MetS), but not in overweight without MetS. In order to extend these data, the objective of the present study was to evaluate the oxidative and nitrosative stress in overweight and obese subjects. Selected subjects were divided into three groups: control group (G1, n=131) with body mass index (BMI) between 20 and 24,9 kg/m^2; overweight group (G2, n=120) with BMI between 25 and 29,9 kg/m^2; and obese group (G3, n=79) with BMI ≥ 30 kg/m^2. MetS was defined following the Adult Treatment Panel III (ATP III) criteria, and later the subjects were divided according to the presence or absence of each MetS component. It was evaluated the plasma total antioxidant capacity by TRAP (Total Radical-Trapping Antioxidant Parameter), chemiluminescence initiated by t-butyl hydroperoxide (CL-LOOH), advanced oxidation protein products (AOPP) and nitric oxide metabolites (NOx). G3 presented higher AOPP in relation to G1 and G2 (p=0.001 and p=0.011, respectively), whereas NO had lower levels in G2 and G3 compared to G1 (p=0.009 and p=0.048, respectively). The results were adjusted for the presence of MetS to evaluate its influence. AOPP did not differ between the groups, whereas significant lower NO maintained its significance. Data adjusted by BMI showed that subjects with higher triacylglycerol levels had higher AOPP (p=0.001) and decreased TRAP/uric acid (p=0.036). Subjects with lower HDL-cholesterol levels and higher blood pressure showed increased AOPP (p=0.001 and p=0.034, respectively) and lower NO levels (p=0.017 and p=0.043, respectively). Subjects who presented insulin resistance had higher AOPP (p=0.024). In conclusion, only nitrosative stress was related to BMI, whereas protein oxidation was related to each component of the MetS. In addition, both NO and AOPP were related to hypertension. MetS components had an essential participation in overweight and obese subjects, whereas hypertriacylglycerolemia was the parameter which showed the highest degree of redox imbalance.

Key words: Overweight. Obesity. Metabolic Syndrome. Oxidative stress. Nitrosative stress.

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ADA	*American Diabetes Association*
AGL	Ácidos graxos livres
AHA	*American Heart Association*
AOPP	*Advanced Oxidation Protein Products*
Apo A-1	Apolipoproteína A-1
Apo-B	Apolipoproteína-B
ATP III	*Adult Treatment Panel III*
AU	Ácido úrico
EDTA	Ácido etilenodiaminotetracético
EGIR	*European Group for Study of Insulin Resistance*
ERN	Espécies reativas de nitrogênio
ERO	Espécies reativas de oxigênio
GLUT-4	Transportador de glicose tipo 4
GPx	Glutationa peroxidase
HDL	*High-density lipoprotein*
HOMA-IR	*Homeostasis Model Assessment of Insulin Resistance*
HU	Hospital Universitário
IDF	*International Diabetes Federation*
IL-6	Interleucina-6
IMC	Índice de massa corporal
LDL	*Low-density lipoprotein*
MCP-1	Proteína quimiotática de monócitos-1
NADPH	Nicotinamida adenina dinucleotídeo fosfato
NCEP	*National Cholesterol Education Program*
NHLBI	*National Heart Lung and Blood Institute*
NO	Óxido nítrico
NOx	Metabólitos do óxido nítrico
OMS	Organização Mundial da Saúde
PAI-1	Inibidor de fator ativador de plasminogênio-1
PCR	Proteína C reativa
QL	Quimiluminescência

RI	Resistência à insulina
SM	Síndrome metabólica
SNS	Sistema nervoso simpático
SOD	Superóxido dismutase
TCLE	Termo de consentimento livre e esclarecido
TNF-α	Fator de necrose tumoral-alfa
TRAP	*Total Radical-Trapping Antioxidant Parameter*
UEL	Universidade Estadual de Londrina
VLD	*Very-low density lipoprotein*

SUMÁRIO

1 INTRODUÇÃO

1.1 OBESIDADE

De acordo com a Organização Mundial da Saúde (OMS), mais de meio milhão de adultos em todo o mundo são classificados como obesos. Em 2014, aproximadamente 11% dos homens e 15% das mulheres com 18 anos ou mais eram obesos. A prevalência de sobrepeso e obesidade é maior entre os países com nível econômico elevado (WORLD HEALTH ORGANIZATION, 2014).

A obesidade aumenta o risco para diversas doenças crônicas, como diabetes mellitus, doenças cardiovasculares, neoplasias, entre outras (PIMENTA et al., 2015). O desenvolvimento da obesidade envolve fatores genéticos e ambientais. O acúmulo de massa gorda parece resultar principalmente de um desequilíbrio entre a ingestão alimentar e o gasto de energia (FARB; GOKCE, 2015). O aumento na ingestão energética devido ao aumento no fornecimento de alimentos processados e a redução na atividade física têm sido apontados como fatores responsáveis pelo ganho de peso (VANDEVIJVERE et al., 2015).

O método mais utilizado para avaliar a obesidade em adultos é o Índice de Massa Corporal (IMC), calculado a partir do peso (em quilogramas) dividido pela altura ao quadrado (em metros) e classificado da seguinte forma: abaixo do peso (IMC < 18,5), peso normal (IMC entre 18,5 e 24,9), sobrepeso (IMC entre 25,0 e 29,9), obesidade grau I (IMC entre 30,0 e 34,9), obesidade grau II (IMC entre 35,0 e 39,9) e obesidade grau III (IMC ≥ 40,0) (PIMENTA et al., 2015).

A distribuição da gordura corporal desempenha um importante papel no desenvolvimento das comorbidades relacionadas à obesidade. Principalmente a obesidade central e o acúmulo de tecido adiposo visceral estão relacionados com as doenças metabólicas e o risco cardiovascular, enquanto indivíduos que apresentam adiposidade periférica têm sido associados a um melhor perfil metabólico (BAYS, 2014; CASTRO et al., 2014).

O tecido adiposo é um órgão endócrino composto predominantemente pelos adipócitos e também por outras células, como fibroblastos, células endoteliais e células do sistema imune, responsáveis pela secreção de adipocinas, que incluem hormônios, citocinas e outras substâncias que apresentam várias funções, tais como regulação do apetite, efeitos na sensibilidade

à insulina e modulação da inflamação (FARB; GOKCE, 2015; MARSEGLIA et al., 2014).

1.2 SÍNDROME METABÓLICA

A Síndrome Metabólica (SM) é definida como um conjunto de fatores de risco para o desenvolvimento de doenças cardiovasculares, que inclui obesidade abdominal, dislipidemia, elevação da pressão sanguínea e resistência à insulina (RI). A SM também apresenta um estado pró-inflamatório e pró-trombótico (GRUNDY et al., 2004).

Organizações como OMS, *European Group for Study of Insulin Resistance* (EGIR), *International Diabetes Federation* (IDF) e *National Cholesterol Education Program* (NCEP) *Adult Treatment Panel III* (ATP III), definiram os critérios de classificação para o diagnóstico da SM (HUANG, 2009).

Em 1998, a OMS estabeleceu pela primeira vez os seus critérios para classificação da SM e reconheceu a RI como principal fator envolvido na patogênese da SM (HUANG, 2009). De acordo com a OMS, para a classificação da SM é necessário apresentar intolerância à glicose, tolerância à glicose diminuída ou diabetes e/ou RI junto com dois ou mais dos seguintes componentes (ALBERTI; ZIMMET, 1998):

a. Elevação da pressão sanguínea (≥ 160/90 mm Hg);
b. Níveis elevados de triglicerídeos (≥ 150 mg/dL) e/ou níveis baixos de HDL (*high-density lipoprotein*) colesterol (< 35 mg/dL para homens e < 39 mg/dL para mulheres);
c. Obesidade central (razão cintura/quadril > 0.90 para homens e > 0.85 para mulheres) e/ou IMC > 30 Kg/m^2;
d. Microalbuminúria (taxa de excreção urinária de albumina ≥ 20 µg/min ou razão albumina/creatinina ≥ 20 mg/g).

O EGIR, em 1999, propôs algumas modificações nos critérios apresentados pela OMS, mas manteve a RI como componente principal da SM (HUANG, 2009). O EGIR sugeriu a classificação da SM pela presença de RI ou hiperinsulinemia de jejum e mais dois fatores (BALKAU; CHARLES, 1999):

a. Hiperglicemia (≥ 110 mg/dL), mas não diabetes;
b. Hipertensão (≥ 140/90 mm Hg ou tratamento para hipertensão);

c. Dislipidemia (triglicerídeos > 177 mg/dL ou HDL colesterol < 39 mg/dL ou tratamento para dislipidemia);

d. Obesidade central (circunferência da cintura ≥ 94 cm para homens e ≥ 80 cm para mulheres).

Em 2005, o IDF apresentou um novo critério para a classificação da SM que exigia a presença da obesidade (HUANG, 2009). O IDF reconheceu a obesidade central como um fator determinante para a SM e que existe associação entre a circunferência da cintura, as doenças cardiovasculares e os demais componentes da SM. Para o diagnóstico da SM os indivíduos devem apresentar obesidade central, definida pela circunferência da cintura com pontos de corte específicos para as etnias (se o IMC > 30 Kg/m^2, a presença de obesidade central pode ser assumida e a circunferência da cintura não precisa ser medida) e mais dois fatores (ZIMMET et al., 2005):

a. Triglicerídeos elevados (≥ 150 mg/dL) ou tratamento específico para esta alteração lipídica;

b. Níveis baixos de HDL colesterol (< 40 mg/dL para homens e < 50 mg/dL para mulheres) ou tratamento específico para esta alteração lipídica;

c. Elevação da pressão sanguínea (≥ 130/85 mm Hg) ou tratamento para hipertensão;

d. Glicemia elevada (≥ 100 mg/dL) ou diagnóstico de diabetes.

O NCEP ATP III apresentou em 2001 os critérios de classificação para SM, que é um dos mais utilizados devido à facilidade de aplicação clínica e epidemiológica. O NCEP ATP III não exigiu nenhum parâmetro específico como requisito para o diagnóstico da SM (HUANG, 2009). Os indivíduos devem apresentar três dos cinco fatores de risco: (JACOBS Jr., 2001):

a. Obesidade abdominal (circunferência da cintura ≥ 102 cm para homens e ≥ 88 cm para mulheres);

b. Triglicerídeos elevados (≥ 150 mg/dL);

c. Níveis baixos de HDL colesterol (< 40 mg/dL para homens e < 50 mg/dL para mulheres);

d. Elevação da pressão sanguínea (≥ 130/85 mm Hg);

e. Glicemia elevada (≥ 110 mg/dL).

Posteriormente, em 2005, esses critérios foram atualizados pelo *American Heart Association* (AHA) *and the National Heart Lung and Blood Institute* (NHLBI) (HUANG, 2009). Os valores de glicemia foram reduzidos de ≥ 110 mg/dL para ≥ 100 mg/dL, de acordo com a modificação apresentada pela *American Diabetes Association* (ADA). Foi observado que o aumento na circunferência da cintura com valores abaixo do ponto de corte proposto (entre 94 e 101 cm para homens e 80 e 87 cm para mulheres) pode ser suficiente para indicar a presença de RI (GRUNDY et al., 2005).

1.2.1 Obesidade Abdominal

A obesidade central, avaliada pela circunferência da cintura, tem sido considerada um importante fator de risco cardiometabólico (MILLAR et al., 2015). O aumento na circunferência da cintura reflete o acúmulo de tecido adiposo visceral e subcutâneo, que estão relacionados com as alterações metabólicas. O tecido adiposo subcutâneo, principalmente a sua porção profunda, é um forte indicador de RI (BAYS, 2014). O tecido adiposo visceral contribui para a sensibilidade à insulina, intolerância à glicose, elevação da pressão sanguínea e dislipidemia (MONTEIRO; AZEVEDO, 2010).

Embora o tecido adiposo subcutâneo seja um importante componente associado ao risco metabólico, o tecido adiposo visceral desempenha um papel mais forte (CASTRO et al., 2014). O tecido adiposo visceral aparenta ser mais suscetível à lipólise do que o tecido adiposo subcutâneo, além de estar relacionado com a produção de fator de necrose tumoral-alfa (TNF-α), interleucina-6 (IL-6), proteína C reativa (PCR) e inibidor do fator ativador de plasminogênio-1 (PAI-1) (MONTEIRO; AZEVEDO, 2010).

O TNF-α é uma citocina que influencia a resposta inflamatória, favorecendo a resposta de fase aguda sistêmica pela liberação de IL-6 e reduzindo a liberação de substâncias antiinflamatórias, como a adiponectina. A IL-6 é uma citocina pró-inflamatória envolvida na transição da doença inflamatória aguda para doença inflamatória crônica (MARSEGLIA et al., 2014). A IL-6 é capaz de estimular a produção de PCR pelo fígado, um importante marcador inflamatório, que em níveis elevados, pode indicar risco cardiovascular (DING et al., 2015; HALCOX et al., 2014).

O aumento nos níveis de citocinas inflamatórias e PCR são responsáveis pelo estado pró-inflamatório observado na SM. Além disso, a SM está fortemente associada à inflamação sistêmica (ESFAHANI et al., 2015). O PAI-1 atua inibindo o sistema fibrinolítico e, consequentemente, níveis elevados de PAI-1 estão associados com o estado pró-trombótico, também característico da SM (GRUNDY et al., 2004; PAPAETIS; PAPAKYRIAKOU; PANAGIOTOU, 2015).

As adipocinas secretadas pelo tecido adiposo, como leptina, adiponectina, resistina e visfatina, influenciam diversos processos fisiológicos e estão envolvidas na patogênese da obesidade e da SM (KURAL et al., 2014). A adiponectina é reconhecida pela sua função protetora no desenvolvimento da SM. Apresenta propriedades antiinflamatórias, antiaterogênicas, antioxidantes e melhora a sensibilidade à insulina. Tem sido observado uma relação inversa entre adiponectina, a SM e os seus componentes (ESFAHANI et al., 2015; HATA et al., 2015).

1.2.2 Resistência à Insulina

A RI é uma condição caracterizada pelo comprometimento na ação da insulina. Ocorre diminuição na absorção da glicose pelos tecidos, como músculo esquelético, fígado e tecido adiposo, devido à redução na sensibilidade à insulina e o aumento na produção de glicose pelo fígado, levando à hiperglicemia (CASTRO et al., 2014; CHEN et al., 2015).

A obesidade abdominal e a RI parecem ser os principais fatores envolvidos na patogênese da SM (PETERSEN et al., 2007). A RI leva ao aumento nos níveis de triglicerídeos e glicose, aumento da pressão sanguínea e redução dos níveis de HDL colesterol (PALANIAPPAN et al., 2004).

A obesidade abdominal está associada ao desenvolvimento da RI e um dos mecanismos sugeridos é o aumento de ácidos graxos livres (AGL). O tecido adiposo visceral, responsável pelo armazenamento de energia principalmente na forma de triglicerídeos, apresenta capacidade de hipertrofia, entretanto, quando os adipócitos atingem o seu limite de expansão sofrem lipólise, resultando na liberação de AGL que são levados para outros tecidos, como o fígado e músculo. Devido à capacidade limitada desses tecidos em oxidar e/ou armazenar os AGL, o acúmulo ectópico de gordura pode levar a RI. No fígado, devido ao aumento de AGL levados

pela veia porta, a presença de RI causa o aumento na produção de glicose. Assim, o aumento nas concentrações de AGL nas células estimula a gliconeogênese (CASTRO et al., 2014; LE LAY et al., 2014; PAPAETIS; PAPAKYRIAKOU; PANAGIOTOU, 2015).

As citocinas inflamatórias TNF-α e IL-6 estão relacionadas com a redução na expressão do receptor da insulina, de seus substratos e do transportador de glicose (GLUT-4). A ação do TNF-α no desenvolvimento da RI ocorre nos adipócitos, hepatócitos e células do músculo esquelético. O TNF-α estimula a lipólise e reduz a oxidação de AGL nos hepatócitos e nas células musculares. A IL-6 apresenta ação nos adipócitos e nos hepatócitos. Os níveis de IL-6 também estão associados ao aumento nos níveis de AGL, entretanto, a IL-6 pode promover a absorção da glicose e a oxidação dos AGL nas células musculares. Aparentemente, a elevação crônica de IL-6 observada em estados de inflamação persistente pode aumentar a RI, enquanto a elevação aguda pode contribuir para a manutenção dos níveis normais de glicose (PAPAETIS; PAPAKYRIAKOU; PANAGIOTOU, 2015).

A leptina atua no hipotálamo regulando a saciedade e pode melhorar a sensibilidade à insulina nas células musculares, estimulando a oxidação de AGL. Entretanto, indivíduos obesos apresentam níveis circulantes elevados de leptina que caracterizam um estado de resistência e que parece ser responsável pelos efeitos da leptina na RI (CHEN et al., 2015; PAPAETIS; PAPAKYRIAKOU; PANAGIOTOU, 2015).

1.2.3 Dislipidemia

A SM apresenta um quadro de dislipidemia aterogênica, caracterizada pelo aumento nas concentrações de triglicerídeos, juntamente com o aumento no número de partículas de VLDL (*very-low density lipoprotein*), e/ou redução nos níveis de HDL colesterol. Também pode ser observado o aumento de partículas menores e densas de LDL (*low-density lipoprotein*), que aparentam ser mais aterogênicas do que as partículas maiores de LDL. Entretanto, a presença dessas partículas menores está relacionada com o aumento no número total de partículas de LDL e, consequentemente, quanto maior a fração de LDL, maior o potencial aterogênico (GRUNDY, 2004; HANSEL et al., 2004).

Enquanto as partículas de VLDL e LDL são consideradas aterogênicas, devido à presença de apolipoproteína-B (Apo-B), o HDL colesterol apresenta atividades antiaterogênicas, antiinflamatórias, antitrombóticas e antioxidantes. A principal função do HDL é o transporte reverso de colesterol, através da remoção do colesterol de tecidos periféricos que são levados para o fígado. Além disso, a Apo A-1, principal apolipoproteína presente nas partículas de HDL, é responsável por inibir a oxidação de LDL. O HDL também apresenta ação no endotélio vascular, estimulando a produção de óxido nítrico (NO) (HANSEL et al., 2004; MINEO et al., 2006).

Dentre as condições associadas à dislipidemia aterogênica está a obesidade central e a RI (MONTALI et al., 2015). A insulina possui ação de supressão da lipólise nos adipócitos, entretanto, na RI a sua ação se torna deficiente, aumentando a lipólise e a liberação de AGL. No fígado, os AGL são substrato para a síntese de triglicerídeos e aumentam a produção e a secreção de VLDL. As partículas de VLDL são as principais transportadoras de triglicerídeos na circulação, resultando no aumento dos níveis de triglicerídeos. Além disso, a VLDL é metabolizada em lipoproteínas remanescentes e em partículas menores e densas de LDL. Os níveis elevados de triglicerídeos reduzem as concentrações de HDL colesterol, devido à transferência dos triglicerídeos contidos nas partículas de VLDL para o HDL. Os triglicerídeos ricos em HDL são substratos para as lipases hepáticas, sendo eliminado da circulação e resultando em um menor número de partículas de HDL (GRUNDY, 2004; KAUR, 2014; TENENBAUM; KLEMPFNER; FISMAN, 2014).

1.2.4 Hipertensão

A hipertensão, caracterizada pela elevação da pressão sanguínea, é um dos principais componentes da SM (ABDILLA et al., 2007). As complicações da hipertensão incluem a doença cardíaca coronariana, acidente vascular cerebral, hipertrofia ventricular esquerda, insuficiência cardíaca e insuficiência renal crônica (GRUNDY, 2004).

Vários mecanismos estão envolvidos no desenvolvimento da hipertensão na SM. Os níveis de pressão sanguínea estão fortemente associados à adiposidade visceral e a RI. A presença de RI está relacionada com a ativação do

sistema nervoso simpático (SNS), que resulta em aumento na reabsorção de sódio pelos rins, aumento do débito cardíaco e vasoconstrição das artérias. No tecido adiposo são expressos angiotensinogênio, enzima conversora de angiotensina e receptor de angiotensina 1. Em indivíduos obesos, pode ocorrer o aumento na produção de angiotensinogênio e angiotensina II pelo tecido adiposo. Os adipócitos produzem aldosterona em resposta á angiotensina II, podendo ser considerado um pequeno sistema renina-angiotensina-aldosterona (KAUR, 2014; YANAI et al., 2008).

O aumento nos níveis de leptina e de AGL tem sido indicado como possíveis fatores responsáveis pelo aumento da ativação do SNS na SM. Os AGL parecem aumentar a pressão sanguínea, a frequência cardíaca e a vasorreatividade dos receptores α1-adrenérgicos, enquanto reduz a sensibilidade do barorreflexo, vasodilatação endotélio-dependente e a complacência vascular. A ação da leptina no hipotálamo aumenta a atividade simpática nos vasos sanguíneos periféricos e nos rins, resultando na elevação da pressão sanguínea (HEAD, 2015; KAUR, 2014; YANAI et al., 2008).

Os mediadores inflamatórios também têm sido relacionados ao desenvolvimento da hipertensão. O TNF-α estimula a produção de angiotensinogênio e endotelina-1 que se liga a receptores específicos na musculatura vascular e causa vasoconstrição. A IL-6 induz o aumento nos níveis plasmáticos de angiotensinogênio e angiotensina II (MAGUIRE; DAVENPORT, 2015; YANAI et al., 2008).

1.3 Estresse Oxidativo

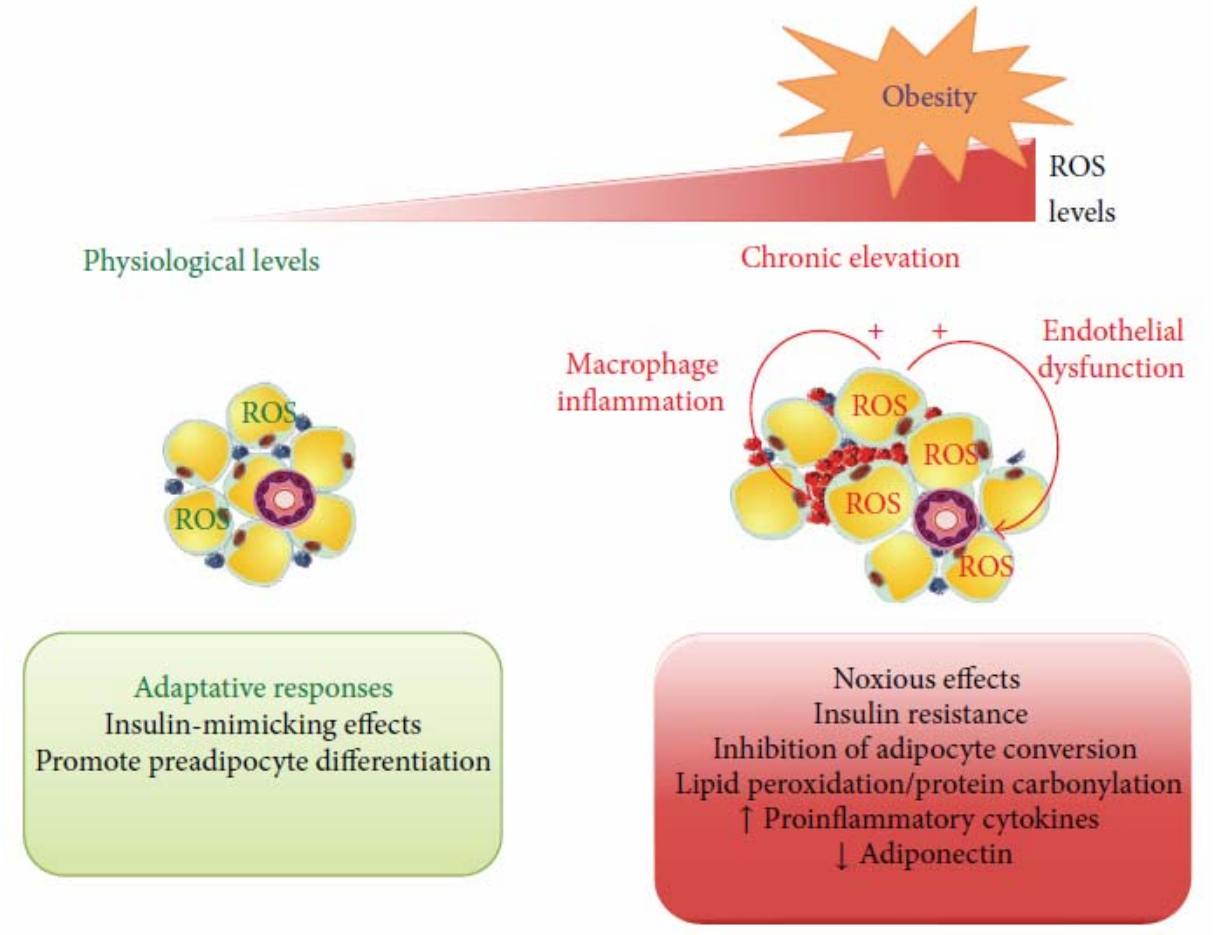
O estresse oxidativo resulta do desequilíbrio entre o aumento na produção de pró-oxidantes e a redução das defesas antioxidantes, podendo causar danos á macromoléculas celulares como lipídeos, proteínas e ácidos nucléicos. Os radicais livres e as espécies reativas de oxigênio (ERO) são as principais moléculas mediadoras da maioria das reações responsáveis pelo estresse oxidativo (SORG, 2004; TANGVARASITTICHAI, 2015).

As ERO são produtoras de radicais livres e podem ser neutralizadas por moléculas endógenas, como ácido úrico e compostos contendo o grupo tiol (-SH), por antioxidantes exógenos derivados da dieta, como o selênio, os

carotenoides e as vitaminas C e E, e por enzimas que catalisam as reações de redução das ERO, como a superóxido dismutase (SOD), catalase e glutationa peroxidase (GPx). Os danos induzidos pelo estresse oxidativo ocorrem quando as defesas antioxidantes não são capazes de neutralizar a produção de ERO (SIMÃO; LOVOZOY; DICHI, 2014; SORG, 2004).

Tem sido sugerido que o estresse oxidativo pode ser um dos mecanismos subjacentes envolvidos nas alterações metabólicas observadas em indivíduos com sobrepeso e obesidade (SIMÃO; LOVOZOY; DICHI, 2014). O aumento no estresse oxidativo pode ser considerado um importante fator envolvido na patogênese da SM. A produção elevada de pró-oxidantes resulta em inflamação, disfunção endotelial e RI (DEMIR et al., 2014).

Figura 1 – Respostas metabólicas adaptativas e efeitos nocivos induzidos pelos níveis de estresse oxidativo no tecido adiposo. ROS: reactive oxygen species. Fonte: Le Lay et al. (2014).



A obesidade está associada ao aumento nos níveis circulantes dos marcadores de estresse oxidativo, assim como a SM, que normalmente acompanha a obesidade (VAN GUILDER et al., 2006). O estresse oxidativo tem sido associado a todos os componentes da SM e ao surgimento das complicações cardiovasculares (BONOMINI; RODELLA; REZZANI, 2015). Tem sido demonstrado que o aumento nos parâmetros de estresse oxidativo em indivíduos com sobrepeso e obesos, parece estar relacionado com a presença dos componentes da SM (SIMÃO; LOVOZOY; DICHI, 2014).

Para a avaliação do estresse oxidativo normalmente são analisados marcadores de peroxidação lipídica, entretanto, as proteínas também são importantes alvos para a ação de oxidantes (CODOÑER-FRANCH et al., 2012). A AOPP (*Advanced Oxidation Protein Products*), formada durante o estresse oxidativo através da reação de proteínas plasmáticas com oxidantes clorados, é considerada um marcador confiável para avaliar o dano oxidativo de proteínas (PIWOWAR; KNAPIK-KORDECKA; WARWAS, 2007).

Várias hipóteses foram propostas para explicar os mecanismos que associam a obesidade e o estresse oxidativo. Um dos mecanismos propostos mostrou que o aumento no estresse oxidativo está associado ao aumento na expressão de NADPH oxidase no tecido adiposo, sugerindo que a NADPH oxidase induz a produção de ERO pelos adipócitos. Além disso, é observada a redução na expressão e na atividade de enzimas antioxidantes no tecido adiposo. Outras hipóteses incluem a oxidação de ácidos graxos que produz ERO e a secreção de adipocinas pelo tecido adiposo, que são capazes regular a atividade de enzimas que geram ERO e espécies reativas de nitrogênio (ERN) (SIMÃO; LOVOZOY; DICHI, 2014; TANGVARASITTICHAI, 2015).

O estresse oxidativo apresenta um importante papel na patogênese da RI, através da interrupção na sinalização da insulina e desregulação das adipocinas (TANGVARASITTICHAI, 2015). Embora o estresse oxidativo tenha sido proposto como uma ligação entre a obesidade e a RI, os mecanismos envolvidos não foram bem esclarecidos (SIMÃO; LOVOZOY; DICHI, 2014). Foi demonstrado que níveis elevados de ácidos graxos aumentam o estresse oxidativo através da ativação da via NADPH oxidase, causando a produção desregulada de adipocinas, como adiponectina, PAI-1, IL-6 e proteína quimiotática de monócitos-1 (MCP-1) (FURUKAWA et al., 2004). Por outro lado, foi demonstrado que a insulina pode

promover a formação de peróxido de hidrogênio (H_2O_2) nas células adiposas (OHMORI et al., 2005), o que torna atraente a hipótese de que o estresse oxidativo pode ser tanto causa como consequência da RI.

O aumento no estresse oxidativo modifica a fosforilação de proteínas sinalizadoras e reduz a expressão de substratos do receptor de insulina. O peróxido de hidrogênio (H_2O_2), por exemplo, apresenta efeitos nos adipócitos e nas células musculares. Citocinas inflamatórias, como o TNF-α, podem prejudicar a sinalização da insulina pelo mesmo mecanismo. O aumento no estresse oxidativo está associado à manutenção do estado pró-inflamatório, devido à ativação crônica de vias pró-inflamatória (BONOMINI; RODELLA; REZZANI, 2015; KEANE et al. 2015).

O estresse oxidativo tem sido relacionado à patogênese da aterosclerose. A ativação de NADPH oxidase nas células cardiovasculares resulta na produção de ERO, que estão envolvidas na adesão e migração de monócitos/macrófagos, proliferação de células lisas da musculatura vascular e fibroblastos e remodelação da matriz extracelular, levando à doença cardiovascular aterosclerótica (FUJITA et al., 2006). Além disso, a produção de ERO pela NADPH oxidase também parece estar envolvida na patogênese da hipertensão induzida pela angiotensina II e hipertrofia da musculatura vascular lisa (HAYASHI et al., 2008).

A disfunção endotelial é um processo observado na patogênese da aterosclerose e o estresse oxidativo tem sido considerado um dos principais mecanismos responsáveis. A disfunção endotelial pode ocorrer devido à redução na biodisponibilidade de NO, que depende do equilíbrio entre a sua produção e a reação com ERO (URAKAWA et al., 2003). O NO reage com radical ânion superóxido ($\bullet O_2^-$) e forma peroxinitrito ($ONOO^-$), um forte oxidante (SORG, 2004).

O NO é um potente vasodilatador, além disso, inibe a adesão de plaquetas e leucócitos e a migração e proliferação celular, que são eventos envolvidos na aterosclerose. A inibição desses eventos pelo NO protege a função cardiovascular. Devido o tempo de vida muito curto é difícil determinar os níveis de NO. Por esse motivo, para avaliar a produção de NO são medidos os produtos circulantes finais estáveis do NO, que são os nitritos e nitratos, denominados metabólitos do NO (ZAHEDI ASL; GHASEMI; AZIZI, 2008).

2 JUSTIFICATIVA

Em um estudo realizado anteriormente pelo nosso grupo, foi verificado um aumento no estresse oxidativo em indivíduos com sobrepeso com SM, mas não em indivíduos com sobrepeso sem SM (VENTURINI et al., 2012). Deste modo, o presente estudo apresenta como justificativa a intenção de ampliar os dados do estudo mencionado, avaliando concomitantemente o estresse oxidativo e nitrosativo em indivíduos com sobrepeso e obesos.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar o estresse oxidativo e nitrosativo em indivíduos com sobrepeso e obesos em comparação com indivíduos eutróficos.

3.2 OBJETIVOS ESPECÍFICOS

1) Avaliar se o aumento no IMC por si só é capaz de aumentar o estresse oxidativo e nitrosativo em indivíduos com sobrepeso e obesos;

2) Avaliar se o aumento no estresse oxidativo e nitrosativo em indivíduos com sobrepeso e obesos está relacionado com a presença da SM;

3) Avaliar se os componentes da SM podem influenciar individualmente o aumento no estresse oxidativo e nitrosativo em indivíduos com sobrepeso e obesos.

4 METODOLOGIA

4.1 ASPECTOS ÉTICOS

O estudo foi aprovado pelo Comitê de Ética e Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina (UEL). O termo de consentimento livre e esclarecido (TCLE) foi obtido de todos os indivíduos que participaram do estudo.

4.2 POPULAÇÃO DE ESTUDO

Foi realizado estudo transversal, onde os participantes foram selecionados entre funcionários e doadores de sague do Hospital Universitário (HU) de Londrina, Paraná, Brasil. No total, 330 indivíduos concordoram em participar do estudo. Os critérios de inclusão foram indivíduos de ambos os sexos, com idade de 18 a 65 anos. Os critérios de exclusão foram doença tireoidiana, renal, hepática, gastrointestinal, infecciosa ou oncológica e o uso de medicamentos hipolipemiantes, hipoglicemiantes, antiinflamatórios, terapia de reposição hormonal e suplementos antioxidantes. Por razões éticas os participantes que estavam tomando medicamentos anti-hipertensivos não foram excluídos do estudo e foi permitido continuar tomando a mesma dose dos medicamentos.

4.3 DELINEAMENTO DO ESTUDO

Os indivíduos foram divididos em três grupos: grupo controle (G1) que foi composto por 131 indivíduos com IMC entre 20 e 24,9 Kg/m^2; grupo com sobrepeso (G2) que foi composto por 120 indivíduos com IMC entre 25 e 29,9 Kg/m^2; e o grupo com obesidade (G3) que foi composto por 79 indivíduos com IMC ≥ 30 Kg/m^2.

A SM foi definida seguindo os critérios de classificação estabelecidos pelo *Adult Treatment Panel III* (ATP III). O diagnóstico da SM foi encontrado para indivíduos com pelo menos três das cinco seguintes características: (1) obesidade abdominal: circunferência da cintura ≥ 94 cm para homens e ≥ 80 cm para mulheres; (2) hipertrigliceridemia: triglicerídeos ≥ 150 mg/dL; (3) níveis baixos

de HDL colesterol: HDL < 40 mg/dL para homens e < 50 mg/dL para mulheres; (4) elevação da pressão sanguínea: pressão sanguínea ≥ 130/85 mm Hg ou uso de anti-hipertensivos; (5) glicemia em jejum elevada: glicose ≥ 100 mg/dL (GRUNDY et al., 2005).

Posteriormente os indivíduos foram redivididos de acordo com a presença ou não de cada um dos componentes da SM: indivíduos com triglicerídeos < 150 mg/dL (n=218) e triglicerídeos ≥ 150 mg/dL (n=108); indivíduos com níveis normais de HDL colesterol (n=186) e níveis reduzidos de HDL colesterol (n=137); indivíduos normotensos (n=217) e hipertensos (n=112); e indivíduos sem RI (n=163) e com RI (n=115).

4.4 Avaliação Antropométrica e da Pressão Sanguínea

O peso corporal foi medido com precisão de 0,1 Kg utilizando uma balança eletrônica, no período da manhã com os indivíduos vestindo roupas leves e sem sapatos. A altura foi medida com precisão de 0,1 cm utilizando um estadiômetro. O IMC foi calculado pelo peso (Kg) dividido pela altura (m) ao quadrado e o resultado foi expresso em Kg/m^2. A circunferência da cintura foi medida com os indivíduos em pé, na distância média entre a última costela e a crista ilíaca.

Foram realizadas três medidas de pressão arterial, com intervalo de 1 minuto entre cada medida, e os indivíduos permaneceram sentados durante a avaliação. Foi calculada a média das medidas de pressão arterial e o resultado foi utilizado nas análises. Os indivíduos foram considerados hipertensos quando a pressão sanguínea sistólica foi ≥ 130 mm Hg e/ou pressão sanguínea diastólica ≥ 85 mm Hg, ou se os indivíduos fizessem uso de medicamento anti-hipertensivo.

4.5 Avaliação dos Parâmetros Bioquímicos

As amostras de sangue foram coletadas após 12 horas de jejum para a avaliação dos parâmetros bioquímicos. A determinação dos níveis de colesterol total, LDL colesterol, HDL colesterol, triglicerídeos, glicemia e ácido úrico (AU), foram efetuadas em um auto-analisador bioquímico (Dimension Dade AR Dade Behring, Deerfield, IL, USA), utilizando kits Dade Behring®. Os níveis de insulina

foram determinados por quimiluminescência (QL) em imunoensaio com micropartículas (Architect, Abbott Laboratory, Abbott Park, IL, USA).

Foi calculado o índice de RI através do *Homeostasis Model Assessment of Insulin Resistance* (HOMA-IR), de acordo com a seguinte fórmula: HOMA-IR = insulina em jejum (U/mL) x glicose em jejum (mmol/L)/22.5 (HAFFNER; MIETTINEN; STERN, 1997). Foi considerado como RI quando HOMA-IR ≥ 2.5.

4.6 AVALIAÇÃO DO ESTRESSE OXIDATIVO

As amostras para avaliação do estresse oxidativo foram coletadas em tubos contendo EDTA (ácido etilenodiaminotetracético) como anticoagulante e antioxidante. Todas as amostras forma centrifugadas a 3.000 rpm por 15 minutos e o plasma foi aliquotado e armazenado em freezer a -70ºC até a realização dos testes.

4.6.1 Capacidade Antioxidante Total do Plasma

A capacidade antioxidante total do plasma foi determinada pela técnica do TRAP (*Total Radical-Trapping Antioxidant Parameter*), descrita por Repetto et al. (1996). Esta técnica quantifica antioxidantes hidro e lipossolúveis presentes no plasma por QL (REPETTO et al., 1996). Os valores de TRAP foram expressos em µM Trolox.

As medidas de TRAP em condições associadas com hiperuricemia, como a SM, podem ser imprecisas devido às concentrações de AU contabilizar cerca de 60% da capacidade antioxidante total do plasma. Alguns estudos têm verificado um aumento inesperado no TRAP em indivíduos com SM (SKALICKY et al, 2008;. SIMÃO et al., 2008). Por esse motivo, foi realizada uma correção do TRAP com base nas concentrações de AU (VENTURINI et al., 2012). Os resultados foram expressos em µM Trolox/mg/dL AU.

4.6.2 Quimiluminescência Iniciada por T-Butil Hidroperóxido (CL-LOOH)

A avaliação da formação de lipoperóxidos por QL foi efetuada em uma adaptação da técnica descrita por Gonzalez Flecha; Llesuy; Boveris (1991). Os resultados foram expressos em contagem por minuto (cpm).

4.6.3 Determinação dos Produtos Avançados de Oxidação Proteica (AOPP)

AOPP foi determinada no plasma, utilizando o método semi-automático descrito por Witko-Sarsat et al. (1996). As concentrações de AOPP foram expressas em mmol/L de equivalentes de cloramina-T.

4.6.4 Determinação de Metabólitos do Óxido Nítrico (NOX)

A concentração de NO da amostra foi estimada através da medição dos metabólitos do NO, nitritos (NO_2^-) e nitratos (NO_3^-), utilizando esferas de cádmio para a redução de nitrato a nitrito. As concentrações destes metabólitos foram depois determinadas de acordo com o método de Griess (NAVARRO-GONZÁLVEZ; GARCÍA-BENAYAS; ARENAS, 1998). Os valores foram expressos em µM.

4.7 Análise Estatística

Os dados foram expressos como mediana (25-75%). Os dados categóricos foram analisados por um teste qui-quadrado ou, quando apropriado, pelo teste exato de Fisher e os dados foram expressos em valor absoluto. As comparações entre os três grupos categorizados pelo IMC foram realizadas utilizando o teste não paramétrico de Kruskal-Wallis com o teste *pos-hoc* de Dunn. As variáveis que apresentaram significância na análise de variância univariada foram incluídas na regressão logística multinomial para verificar quais os parâmetros de estresse oxidativo foram associados com o IMC. Foi utilizado o teste de Mann-Whitney para comparar dois grupos e a análise de regressão logística binária foi realizada para ajustar os dados pela idade, sexo, etnia e IMC. Os resultados foram considerados significativos quando $p<0.05$. O programa de análise estatística SPSS versão 20.0 foi utilizado para as avaliações.

5 RESULTADOS E DISCUSSÃO

Os resultados obtidos foram descritos e discutidos no artigo *Oxidative and Nitrosative Stress in Overweight Subjects and Obesity*, submetido para a revista *Obesity*.

Oxidative and Nitrosative Stress in Overweight Subjects and Obesity

Running Title: Oxidative and Nitrosative Stress in Obesity

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Abstract

Objective: To evaluate the influence of body mass index on oxidative and nitrosative stress in overweight and obese subjects. **Methods**: Individuals were divided into three groups: control group (G1, n=131): BMI between 20 and 24,9 kg/m^2; overweight group (G2, n=120): BMI between 25 and 29,9 kg/m^2 and obese group (G3, n=79): BMI ≥ 30 kg/m^2. **Results:** G3 presented higher AOPP in relation to G1 and G2 (p=0.001 and p=0.011, respectively), whereas NO had lower levels in G2 and G3 compared to G1 (p=0.009 and p=0.048, respectively). Adjusted for the presence of MetS to evaluate its influence, AOPP did not differ between the groups, whereas significant lower NO maintained its significance. Data adjusted by BMI showed that subjects with higher triacylglycerol levels had higher AOPP (p=0.001) and decreased TRAP/UA (p=0.036). Subjects with lower HDL-cholesterol levels and patients with higher blood pressure showed increased AOPP (p=0.001 and p=0.034, respectively) and lower NO levels (p=0.017 and p=0.043, respectively). Subjects who presented insulin resistance had higher AOPP (p=0.024). **Conclusions**: Nitrosative stress was related to BMI and protein oxidation and nitrosative stress were related to metabolic changes and hypertension. MetS components had an essential participation on oxidative and nitrosative stress in overweight and obese subjects.

Keywords: overweight, obesity, metabolic syndrome, oxidative stress, nitrosative stress.

Introduction

Obesity is associated with an increased risk of developing several chronic diseases, such as type 2 diabetes mellitus and cardiovascular diseases (1). Changes in lifestyle and diet have resulted in an increased number of overweight and obese subjects in developed and in developing countries (2). This trend has been verified in practically all ages, genders and ethnicities (2). Therefore, overweight and obesity have emerged as one of the largest public health problem worldwide.

The harmful effects of free radicals mainly represented by reactive oxygen species (ROS) and/or nitrogen reactive nitrogen species (RNS) have been implicated in the physiopathology of overweight, obesity, hypertension, endothelial dysfunction, and metabolic syndrome (MetS) (3,4) suggesting that oxidative and nitrosative stress (O&NS) can be the underlying mechanism of this dysfunctional metabolic picture in obese subjects (5). In addition, high ROS/RNS production and the decrease in antioxidant capacity leads to various abnormalities, among which we find endothelial dysfunction that is characterized by a reduction in the bioavailability of vasodilators, particularly nitric oxide (NO), and an increase in endothelium-derived contractile factors, favoring atherosclerotic disease (6).

In a previous study, we verified an increase in oxidative stress in overweight subjects with MetS (7), but not in overweight subjects without MetS. In order to extend the data of the mentioned study, the objective of the present study was to evaluate the influence of the body mass index on O&NS in overweight and obese subjects and to verify whether the presence of the components of MetS would modify the results.

Patients and Methods

Subjects

Employees and blood donors of the University Hospital of Londrina, Paraná, Brazil were chosen to participate in this cross-sectional study. Three hundred and thirty subjects agreed to participate. Inclusion criteria were subjects of both genders, aged from 18 to 65 years. Exclusion criteria were thyroid, renal, hepatic, gastrointestinal, infectious or oncological diseases and use of lipid-lowering drugs, drugs for hyperglycemia, anti-inflammatory drugs, hormone replacement therapy,

and antioxidant supplements. For ethical reasons, subjects who were taking antihypertensive drugs were not excluded and were allowed to continue taking the same dose of the drugs.

The subjects were divided into three groups: the control group (G1) included 131 subjects with a body mass index (BMI) between 20 and 24,9 kg/m^2. The overweight group (G2) consisted of 120 subjects with a BMI between 25 and 29,9 kg/m^2 and the obese group (G3) consisted of 79 subjects with a BMI ≥ 30.

MetS was defined following the Adult Treatment Panel III (ATP III) criteria. A diagnosis of MetS was arrived at for subjects with at least three of the following five characteristics: (i) abdominal obesity: waist circumference ≥ 94 cm in men and ≥ 80 cm in women; (ii) hypertriglyceridemia: triglycerides ≥ 150 mg/dL; (iii) low levels of high-density lipoprotein (HDL) cholesterol: HDL < 40 mg/dL in men and < 50 mg/dL in women; (iv) high-blood pressure: blood pressure ≥ 130/85 mm Hg or use of antihypertensive drugs; and (v) high-fasting glucose: glucose ≥ 100 mg/dL (8).

Thereafter, the subjects were divided in accordance with the presence or absence of Mets and its components as follows: triglycerides ≤ 150 mg/dL (n=218) and ≥ 150 mg/dL (n=108); normal HDL-cholesterol levels (n=186) and reduced HDL-cholesterol levels (n=137); normal blood pressure (n=217) and high blood pressure (n=112); and without insulin resistance (IR) (n=163) and with IR (n=115).

The Ethical Committee of the University of Londrina, Paraná, Brazil approved all procedures involving human participants. Written informed consent was obtained from all the participants.

Anthropometric and blood pressure measurements

Anthropometric measurements and laboratorial parameters were assessed. Body weight was measured to the nearest 0.1 kg in the morning by using an electronic scale, with individuals wearing light clothing and no shoes; height was measured to the nearest 0.1 cm by using a stadiometer. BMI was calculated as weight (kg) divided by height (m) squared. Waist circumference (WC) was measured on standing subjects midway between the lowest rib and the iliac crest. Three blood pressure measurements taken with a 1-min interval between after the participant had been seated were recorded on the left arm. The mean of these measurements was used in the analysis. We considered the current use of antihypertensive medication as an indication of high-blood pressure.

Biochemical, immunological, and hematological biomarkers

After fasting for 12 hours, the subjects underwent the following laboratory blood analysis: total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triacylglicerols (TG), glucose and uric acid (UA), evaluated by a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA) using Dade Behring® kits. Plasma insulin level was determined by chemiluminescence microparticule immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a surrogate measurement of insulin sensitivity. HOMA-IR = fasting insulin (U/ml) x fasting glucose (mmol/L)/22.5 (9). IR was considered when HOMA-IR ≥ 2.5.

Oxidative stress measurements

Samples for evaluating oxidative stress and total antioxidant capacity were performed with EDTA as anticoagulant and antioxidant. All samples were centrifuged at 3,000 rpm for 15 minutes and plasma aliquots stored at -70ºC until assayed.

Tert-butyl hydroperoxide-initiated chemiluminescence (CL-LOOH)

Lipoperoxydes levels were determined by CL-LOOH, as described previously by Gonzalez Flecha et al. (10). CL-LOOH is considered much more sensitive and specific than the thiobarbituric acid reactive substances (TBARS) method (11), the usual method to determine lipid oxidation. The results were expressed in counts per minute (cpm).

Determination of advanced oxidation protein products (AOPP)

AOPP was determined in the plasma using the semi-automated method described by Witko-Sarsat et al. (12). AOPP concentrations were expressed as micromoles per liter (µmol/L) of chloramines-T equivalents.

Total radical-trapping antioxidant parameter (TRAP)

TRAP was determined as reported by Repetto et al. (13) This method detects hydrosoluble and/or liposoluble plasma antioxidants by measuring the chemiluminescence inhibition time induced by 2,2-azobis (2-amidinopropane). The system was calibrated with the vitamin E analog TROLOX, and the values of TRAP

were expressed in equivalent of μM Trolox/mg/dL UA. TRAP measurements in conditions associated with hyperuricemia, such as MetS, may be inaccurate because uric acid concentration accounts for 60% of total plasma antioxidant capacity. Some reports have verified an unexpected increase in TRAP in MetS subjects (14,15). Thus, a correction of TRAP based on uric acid concentration was performed (7).

Evaluation of nitric oxide metabolites (NOx)

Nitric oxide (NO) concentration in sample was estimated by measuring nitric oxide metabolites nitrites (NO_2^-) and nitrates (NO_3^-) using cadmium beads for the reduction of nitrate to nitrite. The concentrations of these metabolites were later determined according to the method proposed by Griess (16). The values were expressed in μM.

Statistical analysis

Data were expressed as median (25-75%). Categorical data were analyzed by a chi-squared test or when appropriate by Fisher's exact test and data were expressed in absolute value. The comparisons of the three groups categorized by BMI were performed using the non-parametric Kruskal-Wallis test with the *pos-hoc* Dunn test. The variables that presented significance in univariate analysis of variance were included in the multinomial logistic regression to verify which oxidative stress parameters were associated with BMI. Mann-Withney test was used to compare two groups and logistic binary regression analysis was performed to adjust for age, sex, ethnicity and BMI. The results were considered significant when $p<0.05$. A statistical analysis program SPSS version 20.0 was used for evaluations.

Results

There was no statistically significant difference in ethnicity between the three groups. Overweight (G2) and obese (G3) subjects did not differ regarding sex and age. However, the control group (G1) had a higher frequency in women compared to G2 ($p<0.0001$) and G3 ($p<0.05$) subjects and were younger ($p<0.001$) than G2 and G3 groups. The presence of MetS was higher in G3 compared to G1 ($p<0.0001$) and G2 ($p<0.0001$) and G2 compared to G1 ($p<0.0001$). G3 presented higher WC ($p<0.0001$, $p<0.0001$), glucose ($p<0.001$, $p<0.05$), insulin ($p <0.001$, $p <0.001$),

HOMA-IR (p <0.001, p <0.001), triacylglycerol (p <0.001, p<0.01), and decreased HDL–cholesterol (p<0.001, p<0.05) levels compared to G1 and G2, respectively (table 1). Meantime, G2 had higher WC (p<0.0001) and higher glucose (p<0.001), insulin (p<0.001), HOMA-IR (p<0.001), triacylglycerol (p<0.001), and decreased HDL–cholesterol (p<0.001) levels compared to G1. G2 and G3, respectively, showed higher total cholesterol (p<0.05, p<0.01) and LDL cholesterol (p<0.05, p<0.01) levels compared to G1 (table 1).

Table 2 shows the results of oxidative stress in the three studied groups with p values adjusted for sex and age. G3 presented higher AOPP values in relation to G1 and G2 (p=0.001 and p=0.011, respectively), whereas significant lower NO values were found in G2 and G3 when compared to G1 (p=0.009 and p=0.048, respectively). The groups were then adjusted for the presence of MetS to evaluate its influence on the results. In this new analysis, AOPP did not differ between the groups, whereas significant lower NO maintained its significance. Lipid hydroperoxides and TRAP/UA did not have any significant change in the groups.

To verify the association between oxidative stress biomarkers and the presence of MetS, a binary logistic regression was performed adjusted for sex and age. AOPP levels were directly (Wald=16.039, df=1, OR=1.009, 95% CI=1.005-1.009, p<0.0001) and NO values were inversely (Wald=18.941, df=1, OR=0.958, 95% CI=0.940-0.977, p<0.0001) associated to the presence of MetS (data not shown).

Association between oxidative stress parameters and individual components of MetS was measured and the values adjusted by BMI, sex, age and ethnicity and the results are shown in tables 3-6. Subjects with higher triacylglycerol levels had higher AOPP (p=0.001) and decreased TRAP/UA levels (p=0.036) compared to individuals without hypertriacylglycerolemia (table 3). Subjects with lower HDL-cholesterol and patients with higher blood pressure levels showed increased AOPP (p=0.001 and p=0.034, respectively) and lower NO levels (p=0.017 and p=0.043, respectively) compared to individuals without low HDL-cholesterol levels and normal blood pressure (tables 4 and 5). Subjects who presented IR had higher AOPP levels (p=0.024) compared to those without IR (table 6).

Table 1 - Clinical and laboratory characteristics of controls (G1), overweight (G2) and obese subjects (G3)

	G1 (n=131)	**G2 (n=120)**	**G3 (n=79)**	**G1 X G2**	**G1 X G3**	**G2 X G3**
Gender (F/M)	107/24	69/51	54/25	**<0.0001**	**<0.05**	0.1231
Ethnicity	105/26	94/26	65/14	0.8420	0.8525	0.6180
MetS (Y/N)	12/119	65/55	72/7	**<0.0001**	**<0.0001**	**<0.0001**
Age	32.0 (25.0-43.0)	43.0 (34.5-53.0)	43.0 (34.0-50.0)	**<0.001**	**<0.001**	NS
BMI (Kg/m^2)	22.04 (20.90-23.57)	27.12 (25.98-28.33)	32.25 (31.04-34.99)	**<0.001**	**<0.001**	**<0.001**
WC (cm)	82.0 (77.0-88.0)	97.0 (91.0-101.0)	108.0 (103.0-115.0)	**<0.0001**	**<0.0001**	**<0.0001**
Fating glucose (mg/dL)	86.0 (83.0-92.0)	92.0 (85.0-98.0)	98.0 (88.0-107.0)	**<0.001**	**<0.001**	**<0.05**
Insulin (U/mL)	6.4 (4.65-8.80)	8.10 (5.90-12.90)	14.5 (10.9-17.4)	**<0.001**	**<0.001**	**<0.001**
HOMA-IR	1.338 (1,020-1,940)	1.989 (1.285-3.238)	3.234 (2.696-4.781)	**<0.001**	**<0.001**	**<0.001**
Total cholesterol (mg/dL)	186.0 (152.5-209,0)	197.0 (168.0-226.0)	198.0 (180.0-224.0)	**<0.050**	**<0.010**	NS
HDL-cholesterol (mg/dL)	56.5 (48.5-67.0)	46.5 (38.0-59.5)	42.0 (37.0-51.0)	**<0.001**	**<0.001**	**<0.05**

LDL-cholesterol (mg/dL)	109.8 (83.8-130.2)	121.0 (95.2-141.1)	125.3 (100.5-142.0)	**<0.050**	**<0.010**	NS
Triacylglycerol (mg/dL)	74.5 (48.5-107.5)	124.0 (90.0-183.0)	175.0 (127.0-231.0)	**<0.001**	**<0.001**	**<0.01**

F, female; M, male; MetS, metabolic syndrome; BMI, body mass index; IR, insulin resistance; HOMA, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, nonsignificant; WC, waist circumference.

Table 2 - Oxidative stress evaluation in controls (G1), overweight (G2) and obese subjects (G3)

	G1 (n=131)	**G2 (n=120)**	**G3 (n=79)**	**G1 X G2***	**G1 X G3***	**G2 X G3***
Hydroperoxides (cpm)	13900 (10740-17010)	14120 (10950-17350)	13540 (10250-16260)	NS	NS	NS
AOPP (µmol/L)	127.2 (98.2-174.4)	159.5 (124.7-230.3)	195.2 (157.3.257.1)	NS	**0.001**	**0.011**
NO (µM)	25.67 (13.67-40.45)	13.83 (8.17-42.84)	12.10 (7.96-27.63)	**0.009**	**0.048**	NS
TRAP/UA (µM Trolox/mg/dL)	177.1 (147.2-207.5)	158.8 (126.9-190.0)	138.0 (115.3-164.9)	NS	NS	NS

AOPP, advanced oxidation protein products; NO, nitric oxide; TRAP, total radical-trapping antioxidant parameter; UA, uric acid; NS, nonsignificant. *adjusted p value for sex and age. [#]AOPP was not significant after adjusting for the presence of MetS, whereas NO maintained its significance.

Table 3 - Oxidative stress evaluation in subjects with and without hypertriacylglycerolemia

	Tryacylglycerols <150 mg/dL n=218	**Tryacylglycerols ≥ 150 mg/dL n=108**	**p**	***adjusted p**
Gender (F/M)	169/49	58/50	<0.001	----
Ethnicity	176/42	84/24	0.6321	----
Age	36.0 (28.0-47.0)	46.0 (37.0-53.0)	<0.0001	----
BMI (Kg/m^2)	24.42 (21.74-27.68)	29.75 (26.63-32.10)	<0.0001	----
Hydroperoxides (cpm)	14120 (10900-17730)	13570 (8549-18750)	NS	NS
AOPP (µmol/L)	134.2 (101.2-174.7)	225.1 (160.1-275-89)	<0.0001	**0.001**
NO (µM)	23.72 (12.29-42.84)	11.19 (6.60-27.63)	<0.0001	NS
TRAP/UA (µM Trolox/mg/dL)	170.7 (138.4-204.1)	137.8 (118.0-173.0)	<0.0001	**0.036**

F, female; M, male; BMI: body mass index; AOPP, advanced oxidation protein products; NO, nitric oxide; TRAP, total radical-trapping antioxidant parameter; NS, nonsignificant. *Binary logistic regression adjusted for sex, age, ethnicity and BMI.

Table 4 - Oxidative stress evaluation in subjects with normal and reduced HDL-cholesterol levels

	Normal HDL-cholesterol levels n=186	**Reduced HDL-cholesterol levels n=137**	**p**	***adjusted p**
Gender (F/M)	137/49	89/48	NS	----
Ethnicity	148/38	109/28	NS	----
Age	38.5 (30.0-47.0)	42.0 (30.5-50.0)	NS	----
BMI (Kg/m^2)	24.36 (21.91-28.04)	27.99 (25.44-31.60)	<0.0001	----
Hydroperoxides (cpm)	14290 (10800-18110)	13650 (11480-16720)	NS	----
AOPP (µmol/L)	136.8 (102.9-181.2)	183.8 (131.4-256.5)	<0.0001	**0.001**
NO (µM)	25.78 (12.38-43.93)	12.37 (7.53-29.91)	<0.0001	**0.017**
TRAP/UA (µM Trolox/mg/dL)	163.9 (138.0-205.1)	149.6 (122.4-186.3)	0.0011	NS

F, female; M, male; BMI, body mass index; AOPP,,advanced oxidation protein products; NO, nitric oxide; TRAP, total radical-trapping antioxidant parameter; NS, nonsignificant. *Binary logistic regression adjusted for sex, age, ethnicity and BMI.

Table 5 - Oxidative stress evaluation in normotensive and hypertensive subjects

	Normotensive n=217	**Hypertensive n=112**	**p**	***adjusted p**
Gender (F/M)	158/59	72/40	0.1414	----
Ethnicity	174/43	89/23	0.9926	----
Age (years)	35.0 (27.0-44.0)	47.0 (39.0-55.0)	<0.0001	----
BMI (Kg/m^2)	24.80 (21.83-28.04)	28.60 (26.17-31.70)	<0.0001	----
Hydroperoxides (cpm)	13750 (11130-17650)	14550 (10010-18190)	0.7971	NS
AOPP (µmol/L)	134.4 (100.4-183.8)	195.7 (154.3-274.2)	<0.0001	**0.034**
NO (µM)	22.84 (11.90-40.23)	11.95 (6.80-33.73)	0.0010	**0.043**
TRAP/UA (µM Trolox/mg/dL)	166.3 (137.8-200.9)	145.7 (119.5-184.8)	0.0010	NS

F, female; M, male; BMI, body mass index; AOPP, advanced oxidation protein products; NO, nitric oxide; TRAP, total radical-trapping antioxidant parameter; NS, nonsignificant. *Binary logistic regression adjusted for sex, age, ethnicity and BMI.

Table 6 - Oxidative stress evaluation in subjects with and without insulin resistance (IR)

	without IR n=163	**with IR n=115**	**p**	***adjusted p**
Gender (F/M)	151/12	74/41	0.0781	----
Ethnicity	125/38	90/25	NS	----
Age	38.0 (29.0-47.0)	42.0 (30.0-53.0)	0.0230	----
BMI (Kg/m^2)	24.22 (21.76-26.67)	30.05 (27.21-32.92)	<0.0001	----
Hydroperoxides (cpm)	14200 (11070-17890)	13550 (9291-16690)	NS	----
AOPP (µmol/L)	137.4 (104.0-184.9)	182.0 (127.9-256.5)	<0.0001	**0.024**
NO (µM)	23.50 (11.91-41.00)	11.78 (7.07-27.74)	<0.0001	NS
TRAP/UA (µM Trolox/mg/dL)	169.6 (138.0-203.2)	146.3 (116.3-179.6)	<0.0001	NS

F, female; M, male; BMI, body mass index; IR, insulin resistance; HOMA, homeostasis model assessment; AOPP, advanced oxidation protein products; NO: nitric oxide; TRAP: total radical-trapping antioxidant parameter; NS, nonsignificant *Binary logistic regression adjusted for sex, age, ethnicity and BMI.

Discussion

The redox state was similar in controls, overweight and obese subjects when controlled for the presence of MetS and therefore the principal finding of the present study was that oxidative stress evaluated by lipid and protein oxidation in obese patients is mainly related to the presence of MetS and less related to BMI. However, nitrosative stress with decreased NO bioavailability was associated with BMI, independently of the presence of MetS. In addition, this study verified that protein oxidation was associated with several individual components of MetS, including IR.

The present data are partially in agreement with our previous study, which showed in overweight subjects that increases in oxidative stress markers were only verified in the presence of MetS (7). However, in that study, differently from the present one, obese subjects and nitrosative stress were not evaluated.

Although oxidative stress increase in obese patients is an undisputed issue and can be caused by several factors (17), the present study is in line with others, which pointed out to the most importance of the presence of MetS to reinforce this association. Skalicky et al. (14) verified in obese subjects and Krzystek-Korpacka et al. (18) in overweight and obese adolescents that oxidative stress seemed to be increased by a combination of risk factors associated with MetS rather than by obesity per se. In addition, oxidative stress levels increase with the number of components of Mets (19,20). Taken together, these data suggest that, although weight gain or visceral fat may contribute, to some extent, to oxidative stress increase, the presence of MetS is fundamental to change redox status in overweight and obese subjects.

Our data are also in accordance with previous studies, which showed that hypertriacylglycerolemia, hypertension, lower HDL cholesterol values and IR are essential factors to provoke oxidative stress (15,19,21). Hypertriacylglycerolemia and hypertension lead to an increased production of superoxide anion (O_2^-) via nicotinamide adenosine diphosphate oxidase pathway (14). This anion reacts rapidly with NO to form peroxynitrite ($ONOO^-$), thus inactivating NO and leading to endothelial dysfunction, one of the mechanisms responsible for hypertension in these patients (22), whereas HDL cholesterol antioxidant activity, a major mechanism mediating its cardioprotective effect, is impaired (21). Of note, in the current study, hypertriacylglicerolemia showed the highest degree of redox imbalance as it was the

only MetS component, which concomitantly increased protein oxidation and decreased antioxidant capacity.

Several reports have established the importance of IR in the development of both diabetes and cardiovascular disease (23,24) but the precise role of oxidative stress as cause or consequence of IR is still debated. Furukawa et al. (25) demonstrated in cultured adipocytes that elevated levels of fatty acids increased oxidative stress via NADPH oxidase activation, and oxidative stress caused dysregulated production of adipocytokines, including adiponectin, plasminogen activator inhibitor–1, IL-6, and monocyte chemotactic protein–1. In addition, in obese mice, treatment with NADPH oxidase inhibitor reduced ROS production in adipose tissue, attenuated the dysregulation of adipocytokines, and improved diabetes, hyperlipidemia, and hepatic steatosis. NADPH oxidase inhibitors could improve insulin sensitivity via suppression of the effects induced by chronic exposure to ROS. These results suggested that increased oxidative stress in accumulated fat is an early instigator of MetS and that the redox state in adipose tissue is a potentially useful therapeutic target for obesity-associated MetS. In addition, hydrogen peroxide impairs insulin signaling and inhibits glucose transport, two cardinal features of IR (26). On the other hand, insulin itself promotes hydrogen peroxide formation in human fat cells (3). Altogether, it is tempting to speculate that oxidative stress can be both cause and consequence of IR (17,27).

It has been suggested that AOPP is an early marker of MetS (28) and the most appropriate parameter for determination of oxidative stress in MetS patients (28,29). AOPP is formed during oxidative stress by the action of chloraminated oxidants, mainly hypochlorous acid and chloramines, produced by myeloperoxidase in activated neutrophils (12). AOPP is structurally similar to advanced glycation end-products (AGEs) and exert similar biological activities as AGEs, i.e., induction of proinflammatory cytokines and adhesion molecules (12). The present study showing that AOPP was associated with metabolic changes and hypertension is in line with the importance of protein oxidation in patients with these components of MetS, independently of BMI, and confirm our previous finding that protein oxidation is more related to MetS parameters than lipid oxidation (20). Although some studies have reported an association between AOPP and BMI (15,20,30,31), the current study only presented this finding when the groups were not adjusted for the presence of MetS.

Both reduced or overproduction of NO may be a risk factor for development of cardiometabolic disease (32). Although endothelial dysfunction has been considered an important issue in obese patients, the results of studies on NOx levels have been contradictory. Whereas some reports have shown higher NO levels (33-35), others, similarly to the present study, have found the opposite results (36,37). NO is synthesized in endothelial cells by endothelial nitric oxide synthase eNOS activity, and it is responsible for vasodilatation and the maintenance of endothelial function; eNOS is expressed constitutively and synthesizes NO in only small amounts under basal conditions. In contrast, oxidative stress provokes iNOS expression even in low-grade inflammatory conditions, such as obesity and consequently increases NO, which would be consumed in peroxinitrite generation (38). This hypothesis is supported by some authors that demonstrated an increase in nitrotyrosine, a marker of endogenous peroxinitrite generation (39). Thus, the balance between eNOS and iNOS could explain NO increase or decrease in obese subjects. Although oxidative stress may induce NO production, NO decrease associated with BMI found in the present study is probably related to higher NO consumption by oxidative stress, reducing NO bioavailability. Other finding that can explain decreased NO levels in the present study is related to HDL cholesterol reduction as HDL cholesterol is involved in eNOS expression and activity (40).

Overweight is highly associated with arterial hypertension, independently from the occurrence of MetS, and a BMI of 25 kg/m^2 or greater accounted for approximately 34% and 62% of hypertension in men and in women, respectively (41). NO plays a major role in regulating blood pressure, and its deficient bioactivity is an important component of hypertension (42). Hypertensive subjects have increased generation of ROS, which scavenge NO, thereby reducing NO bioavailability (22). This study confirms the well-established relationship between NO decrease and hypertension, independently whether BMI is considered or not.

The following limitations have to be considered in the present study. First, the small number of participants. Second, the antihypertensive drugs the patients were taking, such as angiotensin-converting enzyme inhibitors, which may elevate plasma adiponectin levels, which in turn can increase NO levels (22). Nevertheless, the present study also has several strengths. First, to our knowledge, this is the first study to evaluate concomitantly O&NS in overweight and obese subjects. Second,

we adjusted the results of oxidative stress measurements for the presence of MetS to evaluate its influence on the results.

In conclusion, only nitrosative stress was related to BMI, whereas protein oxidation was related to each component of the MetS. In addition, both NO and AOPP were related to hypertension. In general, MetS components had an essential participation in overweight and obese subjects, whereas hypertriacylglycerolemia was the parameter, which showed the highest degree of redox imbalance. Whereas more studies are warranted to confirm the present data, this study reinforce the importance of analyzing concomitantly O&NS to obtain a more complete picture of overweight, obesity and associated conditions.

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6 CONCLUSÃO

Em conclusão, apenas o estresse nitrosativo foi relacionado ao IMC, enquanto a oxidação de proteínas foi relacionada a cada componente da SM. Além disso, tanto o NO como a AOPP foram relacionados com a hipertensão. De uma maneira geral, os componentes da SM tiveram uma participação fundamental em indivíduos com sobrepeso e obesos, sendo que a hipertrigliceridemia foi o parâmetro que mostrou o maior grau de desequilíbrio redox. Considerando que mais estudos são necessários para confirmar os dados atuais, o presente estudo reforça a importância de se analisar concomitantemente o estresse oxidativo e nitrosativo para obter um cenário mais completo do excesso de peso, obesidade e condições associadas.

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ANEXOS

ANEXO A

Obesity Author Guidelines

AUTHOR GUIDELINES
Everything you need to know about publishing your research in *Obesity*

AIMS AND SCOPE

The Obesity Society's official research journal, *Obesity*, was launched in 1993 as *Obesity Research*. Two decades later, *Obesity* has become the premier journal in the field. *Obesity* is published 12 times per year and is a forum where knowledge on the cutting edge of discovery can be disseminated to medical and health professionals and researchers whose expertise spans a wide swath of disciplines including diabetes, bariatric surgery, nutrition, public health, pediatrics, basic science, exercise, psychology, and genetics. Most published papers are quantitative; however high-quality qualitative studies will be considered.

Article Categories

Articles will be published under one of four categories. Your study may overlap categories, but select the type that best describes your manuscript.

- Obesity Biology and Integrated Physiology
- Clinical Trials and Investigations
- Pediatric Obesity
- Epidemiology/Genetics

Article Types

Manuscripts published by *Obesity* include:

- Original Articles
- Reviews and Invited Reviews
- Brief Cutting Edge Reports
- Editorials
- Perspectives
- Commentaries
- Letters to the Editor

Articles (in English) should be submitted via our electronic system at: http://mc.manuscriptcentral.com/obesity. Please review "How to Prepare Your Manuscript for Submission" (below) before uploading your documents.

For step-by-step guidance on how to submit your manuscript, please see the separate document **How to Submit Your Manuscript.** A checklist is also provided to help you with the submission process. These are available on our Wiley site.

EDITORIAL POLICIES

Manuscript submissions are considered on the following conditions:

- Your manuscript/its essential substance has not been published - except as an abstract - in any format or language and is not being simultaneously offered elsewhere for print or electronic publication.
- All coauthors have read and approved the content of the submission.
- All coauthors have declared all competing interests.
- All work complies with the Ethical Policies of *Obesity* and has been conducted under internationally accepted ethical standards after relevant ethical review. It is imperative that authors read this policy and complete any necessary documentation prior to your submission.

The journal operates a stringent peer-review process. All manuscripts will be reviewed by the Editors, members of the Editorial Board, or other expert reviewers. At the discretion of the Editors, the manuscript may be returned immediately without full review if deemed not competitive (not within the top 50% for originality and quality) or outside the realm of interests of the majority of the readership of the journal. Acceptance of papers is based on the originality of the observation or investigation; the quality of the work described; the clarity of presentation; and the relevance to our readership. All manuscripts are judged in relation to other submissions currently under consideration. The decision (reject, invite revision, accept) letter will be conveyed through the *Obesity* Manuscript Central (ScholarOne Manuscripts) system, coming directly from the Editor who has assumed responsibility for the manuscript's review. On average, manuscripts submitted in 2014 took less than 30 days to receive a decision.

This journal employs a plagiarism detection system. By submitting your manuscript, you accept that your manuscript may be screened for plagiarism against previously published works.

Most obesity-related topics are suitable for submission if they fall within the scope outlined above. Occasionally, authors inquire whether they should submit their manuscript. It is not possible for the Editorial Office to speculate on whether an article is likely to be accepted or not. The only way to know is to submit your manuscript, which can be done at no charge to you. The editors at *Obesity* are committed to providing a fast, but thorough, review of your submission.

Cost of Publication

There are no submission fees to have your manuscript considered for publication in *Obesity*. However, if accepted, you will incur page charges. Members of The Obesity Society (TOS) receive a discount. If you would like to join and receive reduced rates, go to http://www.obesity.org/membership/join.htm

Please be aware, however, that all manuscripts accepted for publication are chosen based on an objective peer-review process and merit alone and are not influenced by TOS membership status.

Publication Ethics

Obesity is a member of the Committee on Publication Ethics (COPE) and subscribes to its recommendations in the COPE guidelines on good publication practice. Best Practice Guidelines and more information about publication ethics are also available

from Wiley, our publisher. The editors reserve the right to reject a paper on ethical grounds. All authors are responsible for adhering to guidelines on good publication practice.

Conflicts of Interest

All authors are required to sign the ICMJE Conflict of Interest Form and provide a copy to the corresponding author before submission. The corresponding author must then compile a full Disclosure statement that accurately reflects what each author has disclosed and include this Disclosure statement on the Title Page of the Main Document. It is the corresponding author's responsibility to store the ICMJE forms and to provide them to the Editorial Office if requested at any time. Do not send these forms to the Editorial Office and do not upload them to Manuscript Central.

Avoiding Pejorative Language and Images

The Obesity Society's policy is that journal content must not use potentially pejorative adjectives or adverbs when describing individuals with overweight or obesity, as well as language that directly or indirectly attributes moral judgments or character flaws to this population. **Importantly, authors should not use "obese" as an adjective or noun to describe an individual person or group of people, but instead use terms such as "people with obesity" and "populations with obesity."** This also includes language and images that could be interpreted as stereotyping, biased, or prejudiced.

HOW TO PREPARE YOUR MANUSCRIPT FOR SUBMISSION

STEP 1: Decide what type of manuscript you wish to submit

(1) Original Article

Original Articles should focus on substantial novel research, findings, and developments from human or animal studies in all areas relevant to the science of obesity (including Clinical Trials). The following features are essential: hypothesis testing, suitable controls, appropriate statistical methods, clear reporting of results, and conclusions supported by the results.

(2) Review (submit a **Review Proposal first,** and then if approved by the Editors, submit a **Review)**

If you are submitting an unsolicited review, your Review Proposal - not the full Review - should *first* be submitted (cover letter, title page, and abstract only) to Manuscript Central. The Editor-in-Chief will determine whether the proposed subject falls within the guidelines and interests of the journal and that a similar Review is not currently in press or under preparation by another author. If your Review Proposal is approved by the Editor-in-Chief, you may then submit the full text of the Review article. Please note that approval of a Review Proposal is no guarantee of acceptance of the full Review article. The full manuscript will go through the same peer-review process as other articles. Conclusions based on personal viewpoints and suggestions for practical applications are welcome.

If your Review Proposal has already been accepted, use the same manuscript number, change the manuscript type to "Review," and resubmit, providing the full Review article.

(3) Brief Cutting Edge Report
Brief Cutting Edge Reports are short reports on "hot" research questions that present important novel results.
(4) Editorial *(only by invitation of Editor)*

(5) Perspective *(only by invitation of Editor)*
Perspectives can provide new ideas on an old problem or commentary/opinion on a hot topic.

(6) Commentary *(only by invitation of Editor)*
Commentaries should highlight the findings of a paper published in *Obesity* and provide insights about how to view the findings in a wider scientific and clinical context.

(7) Letter to the Editor
Letters to the Editor typically should address issues concerning recently published information in *Obesity*. A Letter to the Editor must reference the original source. A response to a Letter to the Editor must reference the Letter to the Editor in the first few paragraphs. Letters to the Editor can use an arbitrary title, but a response must cite the title of the letter: e.g., Response to [title of letter]. The publication of submitted Letters to the Editor is at the discretion of the Editors.

Each type of manuscript must remain within the limits below for word count and number of figures, tables, and references. Please edit your manuscript as needed prior to original submission and prior to the submission of each and every revision to fit all of the requirements.

Manuscript Type	Word Limit *(excluding cover page, abstract, references, tables, and figures)*	Max Number of References	Max Number of Combined Figures/Tables
Original Article	3500	40	8
Review	5000	80	8
Brief Cutting Edge Report	1500	20	3
Editorial	800 to 1600	5	1
Perspective	1000	10	2
Commentary	500	5	1
Letter to the Editor	500	5	1

STEP 2: Is this manuscript a FIRST SUBMISSION? Prepare a cover letter to the editors
OR
Are you submitting a REVISION? Prepare a letter of response to the reviewers

Your cover letter should include a short explanation of why your paper should be published in *Obesity*, as well as verification that your paper is original, unpublished research. You may also want to discuss what is novel about your study design and results.

The letter of response to the reviewers should provide clear details of the changes made and any responses to the reviewer or editor comments. This should be submitted with each revision.

Please be sure to include the full contact information for the corresponding author in both the cover letter and the response to reviewers.

STEP 3: Format your Main Document (Word)

File Name

Name your **FIRST DRAFT** documents as follows:
LastNameofFirstAuthor-KeywordsFromTitle-DocType

For example:
Brown-BMIandGhrelin-MainDocument.doc
Brown-BMIandGhrelin-Figure1.tiff
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If you are submitting a **REVISION**, provide 2 versions of your Main Document titled:
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Headings

If your manuscript is an **Original Article** or **Brief Cutting-Edge Report,** use the following headings:

Introduction
Methods
Results
Discussion
Conclusion and/or **Acknowledgments** (if needed)
References

Other section headings in your manuscript should be subheadings within one of these main sections.

If your manuscript is a **Review**, use the following headings:

Introduction
Text subdivided into titled sections
Discussion and/or Conclusion
Acknowledgments (if needed)
References

Acknowledgments

This section should be used to thank study participants, those who did not meet the authorship criteria but who provided some type of support, and others who may have some way helped with your study.

IMPORTANT: All disclosures, grant or funding information, and details of specific author contributions should be listed on your Title Page, NOT in this Acknowledgments section

References

References should be cited numerically in the order they appear in the text. Identify references in text, tables, and legends by Arabic numerals in parentheses or as superscripts; authors of unpublished work that has not yet been accepted for publication should be included in the text only (e.g., E Ravussin & DH Ryan, unpublished data).

Please give the names of all authors, unless there are more than 6 authors, in which case, please list only the first 6 authors, followed by *et al*. Journal titles should be abbreviated according to the style used by the National Library of Medicine (NLM).

Examples of journal references:

- Stunkard AJ, Allison KC, Geliebter A, Lundgren JD, Gluck ME, O'Reardon JP. Development of criteria for a diagnosis: lessons from the night eating syndrome. *Compr Psychiatry* 2009;50:391-399.
- Fukushima H, Cureoglu S, Schachern P, et al. Cochlear changes in patients with type 1 diabetes mellitus. *Otolaryngol Head Neck Surg* 2005;133:100-106.

Examples of book references:

- Lissner L, Bengtsson C, Lapidus L, Larson B, Bengtsson B, Brownell KD. Body weight variability and mortality in the Gothenburg Prospective Studies on men and women. In: Bjorntorp P, Rossner S (eds). *Obesity in Europe 88: Proceedings of the First European Congress on Obesity.* London: Libbey; 1989. pp. 55-60.
- Paul AA, Southgate DAT, eds. *McCance and Widdowson's The Composition of Foods.* 4th ed. HMSO: London; 1978.

Example of a web reference:

- Outbreak notice: Cholera in Haiti. Centers for Disease Control and Prevention Web site. Available from: http://wwwnc.cdc.gov/travel/notices/outbreak-notice/haiti-cholera.htm

General Formatting

- Submit all text in English
- Set margins that are 0.75 to 1 inch on all sides. Double-space your main text.
- Turn OFF automatic line numbering. Manuscript Central will create a PDF with automatic line numbers.
- Include page numbers as a "footer."
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- If abbreviations are used, they should be defined and written in full at the first mention in the text and in each table and figure. (Exception: Abbreviations can be used if they are a standard unit of measure.) For a list of standard abbreviations, please consult the Council of Biology Editors Style Guide (available from the Council of Science Editors, 9650 Rockville Pike, Bethesda, MD 20814) or similar sources.

- Drug Names: Generic names should be used. Brand names may be inserted in parentheses.
- Express scientific units in SI units.
- For more guidance: International Committee of Medical Journal Editors (ICMJE) Recommendations for Manuscript Preparation: http://www.icmje.org/recommendations/browse/manuscript-preparation/

STEP 4: Create a Title Page

Your Title Page should be included at the beginning of the Main Document file and should contain:

- **TITLE:** The title of the article (no more than 125 characters, including spaces between words).
- **AUTHORS:** The name of each author (first and last names).
- **AFFILIATION:** The name of the department(s) and institution(s) to which the authors belong, with city, state, and country (full address not necessary).
- **KEYWORDS:** Three to six keywords.
- **RUNNING TITLE:** A condensed version of your main title to be used on follow-up pages of the published article (no more than 50 characters including spaces).
- **CONTACT INFO:** The full mailing address and e-mail address of the corresponding author.
- **WORD COUNT:** Include and update the word count for each draft of your manuscript. This count should include only your main text: Introduction, Methods, Results, Discussion/Conclusion, and/or Acknowledgments. It should NOT include the Title Page (including funding and disclosures information), study importance questions, abstract, references, or text from figures or tables. **IMPORTANT:** If your manuscript exceeds the word count quota for your type of manuscript, condense to meet the limit before submitting. Manuscripts will be returned to be shortened, even if they have been accepted, if deemed too be over the quota. This may require a re-review and can delay time to publication.
- **FUNDING:** Financial and material support must always be acknowledged, with a clear statement defining all funding sources. This should include grants, equipment, drugs and other reagents, or gifts of materials. List all grant and funding information (if any) on the Title Page (not in the Acknowledgments section).
- **DISCLOSURE:** List all potential conflicts of interest (if any) on the Title Page (not in the Acknowledgments section). For guidance, please see the Ethical Policies of *Obesity.*
- **AUTHOR CONTRIBUTIONS:** If you wish to specify the contribution of each author to the manuscript (e.g., study design, data collection, data analysis, data interpretation, literature search, generation of figures, writing of the manuscript), please list these on the Title Page (not in the Acknowledgements section). For example:

  > XY and NM conceived and carried out the experiments. AB and GH conceived the experiments and analyzed data. OP carried out experiments. All authors were involved in writing the paper and had final approval of the submitted and published versions.

STEP 5: Answer the Study Importance Questions

For **Original Articles, Brief Cutting Edge Reports, and Review Proposals/Reviews,** following the Title Page, provide no more than 3 short bullet-point answers to these two study importance questions:

- What is already known about this subject? (or for **Review Proposals/Reviews,** what major reviews have already been published on this subject?)
- What does your study add?

STEP 6: Create an Abstract

Create a structured abstract with these headings: **Objective – Methods – Results – Conclusions**

- Abstracts should be 200 words or less.
- **Perspectives** may include a shorter structured *or* unstructured abstract (150 words or less) at the author's discretion (optional).
- **Editorials, Commentaries, and Letters to the Editor** do not include an abstract.
- In all cases, there should be no text before the Objective heading.

STEP 7: Prepare Tables and Figures

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