GISELE CHIBINSKI PARABOCZ

PESQUISA DE MIELOPEROXIDASE E OUTROS MARCADORES LABORATORIAIS NOS DIFERENTES GRAUS DE OBESIDADE

Dissertação apresentada para obtenção do titulo de mestre na Universidade Estadual de Ponta Grossa, Área de Ciências Farmacêuticas.

Orientador: Prof. Dr. José Carlos Rebuglio Vellosa

Co-orientador: Prof. Dr. Najeh Maissar Khalil

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ATA DE DEFESA DE DISSERTAÇÃO – NÍVEL DE MESTRADO, DA PÓS-GRADUANDA **GISELE CHIBINSKI PARABOCZ**, DO PROGRAMA DE PÓS-GRADUAÇÃO *STRICTO SENSU* EM CIÊNCIAS FARMACÊUTICAS, REALIZADA NO DIA 18 DE FEVEREIRO DE 2013, NA UNIVERSIDADE ESTADUAL DE PONTA GROSSA.

Ao décimo oitavo dia do mês de fevereiro de dois mil e treze, às 9 h, no Auditório de Odontologia da Universidade Estadual de Ponta Grossa, sob a presidência do Prof. Dr. José Carlos Rebuglio Vellosa, em sessão fechada, reuniu-se a banca examinadora para a defesa de dissertação da pós-graduanda Gisele Chibinski Parabocz. Área de concentração: Fármacos, medicamentos e biociências aplicadas à Farmácia. Linha de Pesquisa: Avaliação clínico/laboratorial de processos fisiopatológicos e Avaliação química e biológica de produtos naturais. A banca foi constituída pelos pesquisadores: Prof. Dr. José Carlos Rebuglio Vellosa (Presidente) / UEPG, Prof. Dr. Paulo César Ghedini / IFG e Prof. Dr. Giovani Marino Fávero / UEPG, indicados pelo egrégio colegiado do Programa de Pós-graduação em Ciências Farmacêuticas – nível de Mestrado Acadêmico, desta Universidade, em associação ampla com a Universidade Estadual do Centro-Oeste. Iniciados os trabalhos, a presidência deu conhecimento aos membros da banca e à candidata das normas que regem a defesa de dissertação e definiu-se a ordem a ser seguida pelos examinadores para a arguição. O trabalho analisado foi **"Pesquisa de mieloperoxidase e outros marcadores laboratoriais nos diferentes graus de obesidade".** Encerrada a defesa de dissertação, procedeu-se o julgamento, tendo sido a candidata APROVADA. Foi dada ciência à mestranda que, de acordo com o Art. 34 do Regulamento do Programa de Pós-graduação em Ciências Farmacêuticas - UNICENTRO/UEPG, *para a obtenção do título de Mestre em Ciências Farmacêuticas, área de concentração Fármacos, Medicamentos e Biociências aplicadas à Farmácia, o aluno tem o prazo de 30 (trinta) dias após esta defesa para entregar as cópias da versão definitiva, que devem ser aprovadas pelo orientador e homologadas pelo Colegiado de curso.* Nada mais havendo a tratar, lavrou-se à presente ata que vai assinada pelos membros da banca examinadora e por mim, Rubyan Lucas Santos Piazzetta, _____, secretário do Programa de Pós-graduação em Ciências Farmacêuticas pela UEPG. Ponta Grossa, 18 de fevereiro de 2013.

Observação (se necessária):

_____

_____

Prof. Dr. José Carlos Rebuglio Vellosa

Prof. Dr. Paulo César Ghedini

Prof. Dr. Giovani Marino Fávero

Este trabalho foi realizado no Laboratório de Análises Moleculares e Bioquímicas e no Laboratório Universitário de Análises Clínicas, ambos do Departamento de Análises Clínicas e Toxicológicas da Universidade Estadual de Ponta Grossa (UEPG) com apoio Financeiro da Fundação Araucária de Apoio ao desenvolvimento Científico e Tecnológico do Paraná por meio de auxílio à pesquisa (Editais 08/2009–PPSUS e 13/2009-PPP, convênios 180/2010 e 230/2010).

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Descobri como é bom chegar quando se tem paciência. E para se chegar, onde quer que seja, aprendi que não é preciso dominar a força, mas a razão. É preciso, antes de mais nada, querer.

(Amyr Klink)

RESUMO

A obesidade tornou-se a epidemia do século XXI, estimando-se a existência de 1,4 bilhão de adultos com excesso de peso. A inflamação crônica subclínica existente na obesidade é associada com o aumento do risco de doenças cardiovasculares. O tecido adiposo, além de sua função energética, possui papel endócrino por meio da secreção das adipocinas. Na obesidade, os adipócitos tornam-se disfuncionais, produzindo, em excesso, adipocinas pró-inflamatórias, em detrimento das anti-inflamatórias. O desencadeamento do processo inflamatório é a ligação entre a obesidade e a aterogênese. Desta forma, marcadores de inflamação são alvos em potencial para a prevenção, diagnóstico e tratamento da aterosclerose. A mieloperoxidase (MPO) é uma enzima presente principalmente em neutrófilos, participante dos mecanismos de defesa inata do organismo, através da produção de espécies oxidantes que possuem atividade antimicrobiana. Contudo, os oxidantes originados e a própria MPO participam de eventos que apresentam papel fundamental na aterogênese, tais como oxidação do LDL-colesterol, consumo de óxido nítrico e ativação de proteases. Destarte, a MPO tornou-se objeto de pesquisa e muitos estudos demonstram seu poder preditor de risco cardíaco em determinadas situações. Não obstante, seu uso ainda não está padronizado e os valores referenciais não estão bem estabelecidos para o diagnóstico laboratorial. O objetivo deste trabalho foi avaliar os níveis de MPO e outros biomarcadores em indivíduos com diferentes faixas de Índice de Massa Corporal (IMC). Os resultados obtidos indicaram diferenças significativas entre os grupos nos seguintes parâmetros: pressão arterial sistólica e diastólica, HDL colesterol, taxa triglicerídeos/HDL, taxa HDL/LDL e contagem de leucócitos. Níveis elevados de PCR-us foram observados nos grupos com indivíduos obesos, porém não se constataram alterações significativas para a MPO. Portanto, os níveis séricos de MPO não forneceram informações laboratoriais clinicamente significativas na diferenciação dos grupos estudados.

Palavras-chave: Risco cardíaco. Inflamação. Obesidade. Mieloperoxidase.

ABSTRACT

Obesity has become epidemic in XXI century and it is estimated that there are about 1.4 billion overweight adults. The existing subclinical chronic inflammation in obesity is associated with increased risk of cardiovascular disease. Adipose tissue, in addition to its energy function, has endocrine role through the secretion of adipokines. In obesity, adipocytes become dysfunctional, producing excess proinflammatory adipokines at the expense of anti-inflammatory. This inflammatory process is the link between obesity and atherogenesis. Thus, inflammation markers are potential targets for prevention, diagnosis and treatment of atherosclerosis. Myeloperoxidase (MPO), an enzyme found primarily on neutrophils, participates on innate defense mechanisms of the body through the production of oxidizing species that have antimicrobial activity. However, these oxidants and MPO act in events that feature key role in atherogenesis, such as oxidation of LDL-cholesterol, consumption of nitric oxide and activation of proteases. Therefore, the MPO has become subject of research and many studies have shown its value as predictor of cardiac risk in certain situations. However, its usage is not yet standardized and its benchmark values are not well established for laboratory diagnosis. The aim of this study was to evaluate the levels of MPO and other biomarkers in different Body Mass Index (BMI) ranges. The results indicated significant differences between the groups in the following parameters: systolic and diastolic blood pressure, HDL-cholesterol, triglycerides/HDL rate, HDL/LDL rate and white blood cells count. Elevated levels of hs-CRP were observed in the groups with obese individuals, but no significant changes were observed to serum MPO. Therefore, serum levels of MPO did not provide information on clinically significant laboratory differentiation of groups.

Key words: Cardiac risk. Inflammation. Obesity. Myeloperoxidase.

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CAPÍTULO 1

1.1 REVISÃO BIBLIOGRÁFICA

A obesidade pode ser definida como uma doença crônica caracterizada pelo acúmulo excessivo de tecido adiposo no organismo (COUTINHO, 1999). Em termos de etiologia, a obesidade é classificada como primária ou secundária. A iatrogênica ou secundária ocorre em decorrência de tratamentos medicamentosos (antipsicóticos, antidepressivos, antiepiléticos, esteroides, insulina) ou de certas doenças (Síndrome de Cushing, hipotireoidismo, defeitos hipotalâmicos; TIMAR, 1999). A obesidade primária é uma desordem que sobrevém a partir de um balanço energético positivo e compreende a maioria dos casos (ARONNE, 2002).

A obesidade, essencialmente a primária, tornou-se a epidemia do século XXI, estimando-se a existência de 1,4 bilhão de adultos com excesso de peso, dos quais 200 milhões de homens e 300 milhões de mulheres são obesos. O sobrepeso e a obesidade são considerados o quinto maior fator de risco para mortes, resultando em pelo menos 2,8 milhões de mortes de adultos a cada ano (WHO, 2012).

Acompanhando a tendência mundial, o peso dos brasileiros vem aumentando nos últimos anos. A Pesquisa de Orçamentos Familiares (POF) 2008-2009, realizada em parceria entre o IBGE e o Ministério da Saúde, mostrou que a obesidade e o excesso de peso têm aumentado rapidamente nos últimos anos, em todas as faixas etárias. Neste levantamento, 50% dos homens e 48% das mulheres se encontram com excesso de peso, sendo que 12,5% dos homens e 16,9% das mulheres apresentam obesidade. Já o déficit de peso segue no declínio, regredindo de 8% em 1974-75 para 1,8% entre os homens e de 11,8% para 3,6% entre as mulheres, em todos os estratos de renda, retratando o controle nos índices de desnutrição da população adulta brasileira e o surgimento de um novo problema, o excesso de peso (IBGE, 2010).

A classificação mais utilizada para a obesidade é baseada no Índice de Massa Corporal (IMC), obtido pela divisão entre o peso, aferido em quilogramas, e o quadrado da altura, aferida em metros (WHO, 2000). Segundo a Organização Mundial da Saúde, sobrepeso é definido como IMC entre 25 e 29,9 Kg/m^2, e obesidade como IMC igual ou acima de 30 Kg/m^2. A obesidade é classificada como grau I para IMC entre 30 e 34,9 Kg/m^2, associado com risco moderado, grau II para IMC entre 35 e 39,9 Kg/m^2, com alto risco, e grau III com IMC de 40 Kg/m^2 ou acima deste valor, associado com alto risco de mortalidade. Porém, há evidências de que o risco de doenças crônicas aumenta progressivamente a partir do IMC de 21 Kg/m^2 (WHO, 1995).

A obesidade está associada com o aumento da mortalidade, doenças cardiovasculares, diabetes e cânceres (OLUSI, 2002). Além disso, a associação da obesidade à hipertensão e outros fatores de risco cardiovasculares, tais como resistência à insulina, intolerância à glicose e dislipidemia, é forte e freqüente. Esse agrupamento de fatores de risco, caracterizado como síndrome metabólica, tem alta prevalência na população de adultos e tem sido detectado também em crianças e adolescentes (LOPES, 2007).

O tecido adiposo é o principal reservatório energético nos mamíferos. Os triglicerídeos armazenados possibilitam um fornecimento seguro de energia através de seu considerável valor energético e armazenamento com pouca água associada (PEREIRA et al., 2006). Além das funções termogênica e energética, o tecido adiposo passou a acumular mais um papel, o de sinalizador, tendo sido reconhecido o seu desempenho fundamental no controle metabólico, fisiológico e endócrino dos órgãos (TRAYHURN, 2005). Os adipócitos secretam várias citocinas e proteínas de fase aguda, conhecidas em seu conjunto como adipocinas, que, direta ou indiretamente, elevam a produção e a circulação de fatores relacionados com a inflamação (BULLO et al., 2003). Adiponectina e leptina são as adipocinas mais abundantes sintetizadas pelo tecido adiposo, embora existam várias, como o fator de necrose tumoral-α (TNF-α), interleucina-6 (IL-6) e interleucina-1 (IL-1), entre outras (WEISBERG et al., 2003).

Na obesidade, o tecido adiposo torna-se disfuncional, resultando na superprodução de adipocinas pró-inflamatórias em detrimento das anti-inflamatórias. O acúmulo patológico deste tecido disfuncional caracteriza a obesidade como um fator de grande risco para muitas outras doenças, incluindo diabetes tipo 2, doenças cardiovasculares e hipertensão (MAENHAUT; VAN DE VOORD 2011).

Destarte, sabe-se que na obesidade há a presença de uma inflamação crônica moderada (subclínica), representada pelo aumento de várias citocinas e proteínas de fase aguda (PEREIRA et al., 2006). Segundo Volp et al. (2008), o processo inflamatório é o elo entre a síndrome metabólica e a aterogênese. Tal estado de inflamação originado por uma dieta pró-inflamatória e sedentarismo, entre outros fatores, e mediado, em parte, pelo estresse oxidativo e por citocinas pró-inflamatórias, está coligado ao desenvolvimento de doenças cardiovasculares (DUNCAN et al., 2005).

Por conseguinte, pode-se supor que algumas biomoléculas importantes em processos inflamatórios podem encontrar-se alteradas nestas situações, tornando seus marcadores alvos potenciais para a prevenção, diagnóstico e tratamento da aterosclerose.

A mieloperoxidase (MPO) é uma hemeproteína liberada por neutrófilos, monócitos e alguns subtipos de macrófagos teciduais, pela ativação do mecanismo de defesa inata (ARATANI et al., 2004). Por meio da reação com peróxido de hidrogênio, produz radicais livres e substâncias oxidantes difusíveis com atividade antimicrobiana. Porém, além da função de defesa contra patógenos invasores, os mesmos oxidantes derivados das reações enzimáticas da MPO causam disfunção endotelial e tem importante impacto no desenvolvimento de aterosclerose e síndromes coronarianas agudas (BRENNAN; HAZEN, 2003).

A MPO funciona como catalisadora da oxidação lipídica, tendo a habilidade de modificar oxidativamente o aminoácido tirosina da apolipoproteína B-100 (apo B-100), presente nas partículas de LDL. A modificação oxidativa do LDL leva ao aumento de sua recaptação e degradação por macrófagos, resultando na formação das células espumosas das estrias gordurosas, indicador característico da aterosclerose (PODREZ et al., 1999). A MPO também é capaz de limitar a biodisponibilidade do óxido nítrico por meio de seu consumo catalítico, além da inibição da atividade da óxido nítrico sintetase e redução de seus co-fatores, como NADPH, por meio dos oxidantes originados (BALDUS et al., 2001). Outra propriedade da MPO é sua ação moduladora sobre a cascata de proteases, participando das complicações agudas da aterosclerose, estímulo à trombogenicidade e vulnerabilidade da placa (HAZEN, 2004).

Portanto, a MPO apresenta papel fundamental em várias etapas da aterogênese e pode ser um marcador de risco precoce para doenças cardíacas. Brennan e Hazen (2003) demonstraram que uma única medida plasmática de MPO pode predizer independentemente o risco precoce de infarto do miocárdio, assim como o risco de eventos cardíacos adversos, mesmo na ausência de necrose do miocárdio. Há estudos demonstrando que níveis elevados de MPO predizem o risco de doença coronária arterial em grupos considerados de baixo risco (MEUWESE et al., 2007), anunciam de maneira precoce o risco de futuros eventos cardiovasculares em pacientes com síndromes coronárias agudas (BALDUS et al., 2003) e aumentam a predição de risco cardiovascular de forma independente ou associada com outros marcadores, como a proteína C reativa ultrassensível (PCR-us) (HESLOP et al., 2010).

A MPO também pode ser considerada um marcador de estresse oxidativo. Além da geração de vários radicais livres e oxidantes, a atividade da MPO também conduz à gênese de múltiplos produtos de oxidação de ácido araquidônico e linoléico (CHISOLM; STEINBERG, 2000).

Ainda não é consistente o valor preditivo adicional dos níveis de MPO na estratificação de risco cardiovascular para incorporá-la à prática clínica como sinalizadora de

vulnerabilidade de placa. Os valores de referência para sua aplicação laboratorial no auxílio ao diagnóstico e avaliação de risco cardíaco pelo clínico ainda não estão bem estabelecidos. Estudos adicionais são necessários para confirmar seu papel nas diferentes formas de apresentação da cardiopatia isquêmica, além da padronização do ensaio, ponto fundamental para a transição desse marcador do ambiente de pesquisa para uso na rotina clínica.

Sendo assim, trabalhos que visem definir o uso da mieloperoxidase em condições de saúde e doença são muito importantes, especificamente em pacientes obesos, os quais podem não apresentar manifestação sintomática e encontrar-se em estado subclínico de alterações patológicas.

1.2 OBJETIVOS

1.2.1 GERAL

Este trabalho tem por objetivo, determinar os níveis de mieloperoxidase em diferentes graus de obesidade, correlacionando a outros fatores metabólicos e parâmetros laboratoriais no acompanhamento médico.

1.2.2 ESPECÍFICOS

1.2.2.1 Definir se a mieloperoxidase encontra-se elevada em indivíduos obesos;

1.2.2.2 Correlacionar os níveis séricos de MPO aos de outros marcadores inflamatórios;

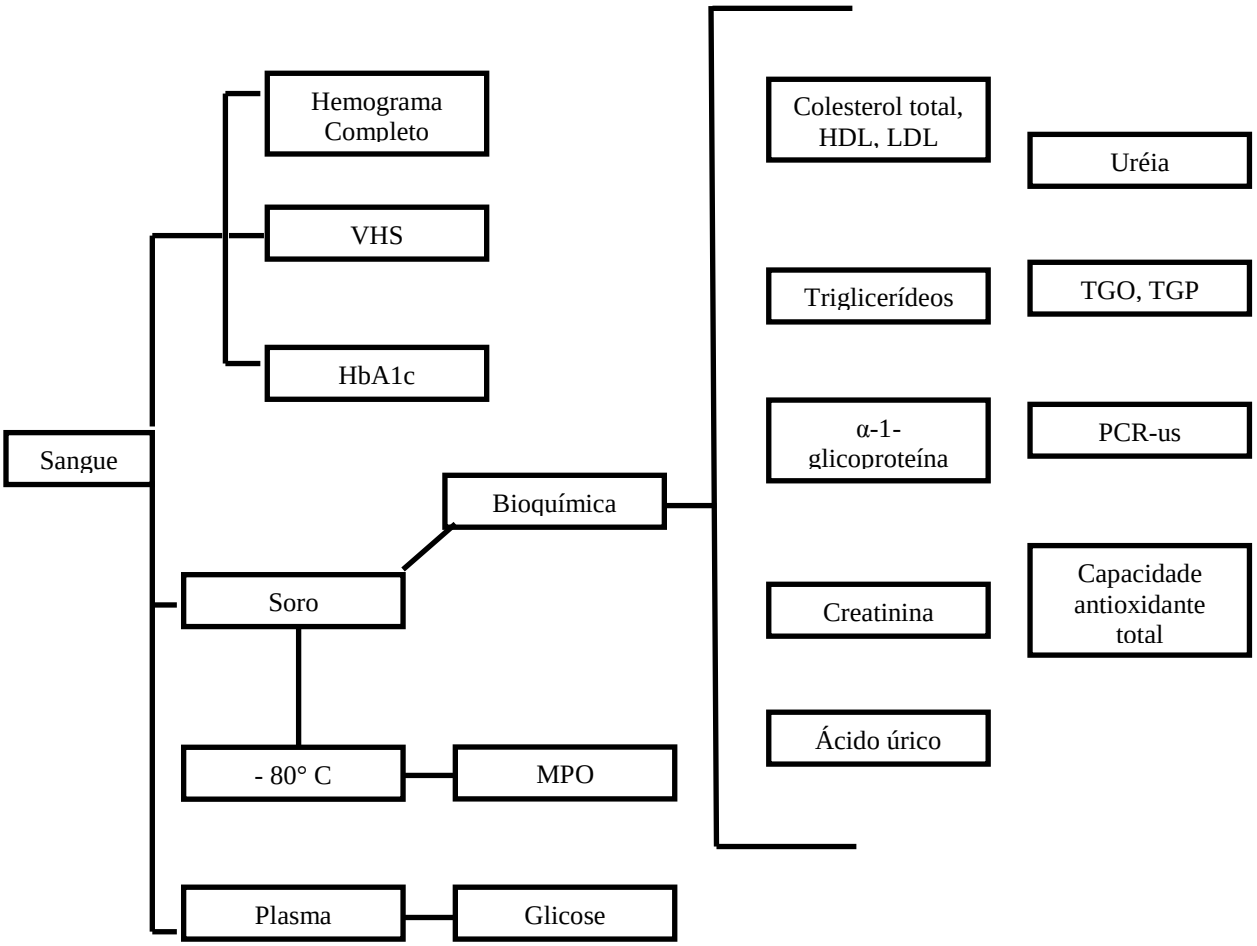
1.2.2.3 Mensurar a capacidade antioxidante total no soro destes pacientes.

1.3 MATERIAIS E MÉTODOS

O protocolo do estudo (nº 12476/09) foi aprovado na Comissão de Ética em Pesquisa da Universidade Estadual de Ponta Grossa, conforme parecer 74/2009. Foram coletadas amostras de voluntários, obtidas no Laboratório Universitário de Análises Clínicas (LUAC). Os pacientes foram esclarecidos sobre a pesquisa e responderam a uma entrevista sobre sua condição clínica e assinaram o termo de consentimento, de acordo com o Comitê de Ética.

Os materiais e métodos utilizados nesta pesquisa, bem como os resultados obtidos, são descritos no artigo originado a partir deste trabalho. A dissertação é apresentada na forma deste artigo no Capítulo 2, de acordo com as opções de formato apresentadas pelo regulamento do Programa de Pós-Graduação em Ciências Farmacêuticas.

Figura 1 – Esquema geral dos métodos utilizados



1.3.1 Artigo: ***Serum myeloperoxidase is not altered in obesity and overweight: inflammatory and biochemical parameters evaluation***

O artigo apresenta a avaliação da mieloperoxidase (MPO) como um potencial biomarcador de risco cardíaco em indivíduos obesos na rotina laboratorial. Este artigo será submetido para avaliação pelo corpo editorial de revista científica *Metabolism, Clinical and Experimental* (Qualis B1 na área de Farmácia e JCR 2011: 2,664).

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CAPÍTULO 2

(ARTIGO)

Serum myeloperoxidase is not altered in obesity and overweight: inflammatory and biochemical parameters evaluation

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Abstract

Objective: Obesity is a low-grade chronic inflammation and it is associated with increased cardiovascular risk. There are many researches looking for new biomarkers that are able to predict the occurrence of future cardiovascular complications. The aim of this study was to assess levels of myeloperoxidase (MPO) and other biomarkers in obese patients.

Materials/Methods: The study included 174 volunteers: 45 individuals with BMI < 25 kg/m^2 (control group), 43 individuals with overweight (BMI = 25.0 to 29.9 kg/m^2), 41 individuals in class I obesity (BMI = 30.0 to 34.9 kg/m^2), 30 individuals in class II obesity (BMI = 35.0 to 39.9 kg/m^2), 15 individuals in class III obesity (BMI ≥40.0 kg/m^2). We analyzed biochemical and hematological parameters, total antioxidant capacity (TAC), MPO and Hs-PCR of all study patients.

Results: There were significant increases in systolic and diastolic blood pressure, HDL cholesterol and the ratios of triglycerides to HDL cholesterol and HDL to LDL cholesterol to the groups in relation to control. Significantly elevated levels of Hs-CRP and TAC were observed in the groups with obesity, but MPO did not show a significant increase. MPO demonstrated a correlation with white blood cell count (0.400) and Hs-CRP (0.262).

Conclusions: Many biomarkers demonstrated a increased cardiovascular risk to obese individuals, but serum levels of MPO did not provide information on clinically significant laboratory differentiation of studied groups.

Abbreviation

MPO, myeloperoxidase; BMI, body mass index; TAC, total antioxidant capacity; Hs-CRP, high-sensitivity C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; EDTA, ethylenediaminetetraacetic acid; GPT, glutamic pyruvic transaminase; GOT, glutamic oxalic transaminase; ABTS, 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate; ESD, erythrocyte sedimentation rate.

1. Introduction

Obesity is becoming a global epidemic. In the year 2008, about 1.4 billion adults were overweight in the world, which over 200 million men and nearly 300 million women were obese [1]. Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health, and both are the fifth leading risk for global deaths [1]. Currently, overweight and obesity are classified using the body mass index (BMI). In adults, overweight is defined as a BMI of 25.0 to 29.9 kg/m^2 and obesity is defined as a BMI $\geq$30.0 kg/m^2 [2]. Body mass index (BMI) provides the most useful population level measure of overweight and obesity as it is the same for both sexes and for all ages of adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2) [1].

Numerous studies have documented that obesity is associated with the presence of systemic hypertension, coronary heart disease, arteriosclerosis, type 2 diabetes mellitus as well as an increased mortality risk [3]. At the same time, obesity has also been associated with the alteration of several metabolic and hormonal parameters, including hyperinsulinemia, increased insulin resistance and elevated plasma glucose [4] as well as dyslipidemia [5].

Obesity is associated with chronic inflammation [6]. The reports indicated that increased pro-inflammatory factors, such as interleukin-6 (IL-6), resistin, and tumor necrosis factor-alpha (TNF-α), are observed in obesity-related diseases [7]. It is necessary the maintenance of a normal amount of adipose tissue because the deregulate adipocytokines releasing may lead to inflammation and vascular disturbances [8]. Leptin, one of the most abundant adipocytokines, have been implicated as direct participant in the regulation of coronary vasomotor function in obesity [9].

It is suggested that the induction systemic oxidative caused by obesity *per se* and the increase oxidative stress in accumulated fat subscribe to dysregulation of adipocytokines and development of metabolic syndrome [10]. As an early generator of obesity-associated metabolic syndrome, increased oxidative stress in accumulated fat should be an important target for the development of new therapies and biomarkers.

Biomarkers play important role in risk stratification of coronary atherosclerosis development, although the underlying pathomechanism has not been completely clarified to date [11]. The interaction between sedentary lifestyle, certain dietary patterns and genetic factors is generally considered to contribute to its pathogenesis [12].

Myeloperoxidase (MPO) is a leukocyte derived enzyme-generating reactive oxidant species with mainly microbicidal properties [13]. This hemeprotein is considered to be a potential marker of cardiovascular disease (CVD). Recent studies have emphasized the importance of MPO in CVD progression [14, 15]. Myeloperoxidase seems to play a potentially important role in promoting the progression of atherosclerosis, thus triggering the occurrence of acute vascular events. Patients with coronary artery disease when compared to healthy subjects present MPO levels significantly higher [16].

MPO can promoting vascular disease through several mechanisms, including the generating dysfunctional high-density lipoprotein (HDL) by selectively modifying apolipoprotein A-1, stimulating conversion of low-density lipoprotein (LDL) into an atherogenic form, promoting vulnerable plaque, and promoting myocardial dysfunction and abnormal ventricular remodeling after myocardial infarction [17]. Myeloperoxidase also promoting endothelial dysfunction, decreasing nitric oxide's bioavailability [18].

The combination of these detrimental effects has culminated in the concept that MPO may be an active mediator of atherogenesis and can predict future cardiovascular events [13]. Considering there is a continuous search for novel, more powerful biomarkers that are able to predict the occurrence of future cardiovascular complications, our study aim was to assess levels of MPO and its correlation with other biomarkers in obese patients.

2. Materials and methods

2.1. Subjects

The volunteers were patients from the University Laboratory of Clinical Analyses (LUAC-UEPG). All volunteers gave informed consent for their participation in this research. In total, 187 individuals were selected for the study. Inclusion criteria were age over 18 years and no infection or chronic inflammatory underlying disease.

Anthropometric measurements were taken using standardized techniques and calibrated equipment. Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in square meters). The following WHO-recommended criteria were used: overweight (BMI = 25.0 to 29.9 kg/m^2); class I obesity (BMI = 30.0 to 34.9 kg/m^2); class II obesity (BMI = 35.0 to 39.9 kg/m^2); and class III obesity (BMI ≥40.0 kg/m^2) [19]. Blood pressure was measured with a standardized sphygmomanometer after 10 minutes of rest in the sitting position.

2.2. Ethics

All participants were informed about the survey and freely signed and dated the consent form. The protocol was approved by the Ethics in Research Committee of the State University of Ponta Grossa (74/2009) and was conducted in accordance with the Helsinki Declaration.

2.3. Laboratory analysis

Blood samples were obtained in the morning after 12 hours of fasting. The clinical histories of the study participants, such as age and sex, current history of infection, systemic arterial hypertension, diabetes mellitus and medicines used, were obtained at the time of blood collection. In total, 10.0 mL of blood was collected in tubes without an anticoagulant and 10.0 mL of total blood was collected in tubes with an anticoagulant (EDTA - ethylenediaminetetraacetic acid). The tubes without anticoagulant were immediately centrifuged to obtain serum and separated into two aliquots, one for the evaluation of biochemical and immunological parameters and the other frozen at −80 °C for the subsequent analysis of myeloperoxidase. The tubes with anticoagulant were used for the analysis of hematological parameters.

2.4. Biochemical parameters

The levels of glucose, total cholesterol, high density lipoprotein (HDL cholesterol), triglycerides, alfa-1 glycoprotein, creatinine, uric acid, urea, glutamic pyruvic transaminase (GPT) and glutamic oxalic transaminase (GOT) were measured using an automated method in Selectra Junior (Vital Scientific, Netherlands). When levels of triglycerides below 400mg/dL were observed, the Friedewald equation (LDL cholesterol = [total cholesterol] − [HDL cholesterol] − ([triglycerides] /5)) was used to determine the levels of low density lipoprotein (LDL cholesterol). Measurements of glycated hemoglobin (HbA1c) were obtained in Dia Fast (Prime Diagnostics) using the method of low pressure chromatography.

The Total Antioxidant Capacity Assay (TAC) of serum was measured by a method based on 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS). The pre-formed radical monocation of ABTS ($ABTS^{\bullet+}$) is generated by oxidation with potassium persulfate. Antioxidants in the sample suppress the formation of the radical cation to a degree which is proportional to their concentration [20]. In our study, we express TAC as percentage total

scavenging capacity (%), comparing the results with a control tube without serum. All samples were measured in duplicate.

2.5. Hematological parameters

The samples were subjected to cell counts using a Cell-Dyn1400 hematology counter (Abbott, Quebec, Canada) to obtain the number of total leukocytes and the absolute neutrophil count. The erythrocyte sedimentation rate (ESD) was obtained by Westergren method.

2.6. High sensitive C reactive protein (Hs-CRP)

The Hs-CRP levels were determined by turbidimetry using the Selectra Jr. device according to the manufacturer's instructions (CRP U-hs, DiaSys, Germany).

2.7. Myeloperoxidase

The myeloperoxidase levels in serum were determined by an enzyme immunoassay technique (ELISA, enzyme-linked immunosorbent assay) in the capture format, according to the manufacturer's instructions (Consultants Immunology Laboratory, USA).

2.8. Statistical analysis

The patient laboratory data were presented as the mean and standard deviation for continuous variables and for categorical variables such as numbers and percentages. The normality of distributions of the some response variables was controlled with the Kolmogorov-Smirvov test. Before statistical analyses, logarithmic transformations of the variables that did not display a normal distribution were made. In this way, the data were presented descriptively using the means and standard deviation. The possible differences between groups were found using the chi-square ($\chi 2$) test for categorical variables and the analysis of variance (ANOVA) followed by the Tukey post test for continuous variables. To determine the possible correlations between the laboratory parameters, we used the Pearson correlation. In all tests, the level of significance was pre-set at $p<0.05$. The analyses were performed using a statistical program (SPSS^{TM} version 18 for Windows, SPSS do Brasil, São Paulo, SP, Brazil).

3. Results

One hundred and seventy four individuals met the inclusion criteria, and the characteristics of the groups are compared in Table 1, which presents the number of cases of hypertension and diabetes, according to an interview with the patients. The age and distribution between males and females was similar among the five groups, demonstrating that the study population was homogeneous. The groups of obese patients, as expected, showed more cases of hypertension and diabetes mellitus in relation to the control.

Table 1 – Characterization of studied groups: control (CON), overweight (OW), class I obesity (OB I), class II obesity (OB II), and class III obesity (OB III).

Characteristics	CON (n=45)	OW (n=43)	OB I (n=41)	OB II (n=30)	OB III (n=15)	Value p
Age (years)*	38.24 ± 15.64	44.12 ± 15.06	45.61 ± 13.29	44.53 ± 13.02	48.87 ± 12.97	0.059[ns]
Gender†						
Female	35 (79)	29 (67)	36 (88)	27 (90)	10 (67)	0.064[ns]
Male	10 (21)	14 (33)	5 (12)	3 (10)	5 (33)	
BMI (Kg/m^2)	21.86 ± 2.14[a]	27.17 ± 1.14[b]	31.73 ± 1.31[c]	37.17 ± 1.43[d]	45.30 ±4.87[e]	<0.001[s]
SAH†						
No	41 (91)	31 (72)	31 (76)	18 (60)	5 (33)	< 0,001[s]
Yes	4 (9)	12 (28)	10 (24)	12 (40)	10 (67)	
DM††						
No	45 (100)	40 (93)	36 (88)	30 (100)	11 (73)	- - -
Yes	0 (0)	3 (7)	5 (12)	0 (0)	4 (27)	

Values are mean ± SD or absolute and relative (%) frequencies.
* ANOVA and Tukey post hoc test.
† $\chi 2$
†† Descriptive statistics only. 5 cells (50%) have expected count less than 5. The minimum expected count is 1.03, $\chi 2$ is not appropriate.
SAH. systemic arterial hypertension
DM. diabetes mellitus
[ns]Non-significant.
[s]Significant.
Horizontally (BMI), different letters indicate statistically significant differences among conditions.

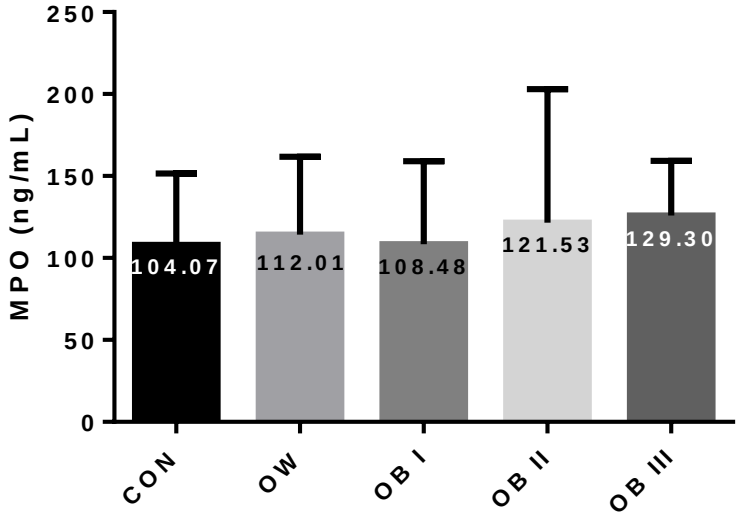
The groups exhibited significant differences compared to the control group (Table 2) to a few parameters, including systolic and diastolic blood pressure, HDL and the ratios of HDL to LDL and triglycerides to HDL. Fasting glucose, urea, creatinine, total cholesterol, LDL, GPT, GOT and HbA1c showed no statistical difference between any groups. Among the inflammatory parameters, alpha-1 glycoprotein demonstrated significant difference for the control group only when related to the class III obesity group. The ESR did not show difference amid overweight and class III obesity to control group. Hs-CRP was different between control and the three classes of obesity groups, revealing an inflammatory status and a possible cardiac risk. However, the MPO did not show a significant increase, as seen in Graphic 1.

Only two subjects, both belonging to the obesity class II group, presented MPO levels above 350 mg/L, considered the cutoff for elevated cardiovascular risk [21]. With regard to Hs-CRP, values above 3 mg/L are associated with future cardiovascular events [22], and levels above this value were observed in up to 28% of the control group, 35% of the overweight group and 49%, 50% and 76% of the class I, II and III obesity groups, respectively.

Table 2 – Mean ± SD of laboratory parameters for the groups

Parameters	CON (n=45)	OW (n=43)	OB I (n=41)	OB II (n=30)	OB III (n=15)	Value p
Systolic blood pressure (mmHg)	121.18 ± 17.53[a]	132.55 ± 17.82[ab]	136.77 ± 24.06[b]	135.77 ± 15.02[b]	158.60 ± 21.66[c]	< 0.001[s]
Diastolic blood pressure (mmHg)	72.59 ± 10.50[a]	82.71 ± 10.84[b]	84.25 ± 14.45[bc]	86.30 ± 11.77[bc]	93.93 ± 16.72[c]	< 0.001[s]
Fasting Glucose (mg/dL)	82.91 ± 10.77	88.60 ± 12.56	91.54 ± 21.40	89.20 ± 17.39	92.33 ± 12.75	0.063[ns]
Urea (mg/dL)	27.31 ± 8.98	29.44 ± 8.22	29.44 ± 10.89	28.50 ± 6.37	33.40 ± 10.89	0.243[ns]
Creatinine (mg/dL)	0.96 ± 0.15	1.00 ± 0.20	0.94 ± 0.16	0.93 ± 0.17	0.99 ± 0.20	0.456[ns]
Total cholesterol (mg/dL)	170.13 ± 35.79	174.84 ± 40.68	186.46 ± 40.75	181.50 ± 45.55	180.13 ± 48.46	0.423[ns]
HDL (mg/dL)	56.18 ± 16.38[a]	45.86 ± 13.56[b]	47.54 ± 16.46[ab]	45.27 ± 14.01[b]	41.00 ± 13.56[b]	0.002[s]
LDL (mg/dL)	95.29 ± 35.03	99.49 ± 37.73	110.66 ± 33.41	115.07 ± 38.05	104.40 ± 44.37	0.133[ns]
LDL/HDL	1.89 ± 0.99[a]	2.42 ± 1,28[b]	2.65 ± 1.21[b]	2.66 ± 1.15[b]	2.88 ± 1.72[b]	0.012[s]
Triglycerides (mg/dL)	93.87 ± 33.63[a]	148.05 ± 76.80[b]	128.78 ± 77.16[ab]	119.87 ± 42.05[ab]	173.20 ± 67.83[b]	<0.001[s]
Triglycerides/HDL	1.82 ± 0.89[a]	3.73 ± 2.68[b]	3.29 ± 2.54[b]	2.98 ± 1.48[b]	5.08 ± 3.40[b]	<0.001[s]
Uric acid (mg/dL)	4.59 ± 1.34[a]	5.50 ± 1.39[b]	4.99 ± 1.10[ab]	5.79 ± 1.47[b]	7.12 ± 1.79[c]	<0.001[s]
GOT (UI/L)	21.73 ± 7.66	22.49 ± 6.32	25.34 ± 14.26	27.47 ± 17.00	26.00 ± 7.73	0.209[ns]
GPT (UI/L)	12.09 ± 11.10	12.88 ± 6.60	14.34 ± 12.73	13.30 ± 11.04	13.73 ± 5.56	0.638[ns]
HbA1c (%)	5.36 ± 1.46	5.53 ± 1.62	5.69 ± 1.10	5.69 ± 0.69	6.02 ± 0.69	0.185[ns]
Alpha-1glycoprotein	49.86 ± 35.94[a]	65.63 ± 37.60[ab]	61.41 ± 46.20[ab]	60.60 ± 44.67[ab]	118.86 ± 91.07[b]	0.012[s]
ESR (mm/h)	9.96 ± 7.66[a]	10.91 ± 9.28[ab]	17.03 ± 13.09[b]	17.55 ± 10.00[b]	16.87 ± 12.85[ab]	0.001[s]
Hs-CRP (mg/L)	2.12 ± 2.10[a]	2.80 ± 2.70[ab]	4.89 ± 4.66b[c]	4.32 ± 4.08[bc]	8.24 ± 8.20[c]	<0.001[s]
MPO (ng/mL)	104.07 ± 40.60	112.01 ± 49.10	108.48 ± 50.53	121.53 ± 81.50	129.30 ± 54.10	0.482[s]

Horizontally, different letters indicate statistically significant differences among conditions (ANOVA and Tukey post hoc test).
[ns]Non-significant; [s]Significant.



Graphic 1 – Means and standard deviation of myeloperoxidase. There were no statistical differences for the groups: control (CON), overweight (OW), class I obesity (OB I), class II obesity (OB II), and class III obesity (OB III).

The hematological parameters are present in Table 3. The biggest difference between the groups was found in white blood cell count, reflecting the increase in monocytes and neutrophils count.

Table 3 – Mean ± SD of hemogram for the groups: control (CON), overweight (OW), class I obesity (OB I), class II obesity (OB II), and class III obesity (OB III)

Parameters	CON	OW	OB I	OB II	OB III	Value p
Erythrocytes (M/μL)	4,75±0,43[a]	4,82±0,44[a]	4,70±0,40 [a,b]	4,54±0,26[b]	4,81±0,41[a]	0,103[ns]
Hemoglobin (g/dL)	13,97±1,34[a]	14,19±1,45[a]	13,72±1,37[a]	13,39±1,14 [a]	14,10±1,23[a]	0,176[ns]
Hematocrit (%)	41,72±3,41[a]	41,91±3,87[a]	40,78±3,81[a,b]	39,36±3,10[b]	41,71±3,76[a,b]	0,069[ns]
MCV (fL)	87,95±3,34[a]	87,45±3,20[a]	86,80±4,11[a]	86,71±5,49[a]	86,44±3,46[a]	0,529[ns]
MCH (pg)	29,43±1,36[a]	29,61±1,36[a]	29,21±1,66[a]	29,49±2,04[a]	29,22±1,25[a]	0,768[ns]
MCHC (g/dL)	33,46±1,22[a]	33,86±0,88[a]	33,54±1,19[a]	34,04±1,07[a]	33,81±0,56[a]	0,161[ns]
White cell count (10^3 cell/mL)	5,8±1,6[a]	6,5±1,6[b]	6,3±1,6[a,b]	6,1±1,6[a,b]	8,1±1,9[c]	<0,001[s]
Lymphocytes (10^3cell/mL)	2,2±0,6[a]	2,2±0,7[a]	2,2±0,6[a]	2,3±0,6[a]	2,1±0,9[a]	0,639[ns]
Monocytes (cell/mL)	273,9±185,8[a]	340,9±278,9[a,b]	311,4±175,4[a,b]	386,5±236,4[b,c]	481,4±281,2[c]	<0,001[s]
Eosinophils (cell/mL)	135,5±117,0[a]	237,4±230,3[b]	180,2±173,5[a,b]	168,5±133,6[a,b]	141,6±153,2[a,b]	0,07[ns]
Neutrophils (10^3cell/mL)	3,2±1,1[a]	3,5±1,4[a,b]	3,5±1,2[a,b]	3,9±1,6[b,c]	4,6±1,9[c]	0,02[s]

Horizontally, different letters indicate statistically significant differences among conditions (ANOVA and Tukey post hoc test).
[ns]Non-significant; [s]Significant.
M/μL – million per microliter; g/dL – grams per deciliter; fL – femtoliters; pg –picogram; cell/mL – cells per milliliter; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration

Correlation coefficients were computed among analyzed parameters (Figure 1). The parameters significantly ($p<0,01$) and most strongly correlated with BMI were systolic (0.435) and diastolic blood pressure (0.434), uric acid (0.407) and Hs-CRP (0.396). Higher levels of MPO were related to white blood cell count (0.400) and Hs-CRP (0.262). Other parameters strongly correlated each other were total cholesterol and neutrophil count (0.646).

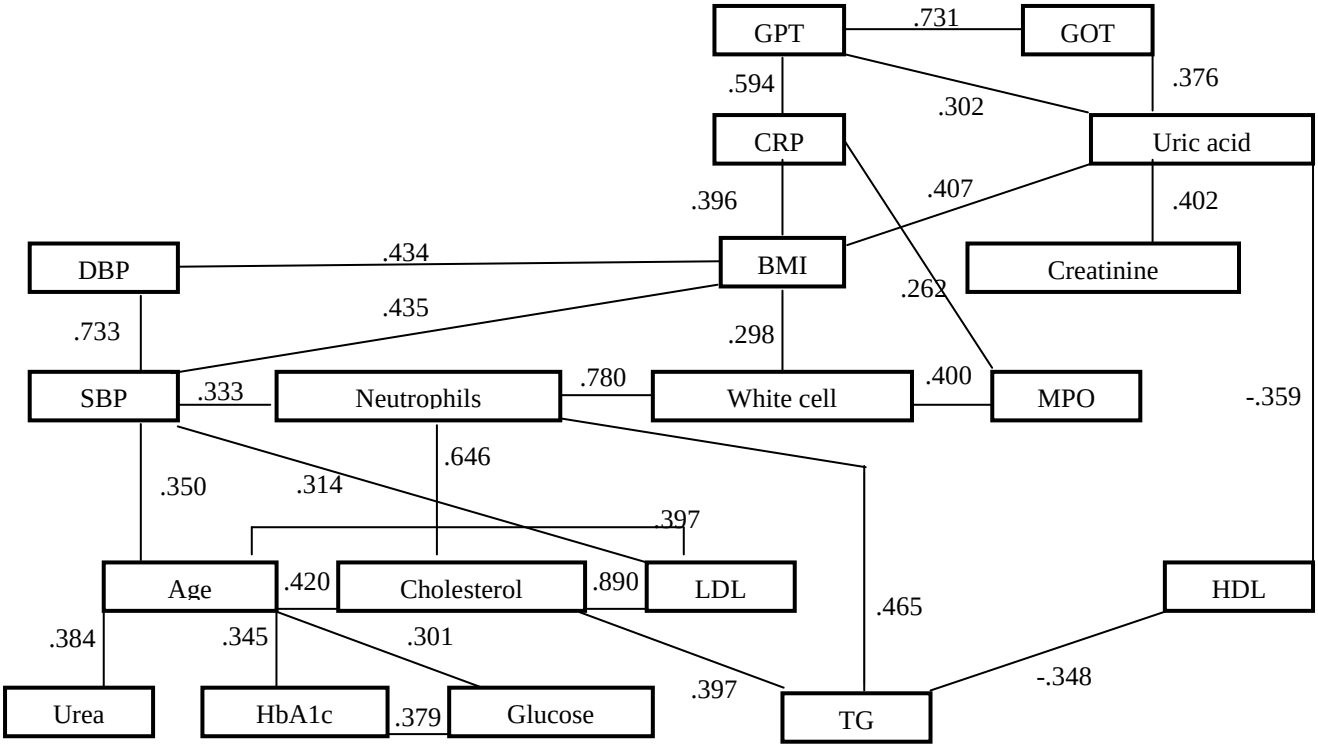
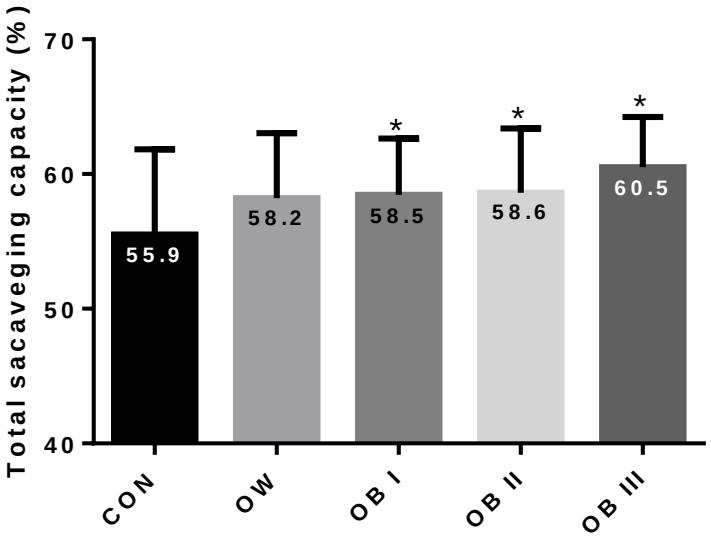


Figure 1 – Correlation coefficients among analyzed parameters.
CRP – C reactive protein; DBP – diastolic blood pressure; GOT – glutamic oxalic transaminase; GPT – glutamic pyruvic transaminase; HDL – high density lipoprotein; LDL – low density lipoprotein; SBP – systolic blood pressure; TG – Triglycerides.

TAC has been used as comprehensive measures of radicals quenching capacity by antioxidants in serum. The groups with BMI≥30 kg/m^2 had a better scavenging capacity than the control (Graphic 2). This could be at least in part explained by the significant increase of uric acid, which is an important contributor to TAC. There is a positive correlation between uric acid and total scavenging capacity (0.240).



Graphic 2 – Total scavenging capacity (%) for the groups control (CON), overweight (OW), class I obesity (OB I), class II obesity (OB II), and class III obesity (OB III).
* statistical difference to control

4. Discussion

MPO has been linked to the initiation and propagation of atherosclerosis by the generation of diffusible oxidants and radical species which result in the oxidation of LDL and generation of other atherogenic lipoproteins, the consumption of nitric oxide and development of endothelial dysfunction [13]. Consequently, the elevation of MPO levels found in the obese population could be regarded as a signal of atherosclerosis and, thereby, a greater propensity towards future cardiovascular events. MPO has been associated with cardiovascular disease in many studies [23, 16] and many conditions may cause its increase [24]. Contrary to expectations, in our study, the MPO did not show an increasing with BMI. Moreover, levels of MPO in the above mentioned studies were higher than in our volunteers. The present findings confirm that MPO is probably more closely related to acute events, rather than to the presence of stable inflammation caused by obesity.

Although, with the present results, the MPO has not been seen as a good indicator of cardiac risk for obese patients, other parameters already consolidated pointed to the increased risk as leukocyte count, Hs-CRP, and rates TG/HDL-C and LDL-C/HDL-C.

The white blood cells count in the overweight and class III obesity groups was higher as compared with control in accordance with previous researches [25]. But despite this, MPO was not significantly higher in obese groups. This fact may be explained by an impaired leukocyte function. At least, a previous study showed lower MPO levels in lymphocytes and neutrophils in patients with *diabetes mellitus* 2 when compared with healthy controls [25].

It is known that higher levels of Hs-CRP is a predictor of cardiovascular disease and may play an important role in the different stages of the development of atherosclerosis [26]. Claire 2010, demonstrated that patients undergoing selective coronary angiography with either MPO or CRP elevated had 5.3-fold higher cardiovascular mortality risk, and patients with high levels of both MPO and CRP had a 4.3-fold risk compared with patients with only elevated marker [27]. In agreement with results obtained in previous studies, our data proved that plasma Hs-CRP level had significant positive correlation with BMI and MPO [26, 28]. The connection between adipose tissue and low-grade chronic inflammation may be explained by the fact of fat cells produce cytokines, in particular IL-6 that induces the synthesis of CRP by the liver. This may be of pathogenic significance as CRP stimulates the uptake of LDL by macrophages, induces complement activation which may cause cellular damage in the artery, and enhances monocyte production of tissue factor, thus enhancing the risk of thrombosis [29].

In accordance with other studies [23, 30, 31] we found a weak negative correlation between MPO and HDL cholesterol. Indeed, MPO binds to apolipoprotein AI of HDL particles and may subsequently be inactivated or excreted [15]. Furthermore, MPO may be involved in generating dysfunctional HDL and thus promote atherogenesis by this pathway [32].

The joint occurrence of high triglyceride level and low HDL-c characterizes the dyslipidemia of Metabolic Syndrome [33]. The ratio of TG/HDL-c serves as a summary measure for either elevated triglyceride level, low HDL-c, or both and has been proposed as an easily obtainable atherogenic marker [34]. A high TG/HDL-c ratio correlates with LDL phenotype B, small HDL particles, and insulin resistance [35].

In recent years, case-control and prospective studies have linked the ratio of TG/HDL-c to CVD incidence, outcomes, and all-cause mortality [36, 37], with improved predictive power in some studies compared with LDL-C or non–HDL-C [38]. In our study, this rate was significantly different between the groups and the control. Whereas the value of this ratio should be below 2, only the control group reached the optimal value, while the class III obesity group got the ratio above 4, a value considered high [39], showing that this group has a higher cardiovascular risk. Another option that can be utilized as an accurate predictor of

cardiovascular risk is the LDL-C/HDL-C ratio, which is more accurate than LDL-C or HDL-C alone [40, 41]. Our results showed a growing trend in the value of this rate with increasing BMI. Using this parameter allows observation of increased cardiovascular risk among groups more consistently than when observed only the LDL-C value. Both rates showed positive correlation with the neutrophil count (TG/HDL-C 0.395; LDL-C/HDL-C 0.520, $p<0.001$), relating the imbalance lipid to the inflammatory state.

Evidence that obesity is related to an increased oxidative stress can be found in the literature [42, 43]. Increased oxidative stress in a situation of fat accumulation is an important pathogenic mechanism of obesity-associated metabolic syndrome and higher cardiac risk. Collective antioxidant capacity measured by TAC represents free radical inhibition by all the antioxidants in a tissue sample such as plasma or serum. Several studies have found lower TAC values in obese people than in nonobese people [44, 45] and animal models of obesity [46]. The inverse was observed in our study. This result may be obtained in part by the significant increase of uric acid, considering that the TAC not only measures the well-known antioxidants, such as vitamins C and E, beta-carotene, glutathione, cysteine, dihydrolipoate, ubiquinol, and polyphenols, but also determines the contributions to defense against oxidative damage by other substances such as albumin, uric acid, bilirubin, and other molecules present in the body [47]. It is already known obese subjects frequently have hyperuricemia and there is a positive correlation between body weight and serum uric acid level [48]. Although uric acid is a non-enzymatic antioxidant, its increased production may increase free radical production due to activation of the xanthine oxidase enzyme system and radical anion superoxide production [49]. Moreover, protein glycation, which occurs in glicose metabolism impairment, may lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase [50].

Finally, assay-related issues such as the use of different detection antibodies limit the transferability of results obtained on one MPO assay to another. A systematic review of their analytical characteristics is needed, for a comparison of results obtained from different MPO assays [51]. In conclusion, MPO provided no relevant clinical information in our study. Given the summarized evidence, it thus is doubtful whether MPO is useful for the clinical work-up of obese patients.

Author Contributions

Author participation: Gisele Chibinski Parabocz and José Carlos Rebuglio Vellosa were responsible for overseeing and executing all phases of the research and were responsible for writing this paper. Danielle Cristyane Kalva Borato helped to make all myeloperoxidase measurements. Harli Pasquini Netto, Fabiane Cristina Erdmann, Lorena Degraf Wiechetck and Lucas Rosolen de Almeida Mello helped to guide, inform and interview all volunteers. Luciana Maria Borba, Francine Alessandra Manente and Josilaine Tonin Ribas helped to execute all hematological and biochemical analyses. Fábio André dos Santos supported all statistical trials.

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Conflict of interest

The authors declare no conflict of interest in any aspect of this report.

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CAPÍTULO 3

3.1 CONSIDERAÇÕES FINAIS

As doenças cardiovasculares representam uma das maiores causas de morbidade e mortalidade em todo o mundo. Um dos fatores de risco para tais doenças e que se encontra em observável ascendência é a obesidade. Hoje, o papel da mieloperoxidase no desenvolvimento da aterogênese, em várias de suas etapas, já está estabelecido, o que a torna candidata a novo marcador laboratorial. O estudo de novos marcadores específicos e mais precoces são relevantes, à medida que permitem ações preventivas e terapêuticas eficientes na promoção da saúde.

Tendo esses apontamentos em vista, buscou-se avaliar os níveis de mieloperoxidase, dentre outros marcadores classicamente utilizados, em diferentes graus de obesidade. Vários dos parâmetros analisados mostraram alterações, acompanhando o aumento do índice de massa corporal, ressaltando o risco elevado ao qual os pacientes obesos estão expostos. Dentre eles, ressalta-se a contagem de leucócitos, a proteína C reativa ultrassensível e as taxas triglicerídeos/HDL e LDL/HDL. Contudo, a mieloperoxidase não demonstrou um crescimento significativo em indivíduos obesos.

Neste contexto, a mieloperoxidase pode ser um parâmetro de grande potencial preditivo de risco cardíaco em pacientes com doenças cardíacas já estabelecidas, como vários estudos demonstram, porém, de acordo com os nossos resultados, não há proficuidade no uso da mieloperoxidase para o prognóstico de risco cardíaco em pacientes obesos. Tal resultado é importante, pois a utilização de um novo marcador na rotina laboratorial exige a padronização e validação de novos métodos e os custos para implantação e compra de materiais e reagentes são elevados. Portanto, a sua aplicação no monitoramento laboratorial de auxílio ao diagnóstico clínico em várias populações deve ser investigada de forma criteriosa e mais aprofundada em estudos prospectivos.

ANEXO A – Parecer COEP UEPG

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COEP
COMISSÃO DE ÉTICA EM PESQUISA

PARECER Nº 74/2009
Protocolo: 12476/09

Em reunião ordinária realizada dia 26 de novembro de 2009, a Comissão de Ética em Pesquisa, **APROVOU** o protocolo de pesquisa intitulado "Determinação dos níveis séricos da enzima mieloperoxidase na saúde e em desordens metabólicas" de responsabilidade do pesquisador José Carlos Rebuglio Vellosa.

Conforme Resolução CNS 196/96, solicitamos que sejam apresentados a esta Comissão, relatórios sobre andamento da pesquisa, conforme modelo (http://www.uepg.br/coep/).

Data para entrega do relatório final: 20/12/2011.

Ponta Grossa, 30 de Novembro de 2009.

UNIVERSIDADE ESTADUAL DE PONTA GROSSA
COMISSÃO DE ÉTICA EM PESQUISA - COEP

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