Universidade de São Paulo
Faculdade de Saúde Pública
Departamento de Nutrição

**Associação da atividade da Lp-PLA₂ e de
antioxidantes lipossolúveis com marcadores
cardiometabólicos em adolescentes**

Isis Tande da Silva

**Tese apresentada ao Programa de Pós-
Graduação em Nutrição em Saúde Pública
para obtenção do título de Doutor em
Ciências.**

**Área de concentração: Nutrição em Saúde
Pública**

**Orientadora: Profa. Dra. Nágila Raquel
Teixeira Damasceno**

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Dedico esta tese

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“Bem é ciência, o esforço do espírito humano para compreender tudo o que nos cerca, sejam gigantescas galáxias estelares, bactérias microscópicas, ou essas partículas elementares. É interessante, e por isso o fazemos. Dizem a curiosidade mata o gato; digo a curiosidade faz o cientista.”

George Gamow

RESUMO

Silva IT. Associação da atividade da Lp-PLA₂ e de antioxidantes lipossolúveis com marcadores cardiometabólicos em adolescentes [tese de doutorado]. São Paulo: Faculdade de Saúde Pública, USP; 2011.

Introdução: A obesidade se caracteriza como um processo oxidativo e inflamatório, que predispõe adolescentes, de modo precoce, a eventos até recentemente pouco frequentes nessa faixa etária. Assim, a ação da enzima Fosfolipase A₂ associada às lipoproteínas (Lp-PLA₂), que reduz fosfolipídios oxidados e gera lisofosfolipídios, bem como a disponibilidade de antioxidantes plasmáticos, representam um importante tema de pesquisa no contexto cardiovascular. **Objetivo:** Verificar se a atividade da Lp-PLA₂ e a concentração de antioxidantes lipossolúveis se associam com os principais marcadores de risco cardiovascular em adolescentes. **Métodos:** Duzentos e quarenta e dois adolescentes (10 a 19 anos), de ambos os sexos foram distribuídos, segundo o índice de massa corporal (IMC), em três grupos: Eutróficos (n=77), Sobrepeso (n=82) e Obesos (n=83). A amostra foi caracterizada através de parâmetros sócio-econômicos, estado de saúde, uso de medicamentos, antedecentes familiares de doenças crônicas e prática de atividade física. Foram avaliados ainda os dados antropométricos (peso, altura e composição corporal - bioimpedância), e o consumo alimentar por meio de três recordatórios 24 h. A partir de uma amostra de sangue coletada após jejum (12h), realizaram-se as análises da atividade da Lp-PLA₂, LDL(-) e seus auto-anticorpos, perfil lipídico (colesterol total, LDL-C, HDL-C e triglicerídeos), tamanho da HDL, proteína transportadora de éster de colesterol (CETP), ácidos graxos não esterificados (NEFAs), adipocitocinas, assim como antioxidantes (retinol, licopeno, α -tocoferol e β -caroteno) no plasma. **Resultados:** **Artigo 1:** “*Lp-PLA₂ maybe an important cardiovascular biomarker in obese adolescents*”. Verificou-se que o perfil lipídico, insulina, HOMA-IR (resistência à insulina) e LDL(-) evidenciaram um maior risco cardiovascular nos adolescentes obesos. A atividade da enzima Lp-PLA₂ mostrou uma variação proporcional ao IMC, circunferência da cintura e porcentagem de gordura. Essa tendência foi, ainda, reforçada pelas associações positivas entre a enzima e HOMA-IR, glicose, insulina e as variáveis lipídicas. Adicionalmente, associação negativa foi encontrada para Apo

AI. As associações mais relevantes foram observadas para ApoB e Apo B / Apo AI ($\beta=0,293$; $P<0,001$, $\beta=0,343$; $P<0,001$, respectivamente). O melhor modelo preditor para a atividade da enzima incluiu Apo B/Apo AI ($\beta= 0,327$; $P<0,001$), tamanho da HDL ($\beta=-0,326$; $P<0,001$), circunferência da cintura ($\beta=0,171$; $P=0,006$) e glicose ($\beta= 0,119$; $P=0,038$). A análise de *Odds Ratio* mostrou que a mudança de uma unidade na razão entre Apo B/Apo AI esteve associada a 73.5 maior risco para elevada atividade de Lp-PLA₂. *Conclusão:* A enzima Lp-PLA₂, em adolescentes, varia em função do estado nutricional e está relacionada a vários marcadores de risco cardiovascular, especialmente a Apo B/Apo AI. Assim, a atividade da enzima pode representar um importante biomarcador de risco cardiovascular na adolescência.

Artigo 2: “*Antioxidant, and inflammatory aspects of phospholipase A₂ associated to lipoprotein (Lp-PLA₂): A review*”. Esta revisão descreve os principais aspectos relacionados à enzima Lp-PLA₂ e seu impacto no perfil inflamatório e oxidativo da doença aterosclerótica. **Artigo 3:** “*Plasma antioxidants, but not the dietary ones, are associated with cardiometabolic risk in adolescents*”. Neste artigo, observou-se que os antioxidantes β -caroteno, licopeno, retinol e α -tocoferol variam em função do IMC. Verificou-se que β -caroteno e α -tocoferol apresentaram valores reduzidos para o grupo obeso em comparação ao eutrófico e ao sobrepeso. Para o β -caroteno, verificou-se que essa diferença pôde ser observada inclusive quando comparados sobrepesos e eutróficos. Quando esses antioxidantes foram ajustados pelo colesterol total ou pelo LDL-C essas diferenças se mantiveram. Correlações importantes foram obtidas entre os antioxidantes ajustados por colesterol ou por LDL-C e as variáveis de risco cardiometabólico (perfil lipídico (colesterol total e frações, CETP), resistência à insulina (HOMA-IR, insulina, glicose) e LDL(-)). A obesidade representou um importante fator para a existência dessas correlações, pois após o ajuste por IMC foram reduzidas. Apesar desses resultados, os antioxidantes avaliados pelo recordatório de 24h não apresentaram nenhuma correlação com os fatores de risco cardiometabólico. *Conclusão:* Antioxidantes plasmáticos estão relacionados ao risco cardiometabólico em adolescentes, sendo que o IMC desempenha um papel importante para a existência dessas correlações. Tais biomarcadores representam

uma melhor maneira de estimar a influência de um padrão dietético sobre o risco cardiometabólico em adolescentes do que a avaliação do inquérito alimentar.

Palavras-chave: Obesidade, adolescente, Lp-PLA₂, antioxidantes, risco cardiovascular

ABSTRACT

Silva IT. Association of Lp-PLA₂ activity and fat-soluble antioxidants with cardiometabolic markers in adolescents [thesis]. São Paulo: Faculdade de Saúde Pública, USP; 2011.

Introduction: Obesity is characterized as an oxidative and inflammatory process, which precociously predisposes adolescents to events until recently uncommon in this age group. Thus, the action of Lipoprotein-associated phospholipase A₂ (Lp-PLA₂), which reduces oxidized phospholipids and generates lysophospholipids, as well as the availability of plasma antioxidants, represent important subjects of cardiovascular research. **Objective:** To evaluate if the activity of Lp-PLA₂ and the concentration of fat-soluble antioxidants are associated with the most important cardiovascular risk markers in adolescents. **Methods:** 242 adolescents (10 to 19 years old), from both sexes, were distributed according to Body mass index (BMI) in three groups: Health Weight (HW, n=77), Overweight (OV, n=82) and Obese (OB, n=83). The sample was characterized by socioeconomic parameters, health condition, use of medications, family history of chronic diseases and physical activity. It were evaluated also the anthropometric data (weight, height, body composition - bioimpedance) and the food intake through three 24h-recall. From a blood sample collected after fasting (12h), were analyzed the Lp-PLA₂ activity, the levels of LDL(-) and its antibodies, the lipid profile (total cholesterol, LDL-C, HDL-C and triglycerides), HDL-size, Non-Esterified Fatty Acids (NEFAs), Cholesteryl Ester Transfer Protein (CETP), adipocytokines, as well as plasma antioxidants (retinol, lycopene, α -tocopherol e β -carotene). **Results:** **Article 1:** “*Lp-PLA₂ as an important biomarker of cardiovascular risk in obese adolescents*”. It was observed that the lipid profile, the insulin, the HOMA-IR (insulin resistance) and the LDL(-) represented a higher cardiovascular risk in obese adolescents. The activity of Lp-PLA₂ has shown a variation proportional to BMI, waist circumference and fat mass percentage. This tendency was reinforced by positive associations of the enzyme with HOMA-IR, glucose, insulin and lipid variables. Additionally, a negative association was observed for Apo AI. The most relevant associations were observed for ApoB and Apo B /

Apo AI ($\beta=0,293$; $P<0,001$, $\beta=0,343$; $P<0,001$, respectively). The best predictor model for the enzyme activity included Apo B/Apo AI ($\beta= 0,327$; $P<0,001$), HDL-size ($\beta=-0,326$; $P<0,001$), waist circumference ($\beta=0,171$; $P=0,006$) and glucose ($\beta=0,119$; $P=0,038$). The *Odds Ratio* analysis showed that the change of one unit in the ratio of Apo B/Apo AI was related to a 73.5 times higher risk of elevated Lp-PLA₂ activity. *Conclusion:* The enzyme Lp-PLA₂, in adolescents, varies in function of the nutritional status and it is related to several cardiovascular risk markers, especially to Apo B/Apo AI. Thus, this enzyme activity may represent an important biomarker of cardiovascular risk in adolescence. **Article 2:** “*Antioxidant and inflammatory aspects of phospholipase A₂ associated to lipoprotein (Lp-PLA₂): a review*”. This review describes the main aspects related to the enzyme Lp-PLA₂ and its impact to inflammatory and oxidative profiles of atherosclerotic disease. **Article 3:** “*Plasma antioxidants, but not the dietary ones, are associated with cardiometabolic risk in adolescents*”. It was observed that the antioxidants β -carotene, lycopene, retinol and α -tocopherol vary in function of BMI. It was verified that β -carotene and α -tocopherol presented reduced values for the OB group in comparison with the HW and OV groups. For β -carotene, it was observed that this difference was also verified when OB and OV were compared. These differences remained alike when the antioxidants were adjusted by total cholesterol or by LDL-C. Important correlations were obtained between the antioxidants adjusted by total cholesterol or by LDL-C, and cardiometabolic risk variables (lipid profile (total cholesterol and fractions, CETP), insulin resistance (HOMA-IR, insulin, glucose) and LDL(-)). The obesity represented an important factor to the occurrence of these correlations, since they were reduced after BMI adjustment. Despite these results, the antioxidants evaluated by 24h-diet recall did not show any correlations with cardiometabolic risk. *Conclusion:* Plasma antioxidants are related to cardiometabolic risk in adolescents, where the BMI represents an important factor. These biomarkers are better than the food intake to estimate the influence of a dietary pattern on the cardiometabolic risk in adolescents.

Keywords: Obesity, adolescent, Lp-PLA₂, antioxidants, cardiovascular risk

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SIGLAS UTILIZADAS

%FM: Porcentagem de gordura

ABESO: Associação brasileira para o estudo da obesidade e síndrome metabólica

AntiLDL (-): Auto-anticorpos anti-LDL eletronegativa

Apo AI: Apolipoproteína AI

Apo B: Apolipoproteína B

Apo CII: Apolipoproteína CII

Apo E: Apolipoproteína E

BIA: Bioimpedância elétrica

CDC: Centro de controle e prevenção de doenças

CETP: Proteína transportadora de éster de colesterol

ELISA: Enzimaimunoensaio

FPLC: Cromatografia líquida de rápido desempenho

HDL: Lipoproteína de alta densidade

HOMA-IR: Avaliação da resistência à insulina

HPLC: Cromatografia líquida de alto desempenho

IBGE: Instituto brasileiro de geografia e estatística

IL-6: Interleucina 6

IMC: Índice de massa corporal

LCAT: Lecitina-colesterol aciltransferase

LDL (-): LDL eletronegativa

LDL: Lipoproteína de baixa densidade

LDLox: LDL oxidada

Lp-PLA₂: Fosfolipase A₂ associada à lipoproteína

MAB: Anticorpo monoclonal

MCP-1: Proteína-1 quimiotática para monócitos

NEFAs: Ácidos graxos não esterificados

OMS: Organização mundial da saúde

OR: Odd Ratio

OxNEFAs: Ácidos graxos não esterificados oxidados

PAF: Fator de ativação plaquetário

PAF-AH: Fator de ativação plaquetário acetilhidrolase

PCR: Proteína C reativa

PMSF: Fluoreto de fenilmetilsulfonila

ROS: Espécies reativas de oxigênio

SD: desvio padrão

SPSS: Pacote estatístico para ciências sociais

TC: Colesterol total

TG : Triglicérides

INTRODUÇÃO

1. INTRODUÇÃO

1.1 Obesidade

1.1.1 Definição e epidemiologia

A obesidade é um importante problema de saúde pública e pelo aumento de sua prevalência, caracteriza-se, segundo a Organização Mundial da Saúde - OMS como uma epidemia global (OMS, 2005). É considerada uma doença crônica, multifatorial, determinada pelo acúmulo excessivo de tecido adiposo no organismo, sendo que tanto o aporte calórico, quanto o gasto energético dependem não só de fatores genéticos e fisiológicos, mas também de variáveis culturais, sociais e psicológicas associadas à quantidade e qualidade da alimentação (MUENNIG, 2008). Atualmente, relaciona-se ao conceito de obesidade a distribuição da gordura corporal (HAMDY et al., 2006), pois a adiposidade visceral e abdominal se correlacionam fortemente com o risco cardiovascular e morbidades associadas (HAMDY et al., 2006; DESPRÉS et al., 2008). Nesse sentido, a Associação Brasileira para o estudo da Obesidade e Síndrome Metabólica (ABESO) recomenda associação do índice de massa corporal e medidas de distribuição de gordura corporal para o diagnóstico da obesidade (ABESO, 2009).

Além do comprometimento à saúde sob o ponto de vista emocional (ANTIPATIS e GILL, 2001), os efeitos do sobrepeso e da obesidade na saúde física têm sido amplamente documentados (ALMEIDA, 2001, NEUMARK-SZTAINER, 2006). A obesidade determina o desenvolvimento precoce e o piora no prognóstico de doenças cardiovasculares, hipertensão, diabetes mellitus, osteoartrite, osteoporose, câncer de mama, de endométrio e de cólon (RODRIGUES et al., 2005; GREENBERG e OBIN, 2006).

Atualmente, a prevalência da obesidade, tanto em países desenvolvidos, como em desenvolvimento vem apresentando números cada vez mais elevados (HASLAM et al., 2005), afetando centenas de milhares de pessoas em todo mundo (WONG-ON e MURILLO-CUZZA, 2004). KELLY et al. (2008) estimaram que, em 2030, a prevalência de sobrepeso e obesidade em adultos será de 2,6 e 1,12 bilhões, respectivamente, em todo o mundo. Essa alta prevalência de sobrepeso e obesidade

está associada com um custo de milhões de dólares gastos no seu tratamento e suas complicações (CHIARA et al., 2003; WONG-ON e MURILLO-CUZZA, 2004; GREENBERG & OBIN, 2006).

De modo semelhante a outros países, o Brasil tem apresentado um intenso crescimento de sua prevalência, sobretudo na última década. Esse perfil está associado a um antagonismo de tendências temporais observado entre desnutrição e obesidade e resultante do processo de transição nutricional, epidemiológica e geográfica (ESCODA, 2002; BATISTA FILHO e RISSIN, 2003; PINHEIRO, 2004).

1.1.2 Obesidade na adolescência

Diante da obesidade e suas implicações, um importante sub-grupo vem despertando interesse especial – os adolescentes. Frutos de uma infância de sobrepeso, se não de obesidade, os adolescentes apresentam uma curva de crescimento de obesidade mais acelerada do que a população em geral. Tal perfil tem refletido diretamente no registro, cada vez mais precoce, de eventos clínicos como dislipidemias, diabetes mellitus tipo II, hipertensão e aterosclerose (HILL et al., 1995; GIL-CAMPOS et al., 2008). Além dos riscos acima, indivíduos obesos, sobretudo crianças e adolescentes, apresentam baixa auto-estima, afetando o desempenho escolar e os relacionamentos, fator que tende a persistir na vida adulta (ABRANTES et al., 2002; PELLANDA et al., 2002). A adolescência e infância caracterizam-se como um momento de grande vulnerabilidade, período onde diversos fatores sociais e ambientais contribuem para a obesidade, onde se destacam os hábitos alimentares e o sedentarismo (ADAIR, 2008).

De acordo com o NHANES (1997 - 2004) a prevalência de excesso de peso em crianças e adolescentes subiu para 17,1% na população americana (OGDEN, 2006). Segundo dados da Pesquisa de Orçamentos Familiares (POF) (IBGE, 2006), a prevalência de sobrepeso nos adolescentes brasileiros entre 2002-2003 foi de 16,7%, enquanto a obesidade atingiu pouco mais de 2% dos adolescentes. Esses dados mostram ainda que o excesso de peso está aumentando significativamente entre adultos e crianças brasileiras (IBGE, 2006). WEISS et al. (2004) relataram em coortes feitas em crianças e adolescentes que a elevação de cada unidade do índice de massa corporal aumenta o risco de síndrome metabólica nesses sujeitos (odds

ratio: 1,55). Em sintonia a esses resultados, VALERIO et al. (2006) descreveram uma prevalência de 41,2% de resistência à insulina em adolescentes obesos, quando comparados aos eutróficos.

Em uma meta-análise FREEMANTLE et al (2008) observaram que a obesidade abdominal, medida das diversas formas, aumentou o risco para diabetes tipo 2 em vários grupos étnicos. Segundo SICREE e SHAW (2007) a epidemia mundial de diabetes tipo 2 está diretamente relacionada ao aumento da obesidade em crianças e adolescentes, embora os adultos ainda sejam os principais atingidos. INVITTI et al. (2007), demonstraram correlações entre obesidade, fibrinogênio e Proteína-C reativa (PCR) em adolescentes italianos, o que caracteriza uma importante relação entre obesidade e risco cardiovascular nessa população. A relação entre PCR e obesidade foi observada da mesma forma em adolescentes brasileiros (SILVA et al., 2010).

YAMAMOTO-KIMURA et al. (2006) identificaram em adolescentes de áreas urbanas do México alta prevalência de fatores de risco cardiovascular. Em concordância, MCGILL et al. (2000) descreveram que a prevenção de doenças cardiovasculares deve se iniciar na juventude, pois observaram associação positiva entre a extensão de aterosclerose nas artérias coronariana de adolescentes e jovens adultos e os valores de VLDL-C e LDL-C, assim como associação negativa com HDL-C.

Portanto, desenvolver pesquisas junto aos adolescentes torna-se extremamente importante, pois a obesidade como processo inflamatório crônico, sistêmico e de baixa intensidade (WARNBERG et al., 2004; LEE e PRATLEY, 2005), predispõe esses indivíduos a eventos até recentemente pouco frequentes nessa faixa etária (HILL et al., 1995; GIL-CAMPOS et al., 2008). Fortalece essas observações estudo realizado pelo Centro de Controle e Prevenção de Doenças (*Center for Disease Control and Prevention* – CDC), que ao avaliar o perfil lipídico de crianças e adolescentes, entre 1999 e 2006, observaram que 20,3% dos jovens apresentavam ao menos um parâmetro lipídico alterado (CDC, 2010). Somando-se a esse perfil, recentemente HALFON and NEWACHECK (2010) analisando a evolução das doenças crônicas na infância observam que a prevalência das doenças

crônicas tem aumentado gradativamente entre as crianças e que há urgência em evidenciar as causas e as formas de prevenção.

Assim, considerando o impacto social do sobrepeso e da obesidade nos adolescentes, torna-se importante avaliar os fatores ambientais que possam modificar os processos associados ao ganho de peso.

1.1.3 Fisiopatologia da obesidade

Evidências demonstram que a obesidade tem sido considerada fator de risco para processos patológicos e inflamatórios crônicos que se iniciam na infância, como a aterosclerose acompanhada ou não de hipertensão e diabetes (ABRANTES et al., 2002; PELLANDA et al., 2002). Esses processos patológicos têm associação direta com a inflamação crônica, que vem sendo considerada ponto central da fisiopatologia da síndrome metabólica e da aterosclerose (ESTEVE et al., 2005; GIL-CAMPOS et al., 2008). De acordo com a revisão de KWITEROVICH et al. (2008), estudos demonstraram que fatores de risco cardiovascular na infância predizem aterosclerose na idade adulta. Embora os mecanismos relacionados à obesidade ainda não estejam totalmente esclarecidos, sugere-se a participação de três importantes eventos: I – Inflamação, II – Resistência à insulina e III - Oxidação (FIEDMAN, 2000; CATERINA et al., 2006; LINNA et al., 2007).

Indivíduos obesos estão caracteristicamente em um estado pró-inflamatório que contribui para lesão endotelial, incremento no risco de doença arterial coronariana e elevação de marcadores inflamatórios (PCR, Fator de necrose tumoral-alfa: TNF- α e Interleucina 6: IL-6), resultantes do desbalanço entre as adipocitocinas. Da mesma forma, adolescentes obesos apresentam concentrações maiores de marcadores pró-inflamatórios, como por exemplo, a PCR (LARKIN et al., 2005; SORIANO-GUILLÉN et al., 2008), o que sugere aumento no risco cardiovascular.

Mediada pelo desbalanço na secreção das adipocitocinas, a obesidade promove danos funcionais e morfológicos locais e sistêmicos, os quais são modulados pelo tamanho da célula adiposa, glicose disponível nessa célula e pelo metabolismo de corticosteroides (SCHMIDT et al., 2005; GREENBERG e OBIN, 2006;). Essas funções permitem uma clara definição do que os adipócitos são para a

saúde e para a obesidade, e como os mediadores inflamatórios atuam nesse processo (ESTEVE et al., 2005; GREENBERG e OBRIN, 2006; GIL-CAMPOS et al., 2008).

Quando em desbalanço, as adipocitocinas tais como adiponectina, leptina e resistina induzem alterações metabólicas, como produção de ácidos graxos não-esterificados e citocinas inflamatórias (TNF- α e IL-6) (SANTOS et al., 2003; COLOMBO e ATALA, 2004; GREENBERG e OBRIN, 2006; GIL-CAMPOS et al., 2008), sendo cada uma delas consideradas fatores-chave na modulação de sensibilidade à insulina.

A resistência à insulina se associa à alta prevalência de componentes da síndrome metabólica, assim como elevado risco cardiometabólico já na adolescência (JUÁREZ-LOPEZ et al., 2010). BARBARROJA et al. (2010) comparando obesos com resistência à insulina e obesos insulino-sensíveis (“saudáveis”) concluiu que o grau de inflamação determina a associação do excesso de peso com as complicações metabólicas.

A inflamação favorece a geração de radicais livres (VANNUCCHI et al., 1998), favorecendo o estado de estresse oxidativo. MARTINO et al. (2008), recentemente demonstraram que o estresse oxidativo na infância tem importante influência na ocorrência precoce de aterosclerose. A obesidade tem sido relacionada ao estresse oxidativo, pois vários estudos têm observado relação positiva entre peso corporal e o conteúdo de LDL oxidada em homens e mulheres (VASANKARI et al., 2001; LINNA et al., 2007). Entretanto, em adolescentes com sobrepeso e obesidade essa relação ainda não está bem estabelecida. Nesse contexto, a presença de LDL eletronegativa (LDL(-)) tem sido monitorada em adultos, crianças e adolescentes como um potencial biomarcador de início e progressão da aterosclerose (HULTHE et al., 2002; OLIVEIRA et al., 2006; BARROS et al., 2006; APOLINÁRIO et al., 2006).

1.2 Fosfolipase A₂ associada à lipoproteína

O processo inflamatório, iniciado na obesidade, contribui significativamente para o início, progressão e ruptura das placas ateroscleróticas ricas em lipídios e componentes inflamatórios (IL-6, TNF- α , linfócitos T e B). Durante esse processo, monócitos diferenciam-se em macrófagos na íntima, alteram seu fenótipo e passam a

secretar um espectro de fatores pró-inflamatórios e protrombóticos, incluindo metaloproteases, espécies reativas de oxigênio, fosfolipases, lipídios bioativos, citocinas pró-inflamatórias e fatores teciduais (TSELEPIS e CHAPMAM, 2002). A ação de radicais livres, das enzimas pró-oxidantes e das fosfolipases, além da glicação não enzimática, de alterações no catabolismo de lipoproteínas, enriquecimento de ácidos graxos não esterificados, reação cruzada com hemoglobina e outros mecanismos possivelmente ainda não identificados favorecem a modificação estrutural e funcional da LDL (SÁNCHEZ-QUESADA et al., 2004; NAKAMURA et al., 2005). A LDL resultante dessas modificações é captada pelos receptores *scavenger* expressos em macrófagos e células musculares lisas, resultando na formação de *foam cells* (TSIRONIS et al., 2004). Nas lesões ateroscleróticas são encontrados ainda auto-anticorpos anti-LDL oxidada (MATSUURA et al., 2006). Atualmente os auto-anticorpos anti-LDL oxidada têm sido descritos como fatores de risco independente para doença aterosclerótica (WADA et al., 2005; CHEN et al., 2008). No entanto, BARROS et al. (2006) propuseram um efeito protetor dos auto-anticorpos, ao observarem valores menores de anticorpos anti-LDL(-) em adolescentes hipercolesterolêmicos ou com história familiar de hipercolesterolemia e doenças cardiovasculares, quando comparados ao grupo controle. Os anticorpos anti-LDL oxidada parecem exercer um efeito protetor nos estágios iniciais de aterosclerose, conforme proposto por GOUNOPOULOS et al., (2007), embora seu papel fisiopatológico ainda exija novas investigações.

Paralelo aos eventos acima, o fator de ativação plaquetário - PAF que é um fosfolipídio ativo envolvido em diversos eventos como a inflamação e anafilaxia passa a ter um papel importante na fisiopatologia da obesidade e aterosclerose (MATSUZAWA et al., 1994). O PAF e os compostos bioativos PAF *like* (fosfolipídios oxidados, éster de colesterol oxidado, ácidos graxos livres oxidados, lisofosfatidilcolina, óxidos de colesterol e isoprostanos) têm sido identificados nas lesões ateroscleróticas e na circulação de indivíduos obesos e com síndrome metabólica (TSELEPIS e CHAPMAM, 2002). DETOPOULOU et al. (2008), observaram que a atividade das enzimas sintetizadoras do PAF apresentam correlação com a inflamação em pacientes com falência cardíaca. Portanto, a

ativação do PAF representa um evento negativo na prevenção ou tratamento da obesidade e morbidades associadas (MCMANUS et al., 2000).

O PAF é inativado por uma enzima específica denominada Fator de ativação plaquetário acetilhidrolase (PAF-AH) (TSIRONIS et al., 2004; CHEN et al., 2007). A PAF-AH tem sido caracterizada como uma fosfolipase que não depende de cálcio para sua ativação, descoberta em 1980 (FAR et al. 1980). Posteriormente, foram descobertas novas ações desta enzima, as quais se diferem das típicas ações das fosfolipases (BLANK et al., 1981). A PAF-AH presente no plasma tem a capacidade de hidrolisar a ligação sn-2 de fosfolipídios com resíduos oxidados, o PAF e os compostos PAF *like*, reduzindo assim, suas atividades biológicas (TSELEPIS e CHAPMAM, 2002). Os resíduos fosfolipídicos oxidados são formados durante a oxidação da LDL e têm papel fundamental nas reações inflamatórias associadas à obesidade e na aterosclerose (TSIRONIS et al., 2004). Portanto, a PAF-AH tendo a habilidade de hidrolisar fosfolipídios oxidados associados às partículas de LDL, pode proteger essa lipoproteína dos efeitos negativos da oxidação, removendo fragmentos de fosfolipídios modificados oxidativamente e, finalmente, contribuir para a redução do processo inflamatório (MATSUZAWA et al., 1994).

A PAF-AH é sintetizada e secretada por macrófagos maduros e ativados, mastócitos ativados e plaquetas (KARABINA et al., 2006) e sua expressão plasmática é regulada por vários fatores, incluindo diferenciação celular e mediadores pró-inflamatórios, tais como os receptores ativadores da proliferação de peroxissomos (PPAR). Embora a secreção de lipoproteínas não pareça influenciar diretamente a síntese e atividade da PAF-AH, o metabolismo intravascular delas e sua meia vida é um fator chave no controle da atividade da PAF-AH (TSELEPIS e CHAPMAM, 2002).

A atividade da PAF-AH no plasma está principalmente associada à LDL (83%), sendo uma pequena porção encontrada na HDL (11%) e sua função é correlacionada fortemente com as concentrações de colesterol associado à LDL (LDL-C) (GUERRA et al., 1997; RIZOS, et al., 2005). Segundo GUERRA et al. (1997), a variação na concentração de colesterol associado à LDL é responsável por um terço das modificações na atividade da PAF-AH. De modo contrário, esses mesmos autores descreveram que a concentração de colesterol na VLDL, um

precursor metabólico de LDL, tem pouca influência na atividade da PAF-AH. Atualmente, sabe-se que mais de 90% da PAF-AH está associada às lipoproteínas. Considerando isso, diversos autores têm proposto denominá-la Fosfolipase A₂ associada às lipoproteínas (Lp-PLA₂) (CASLAKE and PACKARD, 2005), sendo, portanto, a denominação mais aceita atualmente.

1.2.1 Mecanismos de ação da fosfolipase A₂ associada à lipoproteína

Considerando a relação entre fosfolipídios oxidados e aterosclerose, e sabendo-se que o PAF é um mediador da inflamação; a atividade da PAF-AH parece ter um papel importante dentro desse processo (KOSAKA et al., 2001). A PAF-AH plasmática é enzimaticamente ativa e sua especificidade altamente restrita é essencial para prevenir a contínua hidrólise de fosfolipídios presentes nas lipoproteínas nas membranas celulares (TSELEPIS e CHAPMAM, 2002).

Várias evidências demonstram que a modificação oxidativa da LDL está associada com a aterosclerose (VASANKARI et al., 2001; ABUJA, 2001; LAM et al., 2004). WATSON et al. (1995) e SHOHET et al., (1999) mostraram que a PAF-AH é capaz de hidrolisar fosfolipídios oxidados e associaram isso à geração de LDL minimamente oxidada. Esses mesmos autores verificaram que esta enzima está relacionada à prevenção da ligação de monócitos às células endoteliais e a inibição da produção de proteína-1 quimiotática para monócitos (MCP-1).

MATSUZAWA et al., (1994) demonstraram que células com superexpressão de Lp-PLA₂ são resistentes à apoptose induzida por espécies reativas de oxigênio (ROS). Nesse contexto, tem sido proposto que a Lp-PLA₂ pode agir como uma potente enzima antiaterogênica, pois ao hidrolisar fosfolipídios oxidados inibe a modificação oxidativa da LDL. Além disso, a Lp-PLA₂ inibe, *in vitro*, a atividade próaterogênica de fosfolipídios oxidados presentes na LDL oxidada (TSELEPIS e CHAPMAM, 2002). Durante a oxidação da LDL a atividade da Lp-PLA₂ é progressivamente perdida, sugerindo que sua inativação pode facilitar o acúmulo de fosfolipídios oxidados na LDL, o que potencializaria as propriedades próaterogênicas dessa lipoproteína (TSELEPIS e CHAPMAM, 2002). Portanto, o papel básico da Lp-

PLA₂ é prevenir o acúmulo de fosfolipídios oxidados durante a modificação oxidativa da LDL (TSELEPIS e CHAPMAM, 2002). O controle dos lipídios oxidados representa ainda fator de grande importância na prevenção da apoptose mediada pela oxidação celular (MCINTYRE et al., 2009). Nesse sentido, WANG et al. (2010) observaram que a partícula de LDL oxidada estimula a produção de Lp-PLA₂ em monócitos, concluíram ainda que essa regulação se deve aos fosfolipídios oxidados presentes na partícula.

Contrário aos estudos acima e de acordo com STEINBRECHER e PRITCHARD (1989) e SÁNCHEZ-QUESADA et al. (2004) a hidrólise de fosfolipídios oxidados mediada pela Lp-PLA₂, libera lisofosfatidilcolina, um fosfolipídio que participa em vários estágios da formação da placa aterosclerótica. Nesse sentido, CASLAKE et al (2000) propuseram que a Lp-PLA₂, devido sua associação à LDL, representaria um potencial fator de risco para doenças cardiovasculares. Na coorte *West of Scotland Coronary Prevention Study* (WOSCOPS), PACKARD et al, (2000) encontraram maior risco cardiovascular para os maiores quintis de Lp-PLA₂ em indivíduos adultos. Resultados semelhantes foram encontrados nas coortes de Augsburg (KOENIG et al, 1999) e de Malmö (PERSSON et al, 2008). Vários outros estudos de coorte mostraram posteriormente resultados semelhantes, onde a atividade e a concentração da enzima mostraram associação independente com fatores de risco e eventos cardiovasculares (PERSSON et al., 2007; PERSSON et al., 2008; LI et al., 2010). Na primeira, a concentração de Lp-PLA₂ foi diretamente relacionada ao aumento do risco de eventos coronarianos e na segunda observou-se associação entre a atividade da enzima e outros fatores de risco cardiovascular, como forte correlação com o LDL-C e com a espessura da íntima da carótida. Mais recentemente, CASTRO et al., (2007) verificaram que em pacientes com diabetes mellitus tipo 1, brasileiros, a atividade da Lp-PLA₂ foi maior que em pacientes não diabéticos. No mesmo sentido GOMES et al. (2009) demonstraram correlação entre a atividade da enzima Lp-PLA₂ e a susceptibilidade oxidativa da LDL, além de maiores valores de PCR em pacientes diabéticos. OKADA et al. (2006) avaliaram a concentração da Lp-PLA₂ em crianças obesas e observaram que a concentração da enzima apresentou correlação positiva com a gordura abdominal, peso, IMC e LDL-C.

Alguns estudos têm descrito ainda que a ação fisiopatológica da enzima Lp-PLA₂ é determinada pela sua distribuição nas diferentes lipoproteínas; fato que pode ser dependente da espécie. Segundo GARDNER et al. (2008) a enzima Lp-PLA₂ de ratos apresentou maior afinidade pelas partículas mais densas de HDL, enquanto a enzima humana apresenta maior atração por partícula menos densas de HDL.

Diante do potencial papel bimodal da Lp-PLA₂, as revisões de KARASAWA (2006) e KARABINA et al. (2006) demonstraram a partir de vários estudos as vias pró e antiaterogênica que podem ser moduladas pela atividade da Lp-PLA₂. De acordo com esse autor, partículas de Lp-PLA₂ associadas à LDL teriam atividade próaterogênica, enquanto àquelas associadas à HDL seriam antiaterogênicas. No entanto, estudos demonstram que a distribuição da enzima não depende apenas da concentração de colesterol nas lipoproteínas, mas possivelmente de outros fatores ainda não conhecidos, determinando, por exemplo, concentrações maiores da enzima associada à HDL em indivíduos dislipidêmicos e com diabetes, quando comparados aos controles (KUJIRAOKA et al., 2003). TSELEPIS et al. (2001) sugeriram que a um dos principais fatores responsáveis pela redução da afinidade da Lp-PLA₂ à HDL seja a glicação. FAN et al. (2009) demonstraram que quanto maior a relação entre LDL/ Lp-PLA₂ e HDL/ Lp-PLA₂ maiores os níveis de LDL-C, triglicérides, glicose, insulina e menores os de HDL-C em pacientes com síndrome do ovário micropolicístico.

Além desses aspectos, estudos descreveram que populações com alterações genéticas associadas à deficiência de Lp-PLA₂ apresentam um fator de risco independente para a ocorrência da doença coronária (HIRAMOTO et al., 1997; ICHIHARA et al., 1998; YAMADA et al., 1998; YAMADA et al., 2002). De forma contrária, WINKLER et al. (2007) observaram que a atividade da enzima prediz o risco de mortalidade por doença cardiovascular em um período médio de cinco anos e meio e de forma independente indica o risco de morte em paciente com valores baixos e médios de PCR.

Portanto, ainda não se sabe se o aumento na expressão e ativação da Lp-PLA₂ promove aterogênese ou representa um mecanismo de defesa contra a formação de placa e um componente anti-inflamatório (TSELEPIS & CHAPMAM, 2002; RIZOS, et al, 2005). Atualmente, a Lp-PLA₂ tem sido referenciada como um importante

marcador de risco cardiovascular, tanto com relação à sua atividade, quanto sua concentração (BRAUN et al., 2010; The Lp-PLA₂ Studies Collaboration, 2010).

De modo semelhante à Lp-PLA₂, a LDL(-) poderia ter um papel pró e antiaterogênico, pois seu aumento poderia ser resultante da ação protetora da Lp-PLA₂ e, conseqüentemente, seu acúmulo não seria um evento próaterogênico, conforme tem sido demonstrado em diversas publicações (WATSON, 1995; BANCELLS et al., 2008).

1.3 Aspectos nutricionais

O desequilíbrio entre os fatores pró-oxidantes e os antioxidantes, favorecendo os oxidantes, potencializa o dano oxidativo nas células, sendo denominado estresse oxidativo (SIES et al, 1985). A definição mais atual caracteriza o estresse oxidativo de forma mais ampla, ou seja, trata-se do desequilíbrio entre oxidantes e antioxidantes em favor dos oxidantes, determinando uma interrupção da sinalização e controle redox, favorecendo o dano molecular (SIES et al., 1992). O organismo humano utiliza enzimas como superóxido dismutase, catalase, glutathione peroxidase e glutathione reductase, para neutralizar ou reduzir a reatividade de radicais livres. Além desses mecanismos há o sistema de antioxidantes não enzimáticos, onde substâncias não-proteicas de baixo peso molecular provenientes da dieta exercem papel importante no sequestro primário e secundário dos radicais livres (RAMOS et al, 2000). Dentre esses antioxidantes destacam-se alguns componentes dietéticos, tais como o α -tocoferol, β -caroteno, retinol, flavonoides, licopeno, ácido ascórbico, além de outros compostos bioativos.

Desse modo, uma dieta rica em antioxidantes poderia diminuir o estresse oxidativo, a formação e a absorção de peróxidos e seus produtos de decomposição (Yoshida et al., 2005), atuando como ferramenta exógena na modulação da formação de LDL oxidada (SIES & STAHL, 1995). Estudos como o de POLIDORI et al. (2007) demonstraram que antioxidantes dietéticos, avaliados através de sua biodisponibilidade plasmática se encontram reduzidos em pacientes com aterosclerose, quando comparados aos controles. Muitos estudos têm avaliado o efeito dos antioxidantes dietéticos sobre o estresse oxidativo, demonstrando seu efeito benéfico na obesidade (BALESTRIERI et al., 2004; VINCENT et al., 2007;

VINCENT et al., 2009), assim como na doença cardiovascular já estabelecida (DEVARAJ et al., 2007). BECK et al. (2008) avaliando mulheres idosas observaram que o excesso de peso ou a baixa biodisponibilidade plasmática de carotenoides propiciavam maior oxidação das lipoproteínas. CODOÑER-FRANCH et al. (2009) demonstraram que crianças obesas apresentam maior oxidação plasmática, avaliada através de malondialdeído e carbonilas, quando comparadas a crianças magras, apesar de possuírem concentrações semelhantes de α -tocoferol e β -caroteno. VINCENT et al. (2009) observaram que a suplementação com β -caroteno, retinol e ácido ascórbico resultou na redução dos valores de HOMA-IR e concentração de moléculas de adesão em adultos com excesso de peso. No entanto, o efeito da dieta no estresse oxidativo ainda necessita de maiores investigações, pela complexidade do sistema redox e, principalmente, pelo fato da maioria dos estudos apresentar grande heterogeneidade metodológica na avaliação da biodisponibilidade de antioxidantes (BARBOSA et al., 2008). Nesse contexto estudos têm evidenciado que os antioxidantes, especialmente o alfa-tocoferol, podem proteger a partícula de LDL contra oxidação (GRASSMANN et al., 2005; MAFRA et al., 2009).

Além da ação dos antioxidantes dietéticos, outros fatores relevantes são a qualidade e a quantidade dos lipídios dietéticos ingeridos. Estudos mostram que a ingestão de uma dieta rica em lipídios predispõe à dislipidemia, favorecendo a ocorrência de oxidação (SIES et al., 2005). Vale destacar a importância da proporção entre ácidos graxos ômega 3 e ácidos graxos ômega 6, cuja relação tem variado consideravelmente em direção ao maior consumo de ácido graxo ômega 6 (KRIS-ETHERTON et al., 2000). Apesar dessa tendência, tem sido demonstrado que dietas com quantidades mais elevadas de ômega 3 estão fortemente associadas à menor incidência de doenças cardiovasculares (KRIS-ETHERTON et al., 2002). Recentemente, SANDERS et al., (2006) e RUSSO, (2009) descreveram que a redução da proporção entre esses ácidos graxos promoveu redução na concentração de triglicérides e maior proteção cardiovascular, respectivamente. Os ácidos graxos ômega 6 favorecem a ocorrência de trombos, ateromas e inflamação, enquanto os ômega 3 estão associados à redução da inflamação sistêmica e disfunção endotelial, atuando como agente protetor (BROWN, 2001; SIMOPOULOS, 2002). A literatura

apresenta fortes evidências do efeito protetor do ômega 3 no sistema cardiovascular (SCHWALFENBERG, 2006).

RAZQUIN et al. (2009) demonstraram que a dieta do Mediterrâneo, caracterizada pelo elevado consumo de monoinsaturados provindos do azeite de oliva extra-virgem, esteve associada com maior capacidade antioxidante e redução de peso em indivíduos com alto risco cardiovascular, após intervenção de 3 anos.

HATOUM et al. (2010), publicaram recentemente um estudo que associar fatores dietéticos e a atividade da LP-PLA₂. Verificaram que a atividade da enzima se associou inversamente com o consumo de álcool e com o aumento da proteína na dieta. TZOTZAS et al. (2008) observaram que a restrição calórica determinou a redução da concentração plasmática da enzima.

JUSTIFICATIVA

2. JUSTIFICATIVA

De acordo com as evidências científicas, a obesidade é um problema de saúde pública mundial, sendo sua prevalência entre crianças e adolescentes crescente. Além dos aspectos epidemiológicos, sabe-se que a obesidade em adolescentes mantém relação direta com o desenvolvimento precoce de dislipidemia e o aumento dos processos inflamatórios subclínicos e oxidativos. Diante desses pontos a Lp-PLA₂ e os antioxidantes parecem atuar como elementos importantes na saúde cardiovascular de adultos. O papel desses marcadores em adultos tem sido alvo de inúmeros estudos. Entretanto, sua investigação em adolescentes é ainda bastante modesta.

Portanto, avaliar a influência da Lp-PLA₂ e dos antioxidantes lipossolúveis sobre os marcadores cardiometabólicos clássicos (perfil lipídico, IMC, etc) e emergentes (CETP, LDL(-), NEFAs), em adolescentes, pode ampliar os conhecimentos sobre o papel desses componentes na saúde cardiovascular desse segmento da população. A **Figura 1** apresenta uma hipótese de modulação da aterosclerose através das alterações relacionadas à Lp-PLA₂ e a antioxidantes na obesidade em adolescentes.

Figura 1: Hipótese do estudo

- Red box: Variáveis analisadas no presente estudo.
- Dark red box: Destaque para a enzima Lp-PLA₂ e os antioxidantes.

OBJETIVO

3. OBJETIVO

3.1 Objetivo Geral

Avaliar a possível associação da atividade da Lp-PLA₂ e de antioxidantes lipossolúveis com marcadores cardiometabólicos em adolescentes de Escolas públicas localizadas na Zona Oeste da cidade de São Paulo.

3.2 Objetivos Específicos

- Avaliar o perfil lipídico;
- Analisar o tamanho da partícula de HDL;
- Avaliar a resistência à insulina;
- Determinar a concentração das apolipoproteínas;
- Determinar a concentração de adipocitocinas;
- Avaliar a concentração de ácidos graxos não esterificados;
- Determinar a concentração da proteína transportadora de éster de colesterol;
- Avaliar a atividade da enzima Lp-PLA₂;
- Estimar o consumo de antioxidantes lipossolúveis;
- Detectar LDL(-) e seus auto-anticorpos no plasma.

METODOLOGIA

4. METODOLOGIA

4.1 Casuística

O estudo é do tipo corte transversal. A população do estudo foi composta por adolescentes de ambos os sexos, com idade entre 10 a 19 anos, segundo OMS (2005). Os participantes foram selecionados de escolas públicas de ensino médio e fundamental localizadas na Zona Oeste da cidade de São Paulo. Participaram do estudo seis escolas localizadas na Zona Oeste da cidade de São Paulo. A região alvo da pesquisa (Zona Oeste) foi selecionada devido aos fatores logísticos associados, tais como localização próxima à Faculdade de Saúde Pública da USP e facilidade no transporte de amostras. A inclusão de adolescentes no estudo ocorreu de novembro de 2007 a abril de 2009.

Os adolescentes incluídos no estudo foram distribuídos em três grupos de acordo com a classificação do estado nutricional proposta por COLE et al. (2000 e 2007): Eutrófico, Sobrepeso e Obeso. Todos os responsáveis pelos adolescentes passaram pelo processo de esclarecimento, após o qual assinaram o Termo de Consentimento Livre e Esclarecimento (**Anexo 1**). Todos os procedimentos de obtenção de amostras, análises e divulgação dos resultados seguiram as normas do Conselho Nacional de Saúde, no que se refere à Ética em Pesquisa com Seres Humanos (BRASIL, 1999).

4.2 Critérios de inclusão

Foram incluídos no estudo adolescentes de ambos os sexos, que apresentaram as seguintes características:

- Idade entre 10 – 19 anos;
- Regularmente matriculado em uma das escolas participantes do estudo;
- Clinicamente saudável;
- Sem uso frequente de álcool (utilizamos os valores estabelecidos para adultos: consumo $\leq 30,0$ mL de etanol/dia para homens e $\leq 15,0$ mL de etanol/dia para mulheres, segundo IV Diretrizes Brasileira de Hipertensão (SOCIEDADE BRASILEIRA DE HIPERTENSÃO - SBH, 2004);

- Não usuários de medicamentos moduladores do metabolismo lipídico, antioxidantes e hormônios há pelos menos 2 meses.

As informações referentes a esses critérios foram obtidas a partir do auto-relato dos adolescentes ou responsáveis.

4.3 Critérios de exclusão

Foram excluídos do estudo adolescentes com as características abaixo:

- Desnutrição;
- Gravidez ou lactação;
- Presença de doenças crônicas, tais como, neuropatias, endocrinopatias, nefropatias, doenças pulmonares, cardiopatias e doenças reumatológicas, imunodeficiência congênita ou adquirida, além de síndrome de Cushing e hipotireoidismo investigada através do auto-relato;
- Participação em outros protocolos de pesquisa;
- Fumantes (indivíduos que apresentaram o hábito de fumar, não importando a frequência do fumo);
- Situações agudas como gripe, febre e alergias no período da coleta de dados.

4.4 Caracterização da amostra

A amostra foi caracterizada por meio de um questionário estruturado (**Anexo 2**), onde foram avaliados o perfil social, econômico, cultural e de saúde dos adolescentes, assim como os antecedentes de doenças maternos e paternos.

4.5 Delineamento do estudo

Este estudo é do tipo corte transversal e incluiu as seguintes etapas de coleta de dados:

ETAPA I – Identificação de escolares e séries com potenciais adolescentes inclusos no estudo. Entrega do termo de consentimento livre e esclarecido. Contato com os pais e agendamento da coleta de sangue e demais informações.

ETAPA II – Recolhimento do termo de consentimento. Avaliação antropométrica e de composição corporal, coleta de sangue e aplicação do primeiro R24h.

ETAPA III – Aplicação do segundo e terceiro R24h.

Figura 2: Fluxograma de coleta.

4.6 Avaliação antropométrica

O peso atual foi obtido através da medição em balança digital Control® (Plenna, São Paulo, Brasil), com capacidade de 150,0 kg e precisão de 100 g. Através do estadiômetro AlturaExata® (TBW, São Paulo, Brasil), com limite de 2,10 m e precisão de 1,0 mm foi aferida a altura de cada indivíduo. O protocolo de obtenção das medidas de peso e altura seguiu a metodologia descrita por LOHMAN et al. (1988). A partir das medidas de peso e altura foi determinado o índice de massa corporal ($IMC = \text{peso (kg)} / \text{altura}^2 \text{ (m)}$), o qual foi classificado, segundo COLE et al. (2000 e 2007).

Foi coletada a medida de circunferência da cintura, utilizando uma fita inelástica, flexível, com precisão 1,0 mm (TBW, São Paulo, Brasil), localizada no ponto médio entre a última costela e a crista ilíaca utilizado como referência anatômica.

Para obtenção da composição corporal (% de gordura e % de massa magra) foi realizada impedância bioelétrica (BIA), com o uso do aparelho tetrapolar Biodynamics®, modelo 450 (TBW, São Paulo, Brasil), calibrado para a corrente de 800 μA e frequência de 50 KHz .

As medidas foram obtidas por profissional treinado e realizadas com os adolescentes deitados na posição supina, do lado direito do corpo. Dois dispositivos presos por eletrodos foram fixados no dorso da mão direita e dois no dorso do pé direito, conforme protocolo recomendado pela Biodynamics® para realização da impedância bioelétrica.

4.7 Avaliação da maturação sexual

A maturação sexual foi analisada por meio da auto-avaliação, seguindo os critérios de estadiamento puberal propostos por MARSHALL (1969) e MARSHALL & TANNER (1970). Foram fornecidas aos adolescentes figuras com diferentes graus de desenvolvimento da pilosidade pubiana e do desenvolvimento genital. Para as meninas foram apresentadas figuras que mostram diferentes graus de desenvolvimento mamário, além de figuras com diferentes graus de pilosidade pubiana (**Anexo 3**). Cada adolescente realizou sua auto-classificação, analisando as figuras e optando pelo código correspondente ao seu estadio puberal. As meninas foram também questionadas sobre a idade da menarca.

A classificação da maturação sexual como pré-púbere e púbere, para cada sexo, foi realizada segundo a OMS (1995) (**Figura 3**).

Figura 3: Classificação da maturação sexual.

Sexo	Estadio de Tanner	Classificação
Masculino	Genitália no estadio 1 ou 2	Pré-púbere
	Genitália a partir estadio 3	Púbere
Feminino	Mamas no estadio 1	Pré-púbere
	Mamas a partir estadio 2	Púbere

Fonte: Adaptada de OMS (1995).

4.8 Obtenção de sangue

Após jejum de 12h, coletou-se uma amostra de sangue (20,0 mL). O sangue foi coletado em tubos vacutainer contendo ácido etileno-diaminotetraacético-EDTA (1,0 mg/mL), utilizado como anticoagulante e antioxidante, mantido em gelo e protegido da luz até a obtenção do plasma ou soro. Ao plasma acrescentou-se os

seguintes inibidores de proteases: aprotinina (2 µg/mL), benzamidina (2,0 mM) e PMSF (1 mM), assim como o antioxidante BHT (20 mM). Em seguida, o material foi armazenado em temperatura abaixo de -70° até o momento da análise.

4.9 Análise do perfil lipídico

Através da aplicação manual de métodos colorimétricos e enzimáticos foram determinadas as concentrações de colesterol total, colesterol associado à lipoproteína de alta densidade - HDL e triglicérides totais no plasma. Para a determinação das concentrações de colesterol foi utilizado o Kit Colesterol Liquiform® (Labtest, Brasil), para a determinação do colesterol HDL foi utilizado o Kit HDL Liquiform® (Labtest, Brasil) e para a determinação de triglicérides, utilizou-se o Kit Triglicérides Liquiform® (Labtest, Brasil).

O colesterol associado à lipoproteína de baixa densidade (LDL) foi determinado através da equação de FRIEDWALD (1972), a partir da concentração de colesterol total, colesterol na HDL e triglicérides.

Além da análise da concentração de colesterol total, HDL-C, triglicérides e LDL-C foram verificadas as relações entre o conteúdo de colesterol das lipoproteínas. O índice de Castelli I foi determinado pela razão entre o colesterol total e o HDL-C e o de Castelli II pela razão entre LDL- C e HDL-C (CASTELLI et al., 1983).

Na sequência, determinou-se o colesterol não associado à HDL a partir da subtração da fração HDL-C do colesterol plasmático total, a fim de quantificar melhor a concentração de colesterol associado às lipoproteínas aterogênicas.

4.10 Determinações das apolipoproteínas e adipocitocinas

A tecnologia Luminex™ x MAP foi utilizada para a determinação da concentração plasmática das apolipoproteínas AI e B e das adipocitocinas leptina, resistina e adiponectina do soro (ELSHAL et al., 2006).

Essa técnica é realizada por um processo que cora microesferas de látex com dois fluoróforos. Em função de determinadas proporções desses fluoróforos, podem ser criados 100 diferentes conjuntos de microesferas, definidos por um código de

cores que pode ser identificado pelo instrumento Luminex. Os kits LINCOplex™ constituem um imunoensaio (ELISA sanduíche) realizado por meio dessas esferas. Para tanto, anticorpos de captura específicos para ApoAI, B, leptina, resistina e adiponectina imobilizaram as microesferas através de ligações covalentes não reversíveis. Na sequência, a detecção final foi realizada pela adição do anticorpo secundário conjugado a uma substância fluorescente a ficoeritrina (PE). Desse modo o equipamento Luminex 100® realiza a leitura da sequência de microesferas através de feixes de luz emitidos por dois lasers em um citômetro de fluxo. O primeiro feixe de laser detecta (classifica) a microesfera (o código de cor para o ensaio) e o segundo laser quantifica o sinal do conjunto (amostra). As análises foram realizadas em duplicata e os resultados expressos em ng/mL. Essa etapa foi realizada pelo Laboratório da empresa Gênese Produtos e Diagnósticos®.

Os valores de Apo AI e B foram utilizados em sua forma bruta assim como no ajuste das concentrações de HDL-C e LDL-C, respectivamente.

4.11 Determinação dos antioxidantes (α -tocoferol, beta-caroteno, retinol e licopeno)

A concentração plasmática de antioxidantes foi determinada por cromatografia líquida de alta eficiência (HPLC), pela empresa VitaE Cromatografia Líquida em Análises Clínicas.

A extração das vitaminas do plasma foi realizada por meio dos seguintes kits comerciais: retinol e alfa-tocoferol (Cód. KC1600 Chromsystems, Munchen, Germany), beta-caroteno (Cód. 3200, Chromsystems, Munchen, Germany) e licopeno (Cód. 3200, Chromsystems, Munchen, Germany), seguido de posterior aplicação e análise no HPLC (cromatografia líquida de alto desempenho). Todas as análises foram realizadas na presença de padrões externos com múltiplos pontos de concentração e padrão interno. Os resultados foram expressos em: μ mol para todos os antioxidantes analisados.

4.12 Determinação de glicose e insulina

A concentração da glicose plasmática foi obtida através do kit comercial, enzimático e colorimétrico Glicose PAP Liquiform® (Labtest, Minas Gerais, Brasil). Enquanto a insulina plasmática foi avaliada por meio da técnica de radioimunoensaio, através do kit comercial *Human Insulin-Specific* RIA Kit® (Linco Research, St Charles, MO, USA). Essa etapa foi terceirizada junto ao Laboratório da empresa Gênese Produtos e Diagnósticos®. Os resultados foram expressos em mg/dl e uU/mL, para glicose e insulina, respectivamente.

Posteriormente a resistência à insulina foi determinada pelo índice HOMA-IR (*homeostasis model assessment – insulin resistance*), que foi calculado da seguinte forma (Matthews et al., 1985):

$$\text{HOMA-IR} = [\text{Insulina de jejum } (\mu\text{U/mL}) \times \text{glicemia de jejum (mmol/L)}] / 22,5$$

4.13 Determinação dos ácidos graxos não esterificados

O kit comercial *HR Series NEFA-HR(2)*® (WAKO Diagnostics, Texas, EUA) foi utilizado para determinar a concentração dos Ácidos graxos não esterificados (NEFAs) no plasma. O Kit consiste em um ensaio enzimático colorimétrico, no qual é adicionado ao plasma a acil-CoA sintetase, na presença de ATP e CoA. A acil-CoA foi produzida nessa reação e oxidada na sequência pela adição da acil-CoA oxidase. A oxidação gerou o peróxido de hidrogênio, que na presença de peroxidase permitiu a condensação oxidativa de 3 meti-N-etil-N (β-hidroxietil) -anilina com 4-aminoantipirina para formação de um produto final de cor roxa. Dessa forma, a determinação da concentração dos NEFAS pôde ser realizada a partir da densidade óptica desse produto ($\lambda_{\text{primário}} = 560\text{nm}$ e $\lambda_{\text{secundário}} = 670\text{nm}$). As amostras foram analisadas em duplicatas e os resultados foram expressos em Eq/L.

4.14 Determinação da concentração da proteína transportadora de éster de colesterol

O kit comercial *CETP Activity Assay*® (BioVision Research Products, CA, EUA) foi utilizado para a determinação da concentração da Proteína Transportadora de Éster de Colesterol (CETP) no plasma. O método utiliza uma molécula doadora

de lipídios que contém um marcador lipídico fluorescente neutro. Na presença de CETP esse lipídio é transferido para outra molécula receptora. A molécula receptora pela adição do marcador apresenta um aumento de sua fluorescência que pode ser determinado por leitor específico (E_{exc} 465 nm e E_{em} 535 nm) e mantém correlação direta com a concentração da enzima. As análises foram realizadas em duplicata, sendo os resultados expressos em pmol/ μ l/hora.

4.15 Determinação do tamanho da partícula de HDL

O tamanho da partícula de HDL foi determinado pelo método descrito por LIMA and MARANHÃO (2004). Inicialmente a 500 μ L de plasma foi adicionado 500 μ L de polietilenoglicol (8000). Após agitação em vortex por 30 segundos, a HDL foi isolada através de centrifugação (1500g, 10 minutos, 25° C). O sobrenadante foi coletado e se adicionou a ele 1,5mL de NaCl (10mmol/L). Posteriormente, essa mistura foi filtrada através de uma membrana de 0.22 μ m (Millipore Products Division, Bedford, MA) para excluir qualquer impureza. O tamanho da partícula pôde então ser determinado através do aparelho Zeta Potential Analyzer instrument ZetaPALS[®] (Brookhaven Instruments Corporation, NY, USA). O resultado foi obtido pela media $\pm$ desvio padrão das leituras realizadas e expressos em nm.

4.16 Determinação da atividade da enzima Lp-PLA₂

A determinação da atividade da enzima Lp-PLA₂ foi realizada por meio do Kit *PAF – Acetylhydrolase Assay* (catálogo nº 760901, Cayman Chemical Company, USA). Esse ensaio se baseia na utilização do substrato 2-tiol PAF. Após a hidrólise do acetil-tioéster na posição da ligação *sn*-2, tióis livres foram detectados utilizando o ácido 5,5'-ditio-*bis*-(2-nitrobenzóico) (DTNB). Quanto mais elevado o sinal de absorbância encontrado, maior a atividade da enzima Lp-PLA₂. As análises foram realizadas em duplicata, sendo os resultados expressos como nmol/min/mL.

4.17 Avaliação da concentração de LDL(-) no plasma

A concentração de LDL(-) foi detectada no plasma através de ELISA (*Enzyme-Linked Immunosorbent Assay*) sanduíche, seguindo protocolo padronizado por nosso grupo de pesquisa. A sensibilização das placas (Costar[®], modelo 3690, Corning, USA) foi feita com anticorpo monoclonal anti-LDL(-) (MAB-1A3) (0,5 µg/mL, 50 µL/poço), diluído em tampão carbonato-bicarbonato, (0,25 M, pH 9,6), sendo as placas incubadas *overnight* a 4°C. Após esse período, os sítios livres foram bloqueados com leite desnatado (Molico[®], Nestlé, São Paulo, Brasil), diluído a 5% em tampão fosfato salina 0,01mol/L (PBS - pH 7,2) e incubados a 37°C por 2 horas. Em seguida, as placas foram lavadas quatro vezes com PBS-Tween (0,05%). Foi adicionado 50 µL/poço de plasma diluído 1:1600 em tampão PBS, sendo a placa incubada por 2 horas a 37°C. Após essa etapa, a placa foi lavada, conforme descrito acima, e foi adicionado 50 µL/poço de anticorpo monoclonal anti-LDL(-) biotilado (MAB-2C7) (0,12 µg/mL, 50 µL/poço). As placas foram incubadas a 37°C por 1 hora, em seguida, foram lavadas conforme descrito acima. Após essa etapa, foram adicionados 50 µL/poço de estreptoavidina-peroxidase (Sigma[®]) (1:60000) diluída em PBS. As placas foram incubadas por 1 hora, a 37°C e, novamente lavadas, conforme descrito acima. A reação de cor foi desenvolvida através da adição de substrato composto por 3,3',5,5'-tetrametilbenzina (TMB), tampão citrato-fosfato (0,1 M, pH 4,2) e H₂O₂ (30%) (250/12/10, µL/mL/µL). As placas foram incubadas por 10 minutos a 37°C, protegidas da luz. A reação foi bloqueada com 50 µL/poço de H₂SO₄ (2,0 M) e a absorbância monitorada em 450 nm. Os resultados foram expressos a partir da média das absorbâncias das amostras menos o branco aplicadas à curva padrão de LDL(-) (20,0 a 0,00061 ug/mL) e, posteriormente, multiplicadas pela respectiva diluição. O anticorpo utilizado nessa análise foi gentilmente doado pela Profa. Dra. Dulcineia Saes Parra Abdalla, do Laboratório de Bioquímica Clínica da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo (FCF-USP).

4.18 Avaliação da concentração de auto-anticorpos anti-LDL(-)

Os auto-anticorpos anti-LDL(-) foram detectados através de ELISA de captura de anticorpo. A LDL(-), isolada por FPLC, foi diluída em tampão Carbonato-

Bicarbonato (0,25 M, pH 9,6) até concentração final de 0,5 µg de proteína/poço, e incubada *overnight* a 4°C. Os espaços livres foram bloqueados com 5% de leite desnatado diluído em tampão Fosfato-Salina 0,01mol/L (PBS - pH 7,4) e incubados a 37°C por 2 horas. As placas foram lavadas quatro vezes com PBS-Tween (0,05%). As amostras foram diluídas (1:1600) em PBS e incubadas em temperatura de 37°C por 2 horas. As placas foram novamente lavadas, conforme descrito acima. Adicionou-se 50 µL/poço de anti-IgG humana marcada com peroxidase, (1:2000) diluída em PBS. As placas foram incubadas por 1 hora e 30 min, à 37°C, seguida de lavagem, conforme descrito acima. A reação de cor se desenvolveu através da adição de substrato composto por 3,3',5,5'-tetrametilbenzina (TMB), tampão citrato-fosfato (0,1M, pH 4,2) e H₂O₂ (30%) (250/12/10, µL/mL/µL). As placas foram incubadas por 30 minutos à 37°C sob proteção da luz. A reação foi bloqueada com 50 µL/poço de H₂SO₄ (2,0 M) e a absorbância monitorada em 450 nm. Os resultados foram determinados aplicando-se a média das absorbâncias das amostras - branco à equação da curva-padrão de IgG humana (23,4 – 0,19 µg/mL).

4.19 Avaliação do consumo

A avaliação dos hábitos alimentares dos adolescentes foi realizada por meio de três Recordatórios de 24 horas (R24h), cujas informações foram coletadas em dias não consecutivos, incluindo um final de semana (**Anexo 4**). O primeiro R24h foi realizado por meio de entrevista direta, com o auxílio de um Registro Fotográfico Alimentar (ZABOTTO *et al.*, 1996) e os demais por meio de telefone.

A energia (Kcal) e os seguintes nutrientes: carboidratos, lipídios e proteínas (g), retinol (µg), β-caroteno (µg), licopeno (µg), vitamina E (mg), gorduras saturadas (g), gorduras poliinsaturadas (g), gorduras monoinsaturadas (g), ácido oleico (g), ácido linoleico (g), alfa-linolênico (g), colesterol (mg) e fibras (g) foram submetidos à regressão linear para verificar possível correlação com a energia. Nessa análise, as calorias ingeridas foram consideradas variável independente e o valor absoluto do nutriente variável dependente. Todos os nutrientes com distribuição paramétrica que apresentaram coeficiente de correlação (r) próximo de um e significância de $P < 0,05$ foram ajustados pelo Método Residual, proposto por MACKERRAS (1996) e

WILLETT e STAMPFER (1998). Os demais nutrientes foram avaliados na sua forma bruta.

Os pesquisadores de campo receberam treinamento para poderem desenvolver essa atividade, conforme **Anexo 5**.

Os resultados obtidos foram analisados através do programa *NutWin*[®] (UNIFESP, 2005). As tabelas de composição: Tabela Brasileira de Composição de Alimentos - Versão 2 (Núcleo de Estudos e Pesquisas em Alimentação – NEPA/UNICAMP, 2006) e *United States Department of Agriculture* (USDA, 2001); foram utilizadas para inserir novos alimentos ao banco de dados do programa *NutWin*[®] (UNIFESP, 2005).

Além do possível ajuste de nutrientes pela energia, utilizamos o programa PCSide (versão 1.0) para corrigir a variabilidade intrapessoal (NUSSER et al. 1996).

4.20 Análise Estatística

Todas as análises antropométricas e bioquímicas foram coletadas em triplicata, sendo os resultados apresentados sob a forma de média $\pm$ desvio padrão para cada grupo. A análise comparativa e possíveis associações foram avaliadas com o auxílio do programa de computador *Statistical Package for the Social Sciences*[®] (SPSS) versão 15.0 (SPSS INCORPORATION, 2000). O valor de significância considerado foi de 5%.

Para a determinação dos testes utilizados, considerou-se o tipo de distribuição das variáveis, através do teste Kolmogorov-Smirnov ($P > 0.05$). Nos dois artigos para comparação entre grupos foram realizadas utilizando o teste ANOVA (paramétricos) ou Kruskal-Wallis e Mann-Whitney U-test (não paramétricos). Para variáveis categóricas as diferenças foram determinadas pelo teste χ^2 . A maturação sexual e distribuição de sexos entre os grupos se apresentaram estatisticamente similares.

Para o artigo 2 modelos de regressão univariada foram aplicados para avaliação do efeito de variáveis antropométricas/composição corporal e perfil lipídico sobre a variável dependente atividade da Lp-PLA₂. Nesse mesmo sentido, buscando explorar a habilidade dessas variáveis em explicar a atividade da enzima, um teste de regressão multivariado foi aplicado. Os resultados foram expressos

através do coeficiente-b, do R-quadrado e do *P*. O Odd Ratio para o maior quartile de atividade da enzima (≥ 15.9) foi aplicado.

No artigo 3 foram realizadas correlações entre as variáveis de risco cardiovascular e os antioxidantes plasmáticos. Da mesma forma, correlação parcial foi aplicada no sentido de verificar a influência do IMC nas relações encontradas.

RESULTADOS

5. RESULTADOS

ARTIGO 1: “Lp-PLA₂ maybe an important cardiovascular biomarker in adolescents”

Artigo Original

Submetido à European Journal of Clinical Nutrition

Title: Lp-PLA₂ as an important biomarker of cardiovascular risk in adolescents

Sort title: Lp-PLA₂ and obese adolescent's cardiovascular risk factors.

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Summary

Background/Objectives: To evaluate if Lp-PLA₂ activity can be a cardiovascular risk marker in obese adolescents

Subjects/Methods: This cross-sectional study included 242 adolescents (10 - 19 years) of both genders. These subjects were classified in Eutrophic (n=77), Overweight (n=82) and Obese (n=83) groups. Lipid profile, glucose, insulin, HDL size, LDL(-) and anti-LDL(-)antibodies were analyzed. The Lp-PLA₂ activity was determined by a colorimetric commercial kit. Body mass index, waist circumference and body composition were monitored. Food intake was evaluated using a 3-day food record.

Results: HDL-C, insulin, HOMA and LDL(-) values demonstrated the negative profile of the obese group. Moreover, the Lp-PLA₂ activity changed proportionally to BMI, waist circumference and fat mass percentage. It was also positively associated with HOMA-IR, glucose, insulin and almost all variables of lipid profile. Furthermore, it was negatively related to Apo AI ($\beta=-0.137$; $P=0.038$) and strongly positively associated with Apo B ($\beta=0.293$; $P<0.001$) and with Apo B/Apo AI ratio ($\beta=0.343$; $P<0.001$). The better predictor model for enzyme activity included Apo B/Apo AI ($\beta=0.327$; $P<0.001$), HDL size ($\beta=-0.326$; $P<0.001$), WC ($\beta=0.171$; $P=0.006$) and glucose ($\beta=0.119$; $P=0.038$). Logistic regression analysis demonstrated that changes in Apo B/Apo AI ratio were associated with a 73.5 times higher risk to elevated Lp-PLA₂ activity.

Conclusions: The Lp-PLA₂ activity, in adolescence, varies with nutritional status and it is related to markers of cardiovascular risk, mainly with Apo B/Apo AI ratio.

This results confirm that Lp-PLA₂ activity is a marker of cardiovascular risk in adolescence.

Keywords: Adolescent; Lp-PLA₂; cardiovascular risk; obesity

Introduction

Obesity is growing very fast in all age groups, but the growth rate has been much more acute in children and adolescents (NCHS, 2010). According to the National Center for Health Statistics (NCHS), obesity increased from 5% to 18.1% among adolescents between 1976-1980 and 2007-2008 (NCHS, 2010). This profile is consistent with the Brazilian Institute of Geographic and Statistic, which showed that overweight in Brazilian adolescents increased from 16.7% (2002-2003) to 21.5% (2008-2009) (IBGE, 2006, 2010).

The metabolic imbalance in obesity supports inflammation, insulin resistance and oxidation (FIEDMAN, 2000; DANDONA *et al.*, 2004; LINNA *et al.*, 2007). In adolescents, these components favor the early occurrence of atherosclerosis (HOLVOET, 2008) by the emergence of chronic diseases like diabetes and abnormal lipid levels (SEARCH for Diabetes in Youth Study Group, 2006; CDC, 2010).

The incidence of obesity in adolescence may perhaps represents a first step to atherosclerosis in adults (CELIK *et al.* 2009). Many factors can be associated with cardiovascular diseases, and it is known that Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is a possible emerging biomarker (THE Lp-PLA₂ STUDIES COLLABORATION, 2010). Lp-PLA₂ is an enzyme produced by inflammatory cells (KARABINA *et al.*, 2006), that circulates on plasma linked in high proportion with LDL (85%) and less proportion to HDL (15%) (RIZOS, *et al.*, 2005). Afterwards, it was observed that Lp-PLA₂ has the ability to hydrolyse oxidized phospholipids, reducing their biological activity (TSELEPIS e CHAPMAM, 2002). However, this event results on the formation of lysophosphatidylcholine, an inflammatory

component that participates in the development of atherosclerotic plaque (SÁNCHEZ-QUESADA et al, 2004).

Furthermore, it has been observed that Lp-PLA₂, in adults, has association with other cardiovascular risk markers, such as LDL-C and C-reactive protein (PERSSON et al, 2007; Okamura *et al.*, 2007). A recent review showed that Lp-PLA₂ is related to cardiovascular events in adults, even after adjustment by Framingham risk score and C protein reactive (MADJID *et al.*, 2010). Despite these results, the monitoring of Lp-PLA₂ in children and adolescents is scarcely described in literature. In addition, it is not known if obesity is associated to change of Lp-PLA₂ in adolescents. In this context, the aim of this article is to evaluate if Lp-PLA₂ can be a cardiovascular risk marker on obese adolescents.

Material / subjects and Methods

Study subjects

In a cross-sectional study, we evaluated male and female adolescents between ten and nineteen years old (WHO, 2005), selected from public schools located at western area of São Paulo city, SP, Brazil. The inclusion criteria were no smoking, no regular use of alcohol (≤ 30.0 mL of ethanol/day for boys and ≤ 15.0 mL of ethanol/day for girls) (SOCIEDADE BRASILEIRA DE HIPERTENSÃO, 2004), no use of lipid-lowering drugs, no acute disease, no participate on other research protocols and no pregnancy or breastfeeding for at least two months before data collection.

The adolescents included in this study were distributed into 3 groups: healthy weight (HW), overweight (OV), and obese (OB), according to the classification of body mass index (BMI - weight/height^2) proposed by Cole et al. (2000, 2007) for sex and age. The protocol study was approved by the Ethics Committee (School of Public Health, University of São Paulo; Proc. #1668) and

followed the recommendations of the National Council for Health on Ethics in Research with Humans. All the parents of the adolescents signed an informed written consent.

Anthropometric Measurements

Height and weight were determined by a stadiometer (AlturaExata, TBW Brazil, Sao Paulo, SP, Brazil) and by a digital scale (Control, Plenna, Sao Paulo, SP, Brazil), respectively. Waist circumference (WC) was measured using a 1 mm precision flexible and inelastic tape (TBW Brazil[®], Sao Paulo, SP, Brazil). Body composition was evaluated by bioelectric bioimpedance technique, using the instrument tetrapolar Biodynamics[®], model 450 (TBW, São Paulo, Brazil). Sexual maturation was evaluated according to Marshall and Tanner (1970) and Marshall, (1969).

Food Intake

Food intake was evaluated by three 24-hour diet recalls, collected on non consecutive days, including one weekend day. The first recall was conducted by a direct interview and the others by phone interview. Energy (kcal), carbohydrates (g), lipids (g), proteins (g), saturated fat acid (g), monounsaturated fat acid (g), polyunsaturated fat acid (g), oleic acid (g), linoleic acid (g), linolenic acid (g) and fiber (g) were measured through the software *NutWin*[®] (UNIFESP, 2005). These data were adjusted by energy (WILLETT e STAMPFER, 1998 and MACKERRAS, 1996) and by intra-personal variability (NUSSER *et al.*, 1996).

Blood samples and lipid analysis

The levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined by standard methods (Labtest Diagnóstica, MG, Brazil). The LDL-cholesterol (LDL-C) levels were calculated by the Friedewald equation (FRIEDEWALD et al, 1972). Similarly, TC/HDL-C, LDL-C/HDL-C, no-HDL-C and TG/HDL-C ratios were calculated. The apolipoproteins AI (Apo AI) and B (ApoB) were evaluated by commercial kits

(RANDOX®, Co, Antrim, United Kingdom). The concentrations of HDL-C and LDL-C were normalized by Apo AI and B levels, respectively.

Glucose and Insulin measures

The glucose was determined by an enzymatic and colorimetric commercial kit (Glicose PAP Liquiform® - Labtest, MG, Brazil). For insulin levels, the radioimmunoassay was applied using the standard method *Human Insulin-Specific* RIA Kit® (Linco Research, St Charles, MO, USA). Following up, the insulin resistance was estimated by HOMA-IR index (homeostasis model assessment – insulin resistance), where: $HOMA-IR = [\text{fasting insulin level } (\mu\text{U/mL}) \times \text{fasting glucose (mmol/L)}] / 22.5$ (MATTHEWS *et al.*, 1985).

HDL Size

The HDL size was measured by Laser-Light-Scattering method established by LIMA and MARANHÃO (2004).

LDL(-) and Anti-LDL(-)antibodies analysis

The electronegative low density lipoprotein LDL(-) was assessed by ELISA using monoclonal antibodies (MAb 1A3 and MAb 2C7), according to DAMASCENO *et al.* (2006), while the autoantibodies against LDL(-) were evaluated by ELISA, according to DAMASCENO *et al.* (2007).

Lp-PLA₂ activity

Lp-PLA₂ activity in plasma was determined by an enzymatic PAF-Acetylhydrolase Assay kit (catalog no. 760901, Cayman Chemical Company, USA). The analysis were performed in duplicate, with its results expressed in nmol/min/mL.

Statistical analysis

The statistical analysis were performed using the SPSS software, version 15.0. Following the evaluation of the data distribution by Kolmogorov-

Smirnov test ($P > 0.05$), differences between groups were determined by ANOVA test (normally data) or Kruskal-Wallis and Mann-Whitney U-test (not normally distributed data). The χ^2 test was used for comparison of categorical variables and results were expressed as relative frequencies (%). Initially, there were performed sexual maturation- and sex-specific analysis; nonetheless, the groups showed similar profiles. Therefore, these variables were not accepted as confounders.

Univariate regression models were applied in order to determine the effect of anthropometric/body composition and lipid measures (as explanatory variables) on Lp-PLA₂ activity (as dependent outcome). In order to explore the ability of the variables that showed association with Lp-PLA₂, additive multivariate regression models were constructed using the following variables: BMI, WC, %FM, TC, LDL-C, TC/HDL-C, non-HDL-C, Apo AI, Apo B, Apo B/Apo AI, HDL size, insulin, glucose and HOMA. Results from regression models are showed as b-coefficients, R-squared values and P -value.

We have also performed the Odd Ratio (OR) using Lp-PLA₂ activity ≥ 15.9 as cut off point. This point was defined by the highest quartile (Q4) of Lp-PLA₂ activity. Statistical significance was established for P -value < 0.05 .

Results

From the five selected public schools, 2746 adolescents were enrolled in this study. This population was initially invited to participate in the nutritional screening (BMI). After a preliminary nutritional status classification, all adolescents with overweight ($n=481$), obesity ($n=135$) and a randomized sub-sample of eutrophic ($n=756$) adolescents were invited to the second phase of the study. A total of 261 adolescents completed all stage of data collection. After an examination of collected data, 19 subjects were excluded due to the use of lipid-lowering and anti-inflammatory drugs during the collection.

Table 1 shows the characteristics of the adolescents included in the study, according to BMI. The proportion of girls (HW=51%, OV=45% and OB=52%) were statistically similar between groups ($P=0.321$). While the OV group (13.3 ± 1.9

years) was younger than both OB and HW adolescents ($P=0.006$), the OB group was marked by high fat mass percentage ($32.2 \pm 6.9\%$) and elevated central obesity evaluated by waist circumference ($98 \pm 16.5\text{cm}$), confirming previous BMI classification. It is worth to emphasize that food intake was similar between the groups.

As to the lipid profile, HDL-C was lower in OV and OB groups in comparison to HW group ($P=0.027$; $P<0.001$, respectively), and TG was higher in OB group ($P=0.026$). The TC/HDL-C and TG/HDL-C ratios confirmed this tendency, where OB group showed higher values in relation to HW group ($P=0.015$). Regarding the apolipoproteins, there were similarity between groups, except for Apo AI and for the ratio of Apo B/Apo AI, where the OB group showed lower ($P=0.001$) and higher ($P=0.002$) levels, respectively, than HW group.

The insulin levels and HOMA-IR were higher in OV ($P=0.002$ and $P=0.011$, respectively) and OB groups ($P<0.001$; $P<0.001$, respectively) than in HW group. The same pattern was observed for LDL(-), where OB and OV groups were statistically different from HW group ($P=0.049$; $P=0.017$, respectively). An opposite profile was noted for anti-LDL(-) ($P=0.045$; $P=0.010$, respectively).

Figure 1 shows that Lp-PLA₂ activity changed proportionally to BMI. The negative impact of adiposity on Lp-PLA₂ activity was reinforced by tertiles of waist circumference and fat mass percentage.

Table 3 shows the association between Lp-PLA₂ activity and cardiometabolic parameters (**Table 3**). Lp-PLA₂ activity was positively associated with almost all variables of lipid profile, except HDL-C and TG with which there was not correlation. Besides, Lp-PLA₂ was negatively related to Apo AI ($\beta=-0.137$; $P=0.038$) and strongly positively associated with Apo B ($\beta=0.293$; $P<0.001$) and with Apo B/Apo AI ratio ($\beta=0.343$; $P<0.001$). Other evidences are the positive associations with insulin, HOMA-IR and glucose. This enzyme activity was also positively associated with BMI ($\beta=0.195$; $P=0.003$), WC ($\beta=0.270$; $P<0.001$), fat mass percentage ($\beta=0.186$; $P=0.004$). These significant associations explained 64.5% of variability of Lp-PLA₂, while the non significant represented 4.1%.

Given the multiple associations obtained in the univariate test, we analysed the better model of multivariate analysis in order to understand the Lp-PLA₂ activity

in adolescents. The better predictor model featured Apo B/Apo AI ($\beta = 0.327$; $P < 0.001$), HDL size ($\beta = -0.326$; $P < 0.001$), WC ($\beta = 0.171$; $P = 0.006$) and glucose ($\beta = 0.119$; $P = 0.038$) (Table 4). This model explained 26.3% of variability of Lp-PLA₂.

Using logistic regression analysis, we were able to determine the impact of cardiovascular risk factors on Lp-PLA₂ activity. Compared to adolescents with lower Lp-PLA₂ activity (<15.9), those with higher values (≥ 15.9) exhibited significant association with TC (OR 1.012, 95% CI=1.004-1.020, $P = 0.004$), LDL-C (OR 1.012, 95% CI=1.004-1.020, $P = 0.003$), HOMA-IR (OR 1.127, 95% CI=1.010-1.258, $P = 0.032$), Apo B (OR 1.042, 95% CI=1.020-1.064, $P < 0.001$), Apo B/Apo AI (OR 73.53, 95% CI=8.627-626.665, $P < 0.001$). The change in Apo B/Apo AI ratio was related to a 73.5 times higher risk to have a high value of Lp-PLA₂ (≥ 15.9).

Discussion

This study showed that Lp-PLA₂ activity in adolescence is associated with body mass index and central adiposity, and that this relation changes in function of various cardiovascular biomarkers, specially, the Apo B/Apo AI ratio.

Obese and overweight adolescents showed a profile of lower HDL-C, higher TG/HDL-C, insulin and HOMA-IR when compared to HW. Additionally, TC/HDL-C was higher in the OB group than in the HW group. These factors support an increase of cardiovascular risk in obese adolescents and agree with a previous study conducted by Ella *et al.* (2010), which described that the prevalence of metabolic syndrome in obese adolescents is substantial. Lipid profile in adolescents has been widely studied and, according to the Center for Disease Control, the prevalence of abnormal lipid levels reached 20.3% of this population (CDC, 2010). In the same way, LAMBERT *et al.* (2004) verified that the quartiles of BMI increased in unison with increase of LDL-C, triglycerides and HDL-C reduction. The main lipidic alterations in adolescence are hypertriglyceridemia and HDL-C reduction, both associated with insulin resistance (Tomaszewski *et al.*, 2010). In a similar way, REY-YING *et al.* (2009) observed high triglycerides levels in 48.5% of obese adolescents. TAKSALI *et al.* (2008) verified that patients in the higher tertiles of

triglycerides were 5.2 times more likely to present metabolic syndrome. Given these evidences, lipid disorders are amply common in obese adolescents.

In contrast to many investigations of the negative impact of obesity on lipids, there are few studies including children and adolescents that linked nutritional status, lipid profile and Lp-PLA₂. Our results demonstrated the negative effect of weight excess on Lp-PLA₂ activity, which increased according to BMI, waist circumference and fat mass percentage. The waist circumference was the most important anthropometric measure affecting the Lp-PLA₂ activity. Previous studies have shown that Lp-PLA₂ is a good marker for cardiovascular risk in adults (THE LP-PLA₂ STUDIES COLLABORATION, 2010; PERSSON *et al*, 2007; LI *et al*, 2010). For instance, the Lp-PLA₂ Studies Collaboration showed that the enzyme activity was positively correlated with non-HDL-C, LDL-C, Apo B and negatively correlated with HDL-C and Apo AI (THE LP-PLA₂ STUDIES COLLABORATION, 2010). In the same way, SABATINE *et al.* (2007) emphasized that Lp-PLA₂ is an important predictor of coronary revascularization and unstable angina, and can also be treated as a new risk factor.

Despite this, there are only three studies, known by these authors, evaluating Lp-PLA₂ in children and adolescents. First, Okada *et al.* (2006) showed that the Lp-PLA₂ concentration of 17 obese children (11.9 ± 0.7 years old) was positively correlated with weight, waist/height ratio, subscapular/triceps ratio and LDL-C level. Castro *et al* (2007) compared diabetic young adults and adolescents (24.9 ± 7.8 years old) with controls (24.3 ± 9.6 years old), observing higher Lp-PLA₂ activity and elevated susceptibility for oxidized LDL in diabetic patients. Subsequently, Nagel *et al.* in 2008, studying children with 10 years old, observed that overweight was positively associated with cardiometabolic markers, including Lp-PLA₂ concentration. Our study confirms the negative impact of Lp-PLA₂ and amplifies these results in function of sample size, number cardiometabolic markers and the age of the population included. In addition, we demonstrated for the first time the strong impact of Apo B/Apo AI ratio on changes in Lp-PLA₂ activity; more specifically, the changes in these apolipoproteins were associated with a 73.5 times higher risk of elevated Lp-PLA₂ activity. Recently, Hatoum *et al.* (2010), studying patients between 50 and 60 years old, observed that the enzyme was modestly associated with total

cholesterol, LDL-C, Apo B and BMI; however, the lipid adjustment attenuates the relation between BMI and Lp-PLA₂.

The atherogenic role of Lp-PLA₂ was widely associated to LDL-C (Tsimihodimos *et al.*, 2002; Okamura *et al.*, 2007). On the contrary, Okamura *et al.* (2007) suggested that Lp-PLA₂ in HDL plays an antiatherogenic action. This observation was reinforced by a high LDL-Lp-PLA₂ to HDL-Lp-PLA₂ ratio verified in patients with atrial fibrillation. In the same way, Rizos *et al.* (2005) and Lagos *et al.* (2008) observed a high total Lp-PLA₂ activity in patients with metabolic syndrome, however, HDL-Lp-PLA₂ showed low activity. As discussed above, our results confirm the impact of HDL and LDL on Lp-PLA₂ activity given the strong association of this enzyme with LDL-C, Apo B, Apo AI and Apo B/Apo AI ratio. In addition, Apo B and Apo B/Apo AI ratio were the most important parameters related to the risk of increasing of Lp-PLA₂ activity in adolescents. These results suggest that an intervention focused in LDL and HDL could change the atherogenic role of Lp-PLA₂ and, possibly, the cardiovascular risk in adolescents. Therefore, it is likely that changes in LDL and HDL promoted by drugs and life style (diet and exercise) can modify the Lp-PLA₂ activity in adolescents. This possibility was reinforced by the negative association of Lp-PLA₂ with HDL-size, and by the fact that obese adolescents showed lower Apo AI, HDL-C and HDL-size and higher Lp-PLA₂ activity. This scenario confirms the negative impact of obesity in functionality of HDL.

Previously, Pascot *et al.*, (2001) showed that the small size HDL particle has been associated with atherogenic dyslipidemic profile and hyperinsulinemia. More recently, Medina-Urrutia *et al.* (2008) demonstrated that adolescents with small HDL have reduced HDL-C, high triglycerides and HOMA-IR. Rizos *et al.* (2005) verified that patients with metabolic syndrome have higher Lp-PLA₂ than the control group, but the enzyme linked to HDL-Lp-PLA₂ was lower in this group and negatively associated with HOMA-IR. Tsimikas *et al.* (2009), analyzing patients with or without cardiovascular events, observed that Lp-PLA₂ was positively correlated with HOMA-IR in the two groups; nonetheless, the correlation was higher in the cardiovascular event group. In this fashion, our study demonstrated that this enzyme

was positively correlated with insulin and HOMA-IR, reinforcing the notion of the negative impact of obesity on glucose and lipid metabolism.

Many studies have shown that Lp-PLA₂ activity is elevated in hypercholesterolemic, diabetic and metabolic syndrome patients (Campo *et al.*, 2004; Rizos *et al.*, 2005). Nambi *et al.* (2009) associated C-reactive protein and Lp-PLA₂ to traditional risk factors for cardiovascular disease and proposed that these variables can be especially useful in individuals that are in intermediate risk according to these evaluations. Hence, Lp-PLA₂ appears to be an important link between oxidation, inflammation, altered lipid profile and insulin resistance on cardiovascular diseases.

In spite of the important results obtained, we believe that this study shows limitations directly related to the design (cross-sectional), that limits the establishment of the causal impact of our results on prevalence of clinical events.

Finally, we conclude that in adolescence, Lp-PLA₂ changes in function of nutritional status, specially central adiposity, and that it shows important associations with markers of cardiovascular risk, in particular with Apo B/Apo AI ratio. This supports the hypothesis that the enzyme can be a biomarker of cardiovascular risk in adolescence.

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Figure 1:

Figure 1: Total Lp-PLA₂ activity in adolescents according to BMI, tertiles of waist circumference and fat mass percentage.

A: Lp-PLA₂ activity stratified according to BMI. B: Lp-PLA₂ activity stratified according to waist circumference tertiles. C: Lp-PLA₂ activity stratified according to fat mass percentage tertiles. * $P=0.001$ vs HW group or vs Lower tertile. HW, healthy weight; OV, overweight; OB, obese; BMI, Body Mass Index; WC, waist circumference

Table 1. Characteristic of adolescents and food intake, according to BMI.

	BMI groups						
Variables	HW		OV		OB		<i>P</i>
	(n=77)		(n=82)		(n=83)		
Boys, n (%)	26 (35.7)		37 (52.6)		31 (44.4)		0.320
Girls, n (%)	51 (64.3)		45 (47.4)		52 (55.6)		
Age, year	14.4	2.2	13.3 [*]	1.9	14.2 ^{**}	2.6	0.006
Sexual Maturation							0.710
Pre-pubertal	4.0	5.2	7.0	8.5	6.0	7.2	
Pubertal	73.0	94.8	75.0	91.5	77.0	92.8	
BMI, Kg/m ²	20.3	2.1	25.0 [*]	1.9	32.4 ^{*,**}	5.9	<0.001
WC, cm	69	5.8	80.7 [*]	5.7	98 ^{*,**}	16.5	<0.001
Fat mass, %	19.4	7.9	24.5 [*]	6.6	32.2 ^{*,**}	6.9	<0.001
Lean mass, %	76.1	13.9	71.3 [*]	9.8	67.0 ^{*,**}	7.5	<0.001
Food intake							
Energy, Kcal	1522	471	1446	438	1563	521	0.620
Carbohydrates, g	211	20	212	19	207	27	0.706
Proteins, g	64	11	64	10	66	15	0.719
Fat, g	50	8	51	6	51	7	0.313
SFA, g	17.7	3.1	17.4	2.8	17.7	3.3	0.480
PUFA, g	8.5	2.0	9.2	2.0	9.0	2.7	0.213
MUFA, g	17.3	2.8	17.9	2.8	17.7	3.3	0.159
Oleic acid, g	9.2	2.2	9.9	1.7	10.7	2.8	0.050
Linoleic acid, g	3.9	1.0	4.2	1.0	4.0	1.0	0.142
Linolenic acid, g	0.4	0.1	0.5	0.1	0.4	0.1	0.079
Fiber, g	11.1	2.8	10.7	2.3	10.7	2.8	0.948

HW, healthy weight; OV, overweight; OB, obese; BMI, body mass index; WC, waist circumference; SFA, saturated fat acid; PUFA, polyunsaturated fat acid; MUFA, monounsaturated fat acid; BMI, body mass index. ^{*}*P*<0.05 vs HW group. ^{**}*P*<0.05 vs OV group.

Continuous variables were described as mean and standard deviation.

Categorical variables were described as sample (n) and percentage (%).

Table 2. Adolescent's biochemical profile, according to BMI.

BMI groups							
Variables	HW		OV		OB		<i>P</i>
	(n=77)		(n=82)		(n=83)		
TC, mg/dl	143	32.1	140	34.3	142	39.8	0.864
LDL-C, mg/dl	86	33.5	85	35.6	89	40.1	0.800
HDL-C, mg/dl	42	13.5	37*	13.3	35*	12.6	<0.001
TG, mg/dl	75	29.7	87	49.7	91*	45.1	0.07
TG/HDL-C	1.90	0.86	2.69*	1.98	3.07*	2.24	0.001
TC/HDL-C	3.67	1.39	4.02	1.72	4.59*	2.16	0.021
LDL-C/HDL-C	1.81	0.52	1.90	0.90	1.79	0.71	0.051
Non HDL-C	100.9	33.9	102.6	38.3	107.1	43.2	0,575
Apo AI, mg/dl	120.9	18.0	113.7	19.6	109.1*	21.2	0.001
Apo B, mg/dl	62.8	10.6	65.2	15.8	65.2	17.5	0.533
Apo B/Apo AI	0.53	0.12	0.60	0.15	0.61*	0.16	0.003
LDL-C/ApoB	1.38	0.4	1.33	0.48	1.37	0.53	0.810
HDL-C/ApoA	0.35	0.09	0.33	0.12	0.32	0.10	0.122
LDL (-), mg/mL	8.9	5	10.8	4.9*	10.5	5.2*	0.042
Anti LDL(-), mg/mL	8.5	2.6	7.5*	2.7	7.2*	3.0	0.010
HDL size, nm	10.8	2.7	10.8	2.3	10.9	3.2	0.638
Glucose, mg/dl	79.3	12.8	78.0	12.5	82.0	13.2	0.130
Insulin, uU/mL	15.3	7.1	19.8*	8.2	25.9*,**	14.5	<0.001
HOMA-IR	3.0	1.5	3.9*	2.0	5.3*,**	3.1	<0.001

HW, healthy weight; OV, overweight; OB, obese; BMI, Body Mass Index; WC, waist circumference, TC, total cholesterol; BMI, body mass index.

Results were described as mean and standard deviation.

* $P < 0.05$ vs HW group. ** $P < 0.05$ vs OV group.

Table 3: Univariate regression model between Lp-PLA₂ and cardiovascular risk factors.

Variables	R ²	P	β
BMI, Kg/m²	0.038	0.003*	0.195
WC, cm	0.073	0.000*	0.270
Fat mass, %	0.034	0.004*	0.186
TC, mg/dl	0.035	0.004*	0.186
LDL-C, mg/dl	0.016	0.051*	0.128
HDL-C, mg/dl	0.001	0.671	-0.028
TG, mg/dl	0.008	0.163	0.091
TG/HDL-C	0.007	0.199	0.084
LDL-C/HDL-C	0.010	0.121	0.121
TC/HDL-C	0.018	0.042*	0.133
Non HDL-C	0.022	0.022*	0.149
HDL-C/ApoAI	0.001	0.585	0.036
Apo AI, mg/dl	0.019	0.038*	-0.137
Apo B, mg/dl	0.082	0.000*	0.293
Apo B/Apo AI	0.118	0.000*	0.343
LDL-C/ApoB	0.000	0.775	-0.019
LDL(-), mg/mL	0.001	0.706	0.025
Anti LDL(-), mg/mL	0.013	0.078	-0.115
HDL size , nm	0.085	0.000*	-0.291
Insulin, uU/mL	0.039	0.002*	0.198
Glucose, mg/dl	0.019	0.034*	0.138
HOMA-IR	0.047	0.001*	0.216

HW, healthy weight; OV, overweight; OB, obese; BMI, Body Mass Index;
WC, waist circumference; TC, total cholesterol; TG, triglycerides; BMI, body mass index.

Variables in log form, except for percentages. * $P < 0.05$

Table 4: Multivariate linear regressions between Lp-PLA₂ and cardiovascular risk factors.

Variables	Lp-PLA ₂		
	R ²	P	β
<i>Model</i>	0.263		
Apo B/Apo AI		0.000*	0.327
HDL size		0.000*	-0.326
WC		0.006*	0.171
Glucose		0.038*	0.119

WC, waist circumference.

Variables in log form.

* $P < 0.05$

Table 5: Odd Ratio for Lp-PLA₂ activity highest quartile.

Variables	Lp-PLA ₂		<i>P</i>
	<15.9	≥15.9	
TC, mg/dl	1 ^{Ref}	1.012	0.004 [*]
LDL-C, mg/dl	1 ^{Ref}	1.012	0.003 [*]
HDL-C, mg/dl	1 ^{Ref}	0.983	0.162
TG, mg/dl	1 ^{Ref}	1.004	0.178
HOMA-IR	1 ^{Ref}	1.127	0.032 [*]
HDL size , nm	1 ^{Ref}	0.930	0.245
LDL(-), mg/mL	1 ^{Ref}	1.000	0.757
Anti LDL(-), mg/mL	1 ^{Ref}	0.878	0.020 [*]
Apo AI mg/dl	1 ^{Ref}	0.993	0.374
Apo B mg/dl	1 ^{Ref}	1.042	0.000 [*]
Apo B/Apo AI	1 ^{Ref}	73.5	0.000 [*]
LDL-C/ApoB	1 ^{Ref}	1.265	0.443
HDL-C/ApoAI	1 ^{Ref}	0.123	0.168
BMI, Kg/m²	1 ^{Ref}	1.022	0.340
WC, cm	1 ^{Ref}	1.014	0.126
Fat mass, %	1 ^{Ref}	1.015	0.379

HW, healthy weight; OV, overweight; OB, obese; BMI, Body Mass Index; WC, waist circumference, TC, total cholesterol, TG, triglycerides. * $P < 0.05$

ARTIGO 2: “Antioxidant and inflammatory aspects of phospholipase A₂ associated to lipoprotein (Lp-PLA₂): A review”

Artigo de Revisão

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Title: Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A₂ (Lp-PLA₂): a review

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Abstract

The association of cardiovascular events with Lp-PLA₂ has been studied continuously today. The enzyme has been strongly associated with several cardiovascular risk markers and events. Its discovery was directly related to the hydrolysis of the platelet-activating factor and oxidized phospholipids, which are considered protective functions. However, the hydrolysis of bioactive lipids generates lysophospholipids, compounds that have a pro-inflammatory function. Therefore, the evaluation of the distribution of Lp-PLA₂ in the lipid fractions emphasized the dual role of the enzyme in the inflammatory process, since the HDL-Lp-PLA₂ enzyme contributes to the reduction of atherosclerosis, while LDL-Lp-PLA₂ stimulates this process. Recently, it has been verified that diet components and drugs can influence the enzyme activity and concentration. Thus, the effects of these treatments on Lp-PLA₂ may represent a new kind of prevention of cardiovascular disease. Therefore, the association of the enzyme with the traditional assessment of cardiovascular risk may help to predict more accurately these diseases.

Keywords: Lp-PLA₂, Cardiovascular risk, antioxidant, pro-inflammatory.

1. Introduction

The physiopathology of cardiovascular disease (CVD) is marked by the presence of atherosclerosis that involves endothelial dysfunction, inflammation, oxidative stress, insulin resistance and dyslipidemia.

Even considering the early diagnosis and the new variety of treatments for CVD, the American College of Cardiology still predicts that there will be 25 million cases only in USA until the end of 2050 [1]. Furthermore, given the current importance of CVD, thanks to its high worldwide prevalence that accounts for nearly 30% of the global deaths [2], the monitoring of the new biomarkers and risk factors represents an important focus of new researches.

In this context, lipoprotein-associated phospholipase A₂ (Lp-PLA₂) represents a potential cardiovascular risk marker, given its correlations with coronary disease and stroke [3-7]. Initially, Lp-PLA₂ was recognized by its action on hydrolyzing platelet-activating factor (PAF); such characteristic has assigned to it the first name platelet-activating factor acetylhydrolase (PAF-AH) [8].

Despite the other important reviews of Lp-PLA₂ [9-11], the question of whether high activity of Lp-PLA₂ is a causal event or a result of atherosclerosis remains open. Therefore, the main goal of this review is to show the antioxidant and inflammatory role of Lp-PLA₂ and its connection with atherosclerosis, aiming to contribute to the discussions of atherogenic or anti-atherogenic role of Lp-PLA₂. We also discuss possible mechanisms of modulation of Lp-PLA₂.

2. Biochemistry and structural aspects

A brief biological background is necessary to comprehend mechanisms enrolling Lp-PLA₂ and atherosclerosis. Platelet-activating factor (PAF) is an active phospholipid related to many pathologic and physiologic reactions [12]. The PAF is formed through two reactions (**Figure 1**). Firstly, the cytosolic phospholipase A₂ (cPLA₂) acts on membrane phospholipids producing lysophospholipids; then, the lysophospholipids are modified by PAF acetyltransferase, resulting in the formation of PAF [13]. Thus, PAF concentration is modulated by Lp-PLA₂ activity [13,14].

Lp-PLA₂ was discovered in 1980 and it was classified as a Ca²⁺-independent PLA₂ [8], produced by a wide range of inflammatory and non-inflammatory cells [15-17]. It is considered a member of phospholipases family (PLA₂), although exhibits different properties when compared to other PLA₂ [18]. In addition, while Lp-PLA₂ is specific for the breakdown of PAF and oxidized fatty acid residues, PLA₂ is specific for phospholipids containing two long chain acyl groups [18-21].

Another feature of Lp-PLA₂ is that it shows different isoforms, though the more common types are distributed in intracellular [22] and extracellular compartment [8]. Intracellular Lp-PLA₂ shows two variables, I and II [23], while brain tissue exhibits a subtype named Lp-PLA₂-Ib [24]. The Lp-PLA₂ type II consists of a 40-KDa polypeptide chain, and has been associated with antioxidant properties [25]. The extracellular Lp-PLA₂, identified as plasma form, circulates in association primarily with LDL (80-85%) and on minor portion with HDL (15-20%), having its activity strongly correlated with the cholesterol concentrations [26,27]. Lp-PLA₂ has been extracted from human plasma and erythrocytes, bovine brain, liver and seminal plasma, guinea pig peritoneal fluid and plasma, mouse plasma and platelets, cultured rat Kupffer cell- and hepatocyte-conditioned media, rat bile and the parasite *Nippostrongylus brasiliensis* [28]. On the same hand, it was verified that the different isoforms of Lp-PLA₂ define distinct activities for the enzyme [23,29,30].

3. Antioxidant role of Lp-PLA₂

The oxidative stress is closely associated with inflammation and bioactive lipid formation. These bioactive lipids, such as PAF, PAF-like substances, and oxidized phospholipids, have been identified in atherosclerotic plaque [31]. PAF-like products are formed when the phospholipids of the cellular membrane suffers oxidative damage, resulting in compounds that have structures with shorter peroxidized residues at their second carbon and that mimic the action of PAF [32].

In presence of oxidized phospholipids, Lp-PLA₂ removes these fragments acting as an antioxidant. Matsuzawa *et al.* [33], suggested that the over expression of Lp-PLA₂ protects the cells of reactive oxygen species (ROS)-induced apoptosis through oxidized phospholipids hydrolysis.

In addition, oxidized LDL and LDL(-) are known to be important factors on the atherosclerosis initiation and development [34-36]. Heery *et al.* [37] demonstrated that the formation of oxidized phospholipids in LDL stimulates Lp-PLA₂ activity. It is most likely that the Lp-PLA₂ hydrolysis of the lipids present in this particle represents an important antiatherogenic role. In this context, Watson *et al.* [38] showed that the Lp-PLA₂, hydrolyzing oxidized phospholipids, minimizes the generation of highly oxidized LDL, increasing the minimally oxidized LDL content. Subsequently, Benitez *et al.* [39] found that the major portion of Lp-PLA₂ was associated with LDL(-) in detriment to LDL(+), suggesting that the release of chemotactic induced by LDL(-) could be a consequence of the high Lp-PLA₂ activity. Indeed, LDL(-) can be generated by Lp-PLA₂, although the origin of this sub-group of LDL could to be also compatible with oxidative reaction and other mechanisms such as non enzymatic glycosylation, changes on Apo E (apolipoprotein E) and Apo CII (apolipoprotein CII), non esterified fat acids (NEFAS) enrichment or cross linking with hemoglobin [40].

Lourida *et al.* [41] showed that Lp-PLA₂ activity is important for reducing the immunogenicity of oxLDL, a phenomenon that can be attributed to the decreasing of oxidized phospholipids in patients with coronary artery disease and healthy ones. More recently, Noto *et al.* [42] showed in animals that Lp-PLA₂ protects lipoproteins from oxidation, producing less proatherogenic lipoproteins and preserving HDL functions. In this direction, Bazan [43] proposed that recombinant Lp-PLA₂ could be a potential tool directed to antiatherogenic therapy.

4. Inflammatory action of Lp-PLA₂

Despite the antioxidant potential described above, the association of Lp-PLA₂ with inflammatory reactions represents the majority of the studies in literature in the last years.

When Lp-PLA₂ hydrolyzes bioactive lipids, reducing their biological activity, the most generated metabolites are the lysophospholipids. These lipids are involved with atherosclerotic process and show a deleterious role of Lp-PLA₂, contributing to the inflammatory response against oxidized lipoproteins [39,44,45]. These compounds generated by phospholipases A₂ during cell activation, injury, or

apoptosis, are known to affect the function of neutrophils and of a diversity of cell types [46], and can be also produced by phospholipase A1 and by the action of lecithin-cholesterol acyltransferase (LCAT) or endothelial lipase. There are many different lysophospholipids, but the main product of Lp-PLA₂ action is lysophosphatidylcholine [47]; these metabolic processes occur in physiological conditions.

Furthermore, lysophospholipids from apoptotic cells contribute to attract monocytic cells and primary macrophages [48,49]. In this context, Steinbrecher & Pritchard [45] showed that oxLDL, on the presence of phenylmethanesulphonylfluoride (PMSF), an inhibitor of Lp-PLA₂, has lower values of lysophospholipids. In this fashion, Muller *et al.* [50] proposed that lysophosphatidylcholine represents a biomarker of the intensity of the reactive oxygen species production at the inflammatory site. Accordingly, Lavi *et al.* [51] found that patients with early coronary atherosclerosis had higher lysophosphatidylcholine when compared with control subjects. This profile was confirmed by Herrmann *et al.* [52], who showed that carotid artery plaques of patients with cardiac events presented higher Lp-PLA₂, lysophospholipids, macrophage and collagen content when compared to patients without events.

Studying the effects of oxLDL, Kuniyasu *et al.* [53] demonstrated that oxLDL, and particularly, the lysophosphatidylcholine present in this particle, enhances the plasminogen activator inhibitor-1 expression. Vickers *et al.* [54] demonstrated also that lysophosphatidylcholine can contribute to calcify vascular cells on the atherosclerotic plaque, through up-regulation of osteogenic genes and proteins. Hence, many events present in atherosclerotic process involve directly Lp-PLA₂ or its products.

Figure 2 summarizes the possible atherogenic mechanisms involving Lp-PLA₂. In this context, there can be an individual with dyslipidemia, obesity, hypertension, insulin resistance and oxidative stress, and therefore, highly prone to atherosclerosis. These factors contribute initially to the endothelial dysfunction, characterized by the expression of more adhesion molecules and by larger spaces between endothelial cells. Thus, the LDL, macrophages and T lymphocytes can transmigrate more easily to arterial intima. This LDL particle shows a phenotype

more atherogenic, being dense and small, characteristics that make it more susceptible to oxidation. In this site, the reduced content of antioxidants favors the high production of free radicals, and consequently oxidative modifications of LDL. Thus, the Lp-PLA₂ will be activated by oxidized phospholipids present in OxLDL.

The enzyme minimizes modifications of OxLDL, hydrolyzing its oxidized phospholipids; this may be interpreted as an antioxidant action. However, during this process, there are produced high contents of lysophospholipids and oxidized non esterified fat acids (OxNEFAS) that promote adhesion molecules expression and attract macrophages to the arterial intima. The OxLDL, lysophospholipids and OxNEFAS also stimulates cytokines production, like TNF- α and IL-6, which increase the inflammatory profile in the region of the plate. The activated macrophages, through scavenger receptors, phagocyte OxLDL, gradually turning up in foam cells. The muscle cells are also attracted, and migrate to the intima, where they produce collagen, elastin and elastases, involving and stabilizing the lipid plaque. Subsequently, the macrophages become apoptotic, as well as the muscle cells, causing released of lipids in the plaque. In this process, the presence of OxLDL, as well as lysophospholipids and OxNEFAS produced by Lp-PLA₂, is always stimulating the growth of the plaque; these are factors that can be decisive to plaque rupture susceptibility that can culminate in a cardiovascular event.

5. Lp-PLA₂ and Cardiometabolic Risk

Taking into account the mechanisms described, Lp-PLA₂ could influence the cardiometabolic risk; the **Figure 3** expresses the two main possibilities for action of the enzyme in cardiovascular disease context; the antioxidant action, where these hydrolysis reduce the oxidized phospholipids in plasma and the oxLDL contributing to generation of LDL(-); the inflammatory action, where the hydrolysis of PAF, PAF-like products or oxidized phospholipids generate lysophospholipids that stimulate inflammation, so the atherogenic process is stimulated.

According to Campo *et al.* [55], Lp-PLA₂ activity was significantly associated with LDL-cholesterol in hypercholesterolemic patients. As a matter of fact, dyslipidemia promotes an increase in plasma Lp-PLA₂ activity and alters the enzyme distribution between apo B- and apo AI-containing lipoproteins, as observed by

Tsimihodimos *et al.* [56]. The role of LDL-associated Lp-PLA₂ remains controversial, possibly because of the difficulty in analyzing the actions of the enzyme in the dense LDL particle [57].

On the other hand, studies also have showed that the enzyme activity associated with HDL particles can play an antiatherogenic action. Theilmeier *et al.* [58] demonstrated by *in vitro* and *in vivo* models that HDL-Lp-PLA₂ (HDL-Lipoprotein-associated phospholipase A₂) activity was linked to reduction of endothelial adhesiveness and of macrophage recruitment to lesion prone sites. Afterwards, the same group demonstrated that atorvastatin induced the increase of HDL-Lp-PLA₂ activity and the reduction of LDL-Lp-PLA₂ (LDL-Lipoprotein-associated phospholipase A₂) activity [56].

Papavasiliou *et al.* [59], investigating chronic kidney disease patients, found an increase in plasma Lp-PLA₂ activity and a reduction of the ratio of HDL-Lp-PLA₂ to plasma when compared to controls. In the same way, Rizos *et al.* [26] demonstrated that patients with metabolic syndrome have higher Lp-PLA₂ activity than controls. Nevertheless, the Lp-PLA₂ content in HDL was lower; these results were confirmed by Lagos *et al.* [60], who observed that the HDL-Lp-PLA₂ activity was lower in patients with metabolic syndrome. Okamura *et al.* [31] suggested that even the Lp-PLA₂ having an important function in atherogenesis, its association with HDL plays the opposite role, as observed by high LDL-Lp-PLA₂ to HDL-Lp-PLA₂ ratio in patients with atrial fibrillation.

In this fashion, Noto *et al.* [61] showed that diabetic patients with metabolic syndrome have significantly higher Lp-PLA₂ activity than those without this disease, reflecting its importance to metabolic risk. Following up, in a cohort with 299 subjects, Allison *et al.* [62] demonstrated that an increment of one standard deviation in Lp-PLA₂ activity was associated with a higher risk of CVD in five years, but not with mortality. Kiechl *et al.* [63], in a prospective study, demonstrated that oxidized phospholipids/apo B ratio predicted the cardiovascular risk, being the Lp-PLA₂ activity an amplifier of this risk.

Accordingly, Sabatine *et al.* [4] observed that an elevated level of Lp-PLA₂ is a predictor of adverse cardiovascular outcomes, independently of the traditional clinical risk factors in patients with stable coronary artery disease. Persson *et al.* [64]

observed that this enzyme was strongly correlated with lipid fractions and the degree of carotid artery atherosclerosis; this study showed that the association with cardiovascular risk is stronger for activity than for mass, reinforcing the impact of activity in atherogenesis [64].

In a prospective population-based survey, which occurred between 1990 and 2005, it was verified that Lp-PLA₂ was higher in subjects with incidence of CVD [5]. In the same year, Jenny *et al.* [65] showed that subjects with heart failure have the elevation of Lp-PLA₂ levels associated with an increase in the mortality risk. It was detected also that subjects aged > 65 years presented an association between the Lp-PLA₂ and myocardial infarction [65]. An increasing risk of major adverse cardiac events associated with elevated Lp-PLA₂ was also observed in community-based cohort of patients with acute coronary syndrome [6].

More recently, the Lp-PLA₂ Studies Collaboration, analyzing 32 prospective studies, confirmed that the enzyme activity and mass were related to proatherogenic lipids and vascular risk [66]. The study showed also that the association of the enzyme activity with lipid markers is stronger than the association with mass [66]. Recently, the authors of this review verified that the Lp-PLA₂ activity in adolescents is positively associated with total cholesterol, LDL-C, insulin, glucose, HOMA-IR, Apo B (apolipoprotein B)/Apo AI (apolipoprotein AI) ratio and negatively related to HDL size.

In contrast with the studies above, Tsironis *et al.* [67] showed that patients with coronary disease exhibit reduced LDL-Lp-PLA₂ mass and catalytic efficiency, suggesting a diminished ability to degrade pro-inflammatory phospholipids.

Therefore, it is probably that Lp-PLA₂ shows a dual action, directly dependent on its association with LDL (proatherogenic) or HDL (antiatherogenic). **Table 1** summarizes the antioxidant, inflammatory and neutral links between Lp-PLA₂ and cardiometabolic risk.

6. Modulation of Lp-PLA₂

Studies focused on Lp-PLA₂ modulation are little explored in literature, despite of its possible manipulation. Regarding that Lp-PLA₂ is associated with cholesterol and oxidized lipids in LDL and HDL, it is probable that drugs and

environment factors, capable of modulating the lipid metabolism, may change the mass and the activity of this enzyme.

Gerra *et al.* [68] showed that lovastatin was responsible for the simultaneous decrease of LDL-C level and Lp-PLA₂ activity. Similarly, Tsimihodimos *et al.* [56] found reduced Lp-PLA₂ activity in plasma of hypercholesterolemic patients under atorvastatin therapy, with a reduction in LDL-Lp-PLA₂ activity; in contrast, there was no modification in HDL-Lp-PLA₂ activity. The same authors, in an investigation of the effect of fenofibrate on hypercholesterolemic patients, observed a reduction in the LDL-Lp-PLA₂ activity and an increase of the HDL-Lp-PLA₂ activity [69]. Schaefer *et al.* [70], comparing the effect of atorvastatin with placebo in coronary heart disease patients observed a reduction of Lp-PLA₂ under therapy.

Studying the effect of cholesterol feeding and simvastatin treatment on rabbits, Zhang *et al.* [71] found that the LDL-Lp-PLA₂ activity increased with cholesterol feeding and decreased after the treatment. In this way, O'Donoghue *et al.* [72] found that an intensive statin therapy was responsible for 20% of reduction in LDL-Lp-PLA₂, in average. Likewise, Schaefer *et al.* [70] observed that simvastatin determined a reduction of the Lp-PLA₂ mass in 26%.

In the same way, atorvastatin or fenofibrate therapies can increase the ratio of HDL-Lp-PLA₂ to plasma Lp-PLA₂ (or to LDL-Lp-PLA₂) [57]. Also, the effect of gemfibrozil was monitored in men with low HDL-C, and it was verified that individuals in highest quartile of Lp-PLA₂ showed reduction of cardiovascular events [73]. The use of darapladib (oral Lp-PLA₂ inhibitor) by coronary patients caused a reduction of 59% of the enzyme activity after 12 months of treatment; concomitantly, the placebo group presented a significant increase of necrotic core volume when compared to the therapy group [74]. In a complementary study, the combined effect of atorvastatin and darapladib was evaluated in patients with coronary heart disease in the course of 12 weeks; the individuals under darapladib showed a reduction of approximately 54% in the Lp-PLA₂ activity when compared with controls [75].

Investigating patients under low-fat-diet and orlistat treatment, fenofibrate or both drugs during six months, Filippatos *et al.* [76] observed a significant reduction of Lp-PLA₂ activity in all groups (14%, 22% and 35%, respectively) when compared

to basal time. The results suggested the combination of the two treatments as the optimal therapy.

Hence, the direct influence of lipid metabolism on Lp-PLA₂ was confirmed by the efficiency of hypocholesterolemic drugs. Nonetheless, a similar profile was not observed in patients under anti-hypertension treatment: Spirou *et al.* [77] and Rizos *et al.* [78] verified that anti-hypertensive was not able to change Lp-PLA₂ activity.

Despite the positive effect on Lp-PLA₂ demonstrated by application of drugs, many studies have also investigated the influence of diet and other environment factors on the enzyme. In this context, Pedersen *et al.* [79] compared the effects of high (6.6g), low (2.0g) and control doses of n-3 polyunsaturated fatty acids in some metabolic parameters; they did not observe any effect on Lp-PLA₂ activity. Recently, in a sub-sample (n=150, follow up=1 y) of PREDIMED study, the authors of the present work, comparing diets enhanced with a mix of nuts (30 g/d), olive oil (50 g/d) or with low concentration of saturated fat (<7%), observed a reduction in Lp-PLA₂ only in the nuts group [NRTD, personal communication].

The effect of selenium on Lp-PLA₂ was recently evaluated [80] on rats, subject to three different diets (control, high fat and high fat enhanced with selenium). The results showed that the Lp-PLA₂ levels in control group were lower than the other groups, and that the selenium did not affect this enzyme.

The Nurses' Health Study demonstrated that the replacement of energy from carbohydrates for proteins, as well as the alcohol consumption or use of cholesterol-lowering drugs, were associated with a reduction in the Lp-PLA₂ activity. Smoking, overweight, aspirin use, hypercholesterolemia and age were, nevertheless, related to the elevation of Lp-PLA₂ activity [81]. In addition, obese and non-diabetic women submitted to a weight reduction program showed a significant reduction in Lp-PLA₂ activity, directly associated with VLDL-C [82]. The influence of the nutritional status on Lp-PLA₂ activity was also evaluated in adolescents where it was positively associated with body mass index, waist circumference and fat mass percentage [83].

Finally, Chen *et al.* [84] compared vegetarians with omnivores and observed that vegetarians presented lower Lp-PLA₂ activity, with lower total cholesterol and LDL-cholesterol, but with increased chances of higher C-reactive protein.

7. Conclusion

Initially, the discovery of the enzyme Lp-PLA₂ was associated with its ability to hydrolyze PAF and phospholipids, what was seen as a protective function. The enzyme acts as an antioxidant in the presence of oxidized phospholipids. Thus, Lp-PLA₂, in this sense, represents an important factor, reducing the oxLDL atherogenicity. Nowadays, however, its association with cardiovascular events is the most outstanding characteristic observed.

In addition, associations with several cardiovascular risk markers were also described in the literature. The enzyme hydrolyzes bioactive lipids, reducing their biological activity; the major metabolites generated in the process are the lysophospholipids. Given these results, the enzyme has been associated with a pro-inflammatory action, explained mainly by the production of these compounds that stimulate the inflammatory process in the region of the atherosclerotic plate.

Focusing on the enzyme antiatherogenic function, several studies have been evaluating the distribution of Lp-PLA₂ in the lipid fractions. Surprisingly, the HDL-Lp-PLA₂ enzyme has proven beneficial results to the atherosclerotic process. In the same sense, LDL-Lp-PLA₂ is linked to higher cardiovascular risk. Drugs and diet components that alter the lipid profile, the insulin resistance and the inflammatory markers also affect the enzyme activity and its concentration. Possibly, the effects of these components on the Lp-PLA₂ activity, according to the lipid fraction, represent a new kind of prevention of CVD.

The traditional assessment of cardiovascular risk is based on lipid profile, inflammation and body composition. Since the control of these variables seeks to reduce cardiovascular events and this enzyme is strongly related to them, it is probable that the monitoring of its activity and its distribution on lipoproteins will predict better the cardiovascular risk.

List of abbreviations

Apo AI: Apolipoprotein AI

Apo B: Apolipoprotein B

Apo CII: Apolipoprotein CII

Apo E: Apolipoprotein E

cPLA₂: Cytosolic phospholipase A₂

CVD: Cardiovascular disease

HDL-C: High density lipoprotein cholesterol

HDL-Lp-PLA₂: HDL-Lipoprotein-associated phospholipase A₂

LCAT: Lecithin-cholesterol acyltransferase

LDL(-): Electronegative low-density lipoprotein

LDL-C: Low density lipoprotein cholesterol

LDL-Lp-PLA₂: LDL-Lipoprotein-associated phospholipase A₂

Lp-PLA₂: Lipoprotein-associated phospholipase A₂

NEFAs: Non esterified fat acids

oxLDL: Oxidized low-density lipoprotein

OxNEFAs: Oxidized non esterified fat acids

PAF: Platelet-activating factor

PAF-AH: Platelet-activating factor acetylhydrolase

PAF-like: Platelet-activating factor like

PLA₂: Phospholipases family

PMSF: Phenylmethanesulphonylfluoride

ROS: Reactive oxygen species

VLDL-C: Very low density lipoprotein cholesterol

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Authors' contributions

ITS wrote the manuscript, APQM reviewed the manuscript and NRTD designed, drafted and critically reviewed the manuscript. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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Table 1: Potential action of the Lp-PLA₂, according to studies with distinct design.

Study design	Action	Reference
Experimental	Cells ROS protection.	33
Experimental	↓ bioactivity phospholipids in oxLDL	37
Experimental	↓ oxidized phospholipids in mildly oxLDL	38
Case/Control	≈ Oxidized phospholipids and anti-Lp-PLA ₂	41
Case/Control	↓ HDL oxidation, foam cell and autoantibodies titers.	42
Case/Control	↓ HDL-Lp-PLA ₂ activity	25
Case/Control	↓ HDL-Lp-PLA ₂ activity	60
Case/Control	↑ LDL-Lp-PLA ₂ to HDL-Lp-PLA ₂ ratio	31
Case/Control	↓ HDL-Lp-PLA ₂ and ↑ of LDL-Lp-PLA ₂	56
Case/Control	≈ Lp-PLA ₂ activity	55
Case/Control	↑ Lp-PLA ₂ activity	61
Cohort	↑ Lp-PLA ₂ activity in CHD mortality	62
Case/Control	↑ Lp-PLA ₂ activity	63
Cohort	Predictor of cardiovascular outcomes	4
Cohort	Lp-PLA ₂ correlated with cardiovascular risk factors	64
Cohort	Lp-PLA ₂ activity associated with MS and CVD	5
Cohort	Lp-PLA ₂ mass and activity associated with CVD	65
Cohort	↑ Lp-PLA ₂ activity associated with CVD	6
Meta-analysis	Lp-PLA ₂ mass and activity associated vascular risk	66

CVD: Cardiovascular Disease; CHD: Coronary Heart Disease; PAD: Peripheral Arterial Disease; ROS: Reactive Oxygen Species, MS: Metabolic Syndrome

Figure 1: Role of Lp-PLA₂ on the generation of lysophospholipids.

Figure 2: Possible actions of Lp-PLA₂ in the atherosclerotic process.

Figure 3: Lp-PLA₂ action on cardiovascular disease

ARTIGO 3: “Role Plasma antioxidants, but not the dietary ones, are associated with cardiometabolic risk in adolescents”

Artigo Original

Submetido ao Journal of the American Dietetic Association

Title: Plasma antioxidants, but not the dietary ones, are associated with cardiometabolic risk in adolescents

Sort title: Antioxidants and cardiometabolic risk in adolescents

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Summary

Background: In the context of cardiometabolic it has been proposed that antioxidants play a protective role against oxidative damage, despite some controversies. **Objective:** Access the impact of obesity in the associations of plasma and diet antioxidants with cardiometabolic risk factors in adolescents. **Design/Methods:** A cross-sectional study was used to include adolescents from public schools of São Paulo city. These subjects were classified in Health weight - HW, Overweight - OV and Obese - OB groups. Anthropometric Measurements and Sexual Maturation were determined. Food Intake was evaluated by 24-hour diet recalls. β -carotene, α -tocopherol, lycopene and retinol were analyzed. Additional biochemical analysis were performed to access lipid profile, glucose and insulin levels, oxidation status (LDL(-)), adipocytokines, Non-Esterified Fatty Acids (NEFAs) and Cholesteryl Ester Transfer Protein (CETP). **Statistical analysis:** Correlations of cardiometabolic variables with plasma and with diet antioxidants were performed through Spearman test and Partial correlation (BMI). **Results:** 261 adolescents were included in the study. Food intake was similar between the groups and was not correlated with cardiometabolic risk. The OV and OB groups showed lower values of β -carotene, α -tocopherol and of the sum of all antioxidants than HW group. A similar profile was observed for antioxidants adjusted by TC or LDL-C. Plasma antioxidants adjusted by TC or by LDL-C showed significant associations with the majority of the cardiometabolic parameters. Obesity was able to reduce the previous associations. **Conclusions:** Plasma antioxidants are related to cardiometabolic risk, where BMI plays an important role. These biomarkers represent a better way to estimate the influence of a diet pattern on cardiometabolic risk in adolescents than the dietary ones.

Keywords: Cardiometabolic risk, adolescents, 24h-recall, plasma antioxidants

Introduction

Obesity has been related to the development of atherosclerosis and other symptoms accompanying cardiovascular diseases. In the last years, the accelerated curve of obesity growth in children and adolescents has been the focus of many investigations (1,2). Nowadays, it is accepted that obesity in life time is a strong risk factor for occurrence of cardiovascular disease in adulthood (3). Although the mechanisms linking obesity to atherosclerosis are not yet fully understood, it is clear that three major events are involved: inflammation, insulin resistance and oxidation (4,5). The unbalance of these parameters has been observed in obese adolescents, contributing to the early development of clinical events like diabetes, dyslipidemia, hypertension and metabolic syndrome (6,7,8,9).

In this context, the oxidative stress represents an important factor for initiation and development of atherosclerosis and, therefore, it is strongly related to cardiovascular disease (10). A plausible connection between oxidative stress and atherosclerosis is related to the generation of oxidized LDL by free radicals. This modified particle is heavily involved with inflammation and represents an essential factor for the formation of foam cells in the arteries (11). The production of free radicals occurs naturally in human body; however, environment factors and diseases (obesity, cancer, and hypertension) are able to modify the intensity of these reactions, favoring or reducing the oxidative stress. Thus, it is probable that changes in life style (exercise, diet, smoking) exert great impact on oxidative reactions. Accordingly, dietary antioxidants have been suggested to protect from oxidative damage and, possibly, modify the development of cardiovascular disease and its morbidities.

Recent studies reinforce this possibility demonstrating benefic effects of antioxidant on obesity and cardiovascular disease (12,13). In a similar way, VINCENT and colleagues (14) observed that supplementation with β -carotene, retinol and ascorbic acid promoted decreasing of HOMA-IR and adhesion molecules levels in overweight adults. Nevertheless, the effect of diet on oxidative stress requires further investigations, given the complexity of the redox system (15). According Eidelman and colleagues (16), vitamin E supplementation didn't affect

the cardiometabolic risk in adults. Afterwards, Miller and colleagues (17), on a meta-analysis, demonstrated also that high-dosages of vitamin E supplements increased all-cause mortality. Gerss and Köpcke (18) reinforced, however, that different methodological approaches of meta-analysis yield contradictory results. These controversies support the hypothesis that antioxidants vitamins could reflect a dietary pattern and not necessarily the isolated positive effect of a single nutrient on cardiovascular risk factors.

Taking into account the precedent discussion, the aim of this study was to access the impact of obesity in the associations of plasma and diet antioxidants with cardiometabolic risk factors in adolescents.

Methods

Study subjects

A sample of adolescents between ten and nineteen years old, from both sexes and from public schools located at west area from São Paulo, SP, Brazil, were evaluated in a cross-sectional study (19). The inclusion criteria were not smoking, do not consume alcohol regularly (≤ 30.0 mL of etanol/day for boys and ≤ 15.0 mL of etanol/day for girls) (20), do not use lipid-lowering drugs, do not have any acute disease, do not participate in other protocols research and not pregnancy or breastfeeding for at least two months before data collection.

Adolescent's characterization, age, ethnicity and family history of cardiovascular diseases were collected by a structured questionnaire. The adolescents were divided into 3 groups: healthy weight (HW), overweight (OV), and obese (OB). The study protocol was approved by the Ethics Committee (School of Public Health, University of Sao Paulo; Proc. #1668) and followed the recommendations of the National Council for Health on Ethics in Research with Humans (21). All the parents of the adolescents signed an informed written consent.

Anthropometric Measurements, Body Composition and Sexual Maturation

Height and weight were evaluated by a stadiometer (AlturaExata, TBW Brazil, Sao Paulo, SP, Brazil) and a digital scale (Control, Plenna, Sao Paulo, SP, Brazil), respectively. The nutritional status was classified by distribution of body mass index (BMI, weight/height²) for sex and age, according to Cole and colleagues (22,23). Waist circumference (WC) was measured using a 1 mm precision flexible and inelastic tape (TBW Brazil[®], Sao Paulo, SP, Brazil). The instrument tetrapolar Biodynamics[®], model 450 (TBW, São Paulo, Brazil), was used to obtain body composition. Sexual maturation was determined by a self-administered questionnaire proposed by Marshall and Tanner (24,25).

Food Intake

Three 24-hour diet recalls collected on nonconsecutive days, including one weekend day, were used to evaluate habitual food intake. The first recall was conducted by direct interview and the others by phone interview. In the first interview, it was used a Food Photographic Record (26) to present to adolescents the possible food portions sizes. Energy (kcal), carbohydrates (g), lipids (g), proteins (g), fiber (g), retinol (mg), β -carotene (mg), lycopene (μ g), α -tocopherol (mg) were evaluated by the software *NutWin*[®] (27). Additional nutritional information was included in database of software using food composition tables: Brazilian Table of Food Composition - Version 2 (28) and United States Department of Agriculture (29). The data were adjusted by energy (30,31) and intra-personal variability (32).

Antioxidants analyses

The levels of β -carotene, α -tocopherol, lycopene and retinol were measured on plasma by commercial kits (Chromsystems, Munchen, Germany). The concentration was determined by a run on an isocratic high performance liquid chromatography (HPLC) system with UV/VIS detection. The antioxidants were quantified by the inclusion of an internal standard. The analysis was conducted in duplicate.

Lipid analysis

The levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined by standard methods (Labtest Diagnóstica, Lagoa Santa, MG, Brazil). The low density lipoprotein-cholesterol (LDL-C) level was calculated by Friedewald equation (33). The apolipoproteins Apo AI and B were evaluated by standard methods (RANDOX®, Co, Antrim, Reino Unido). Afterwards, the concentration of HDL-C and LDL-C were normalized by Apo AI and B content, respectively.

Glucose and Insulin measures

While the glucose level was determined by an enzymatic and colorimetric commercial kit (Glicose PAP Liquiform® - Labtest, MG, Brazil), the insulin level was measured by radioimmunoassay *Human Insulin-Specific* RIA Kit® (Linco Research, St Charles, MO, USA). After, from these parameters, the HOMA-IR index was calculated (homeostasis model assessment – insulin resistance), where $HOMA-IR = [\text{fasting insulin concentration } (\mu\text{U/mL}) \times \text{fasting glucose (mmol/L)}] / 22.5$ (34).

LDL(-) analyses

The electronegative low density lipoprotein LDL(-) was determined by ELISA using monoclonal antibodies (MAb 1A3 and MAb 2C7), according to Damasceno and colleagues (35).

Adipocytokines

Resistin, adiponectin and leptin levels were evaluated by ELISA method using lincoplex system (Luminex™ xMAP), which involves a process that marks latex microspheres with different fluorophores.

Non-Esterified Fatty Acids (NEFAs)

NEFA was evaluated by the commercial kit HR Series NEFA-HR(2)® (WAKO Diagnostics, Texas, EUA).

Cholesteryl Ester Transfer Protein (CETP)

CETP was measured by the commercial kit CETP Activity Assay[®] (BioVision Research Products, CA, EUA).

Statistical analysis

The statistical analysis was performed using the SPSS software, version 15.0. The data distribution were evaluated by Kolmogorov-Smirnov test ($P > 0.05$), with the differences between groups, determined by ANOVA (normally data) or Kruskal-Wallis and Mann-Whitney U-test (not normally distributed data). The χ^2 test was used for comparison of categorical variables, which results were expressed as relative frequency (%). The antioxidants values were normalized by total cholesterol and LDL-C.

Correlations of cardiometabolic variables with plasma and with diet antioxidants were performed through Spearman test. Partial correlation was used to verify the influence of BMI on obtained correlations. Statistical significance was established for P -value < 0.05 .

Results

2746 adolescents from the five public schools attended the nutrition screening. Following, all adolescents with OW ($n=481$), OB ($n=135$) and a randomized sub-sample of HW ($n=756$) group were invited to the second phase of the study. A total of 261 adolescents reached this stage, among which 19 adolescents were excluded due to the use of lipid-lowering and anti-inflammatory drugs during the data collection. It were performed a sexual maturation- and a sex-specific analysis. However, the groups showed similar profile, and therefore, these variables were not accepted as confounders.

The characteristics of adolescents included in the study, according to BMI, are presented on **Table 1**. It can be observed that the gender proportion and ethnic characteristics were statistically similar between groups ($P = 0.321$; $P = 0.180$). However, the OB and HW groups were older than OV group (13.3 ± 1.9 years) ($P =$

0.006). Similarly, the prevalence of hypertension in the family was higher in OB group when compared to HW group, while obesity prevalence in the OB group family was higher than OV group.

The BMI classification was reinforced by the high fat mass percentage ($32.2 \pm 6.9\%$), low lean mass ($67 \pm 7.5\%$) and elevated central obesity evaluated by waist circumference ($98 \pm 16.5\text{cm}$) detected in the OB group.

Food intake were similar between groups for almost all variables (**Table 1**), except for vitamin E and lycopene, that were higher in the OV group when compared to the HW group; for vitamin E it was also observed that the values were higher in OB than in the OV group.

Figure 1 shows that crude plasma antioxidants were similar between the groups, except for β -carotene and α -tocopherol. β -carotene level was lower in OV and OB groups when compared to HW group ($P = 0.005$, $P < 0.001$; respectively) and higher for OV when compared to OB ($P = 0.001$). Regarding the content of α -tocopherol, less values were observed in OB group in comparison with HW ($P = 0.010$) and OV ($P = 0.033$) groups. Afterwards, the sum of all antioxidants monitored showed that the OB group had lower values than HW group ($P = 0.013$). When antioxidants were adjusted by TC or LDL-C, the groups showed similar profile to the crude data, except for the sum of antioxidants adjusted by TC, which showed lower values in OB group when compared to OV group ($P = 0.032$).

The biochemical profile can be observed on **Table 2**. The OV and OB groups showed lower values of HDL-C when compared to the HW group ($P = 0.027$; $P < 0.001$, respectively), while TG was higher in OB than HW ($P = 0.026$). In this way, Apo AI and Apo B/ Apo AI ratios were lower ($P = 0.002$) and higher ($P = 0.001$), respectively, in the OB group versus the HW group. Following, insulin and HOMA-IR increased in function of BMI ($P < 0.001$, $P < 0.001$). Likewise, LDL(-) was higher in the OV and OB groups compared to the HW group ($P = 0.049$; $P = 0.017$, respectively). CETP was higher in the OB group when compared to the OV and HW groups ($P = 0.016$; $P = 0.002$, respectively). Analyzing adipokynes, it was verified that leptin has increased in function of BMI. The opposite profile was observed for the adiponectin level.

The analysis of the correlations of the plasma antioxidants adjusted by TC showed significant negative associations with waist circumference, fat mass percentage, TG, LDL-C, NEFAs, CETP, insulin, leptin, resistin, LDL(-), Apo B and LDL-C/Apo B. Positive correlations were observed between antioxidants and HDL-C, Apo AI and adiponectin (**Table 3**). When the impact of obesity on these correlations was evaluated, it was verified that partial correlations adjusted by BMI were able to reduce the previous association. The adjustment of the plasma antioxidants by LDL-C confirmed the relation between these vitamins and cardiometabolic variables. In a similar way, correlations adjusted by BMI were maintained only for the sum of antioxidants, lycopene, retinol and α -tocopherol with TC and HDL-C, and for β -carotene with TC.

On the other hand, diet antioxidants did not show any significant correlation with cardiometabolic parameters.

Discussion

The results presented in this study demonstrate that plasma antioxidants are associated with markers of cardiometabolic risk in adolescents and that the obesity is able to reduce these correlations. In addition, it was observed that the consumption of these antioxidants, estimated by 24-hour diet recalls, does not show any correlation with these markers.

This study verified also lower values of the sum of antioxidants, β -carotene and α -tocopherol in the OB group, which was reinforced by cholesterol and LDL-C adjustments. Previous studies demonstrated that the obesity exerts an important influence on plasma antioxidants availability. Neuhouser and colleagues (36), examining serum concentration of retinol, carotenoids and α -tocopherol, verified that obese adolescents presented lower values of all antioxidants. Suzuki and colleagues (37) observed in a population of adults and elderly that the odds ratio for high serum levels of β and α -carotene was lower in obesities when compared to non-obesities. Similarly, Galan and colleagues (38) observed that β -carotene was lower in men with higher BMI. On the contrary, Codoñer-Franch and colleagues (39) did not verified differences in β -carotene and α -tocopherol in obese adolescents compared to lean.

Many correlations were observed between plasma antioxidants and cardiometabolic risk. It is important to note that these correlations were influenced by obesity, since after BMI adjustment, most of them were lost. This study goes in the same direction of the research of Molnár and colleagues (40), who observed that obese adolescents with metabolic syndrome had lower values of β -carotene and α -tocopherol levels when compared to obese or controls. These authors found inverse correlations for β -carotene and α -tocopherol adjusted by lipids (total cholesterol and triglycerides) with insulin and body fat mass. In the present work, fat mass is negatively correlated with β -carotene ($r=-0.227$) and Lycopene ($r=-0.206$), while insulin concentration is negatively correlated with all antioxidants. The administration of vitamin E, vitamin C and β -carotene significantly reduces lipid hydroperoxides, adhesion molecules and increases adiponectin (14). Accordingly, Devaraj and colleagues (13) verified that high-doses of α -tocopherol reduced the C-reactive protein and the cytokine release in cardiovascular patients. In this direction, this work showed the existence of negative correlations of antioxidants with leptin, resistin, LDL(-), and positive correlation with adiponectin.

In opposition to this work, Vivekananthan and colleagues (41) verified, in a meta-analysis of randomized trials, that vitamin E supplementation did not provide a significant decrease of the risk of death by cardiovascular disease, and that β -carotene even increased slightly this risk. In agreement, Knekt and colleagues (42) observed that the intake of vitamin E and C was only weakly related to a reduced coronary heart disease, and that supplemental vitamin E was not significantly related to the reduction of this risk. In another meta-analysis, it was observed that individuals taking vitamin E presented an increased risk of haemorrhagic stroke by 22% (43).

The results obtained by Wannamethee and colleagues (44) demonstrated that fruit intake was significantly and inversely associated with the C-reactive protein and the tissue plasminogen activator antigen. Certainly, as stated by Lichtenstein and Russell (45) and in agreement with Catania and colleagues (46), the positive health outcomes are related to dietary patterns, not to nutrient supplements.

Thus, the use of biomarkers to estimate the effect of this dietary pattern represents an important strategy to evaluate the bioavailability of minerals, vitamins

and antioxidants. Accordingly, bioavailability can be defined as the fraction of an ingested nutrient or compound that reaches the systemic circulation and specific sites where it can exert its biological action (47). In agreement with Stahl (48), Porrini and Riso (47) affirm that bioavailability includes bioactivity. Antioxidants bioavailability can be affected by nutritional status, interactions of nutrients and/or the genetic characteristics and environmental factors, altering its absorption and excretion (47,49); hence, biomarkers show less limitations than the indirect tools used to evaluate diet consumption. In this way, the significant association observed between plasma antioxidants and the cardiometabolic risk in the present study confirms the superiority of these biomarkers in comparison with the 24h recall, in terms of the evaluation of the antioxidants influence on cardiovascular disease; this conclusion is due to the fact that biomarkers access the plasma bioavailability.

Previously, other studies observed that the diet antioxidants were negatively correlated with cardiometabolic factors. On a cohort, Helmersson and colleagues (50) demonstrated that the intake of ascorbic acid and tocopherol were negatively associated to inflammation and oxidative stress biomarkers. On the other hand, Puchau and colleagues (51) verified that the total dietary antioxidant capacity evaluated by 24 hour recall wasn't correlated to cardiometabolic variables. This result is similar to the results obtained in this work and reinforces the observation that the 24 hour recalls and other food questionnaires do not represent an accurate measure of the dietary intake of micronutrients and antioxidants. Many factors are involved; among them, the highly variable composition of nutrients available in composition tables (affected by seasonal and agronomic factors, plant variety, age and part of the plant used) and the food preparation conditions (49). In this sense, Schatzkin and colleagues (52) verified that the food frequency questionnaire and 24-hour recalls cannot be recommended for evaluating intakes of energy and protein, when compared to doubly labelled water and urine nitrogen.

Burrows and colleagues (53) suggested that the 3-day period 24-hour recall is the most accurated method to estimate total energy intake in children, but also emphasized that this group showed misreported intakes, a fact that could be related to ethnicity and weight status. In addition, it weren't observed, in the present study, differences in dietary consumption in the three groups (HW, OV and OB). In this

sense, Fisher and colleagues (54) demonstrated that children with higher body mass and higher BMI tended to under-report more the macronutrient intake. Similarly, Savage and colleagues (55) demonstrated that the under-report is common in obese children and adolescents and tends to increase as weight status increases.

In spite of the cross-sectional design of this study, which represents a limitation for the establishment of the causal impact of results obtained on prevalence of cardiometabolic risk, this study shows many important aspects: I – antioxidants are better evaluated by the use of biomarkers; II – plasma antioxidants, but not 24h recalls, are associated with cardiometabolic risk and III – misreport and underreport observed in adolescents with overweight and obesity compromises the validity of information obtained by 24h recall.

In conclusion, plasma antioxidants are related to cardiometabolic risk and represent a better way to estimate the influence of a diet pattern on cardiometabolic risk in adolescents than the dietary ones. It is worth noting that the obesity plays an important role in these correlations.

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Figure 1: Plasma levels of β -carotene, lycopene, retinol and α -tocopherol in adolescents, according BMI.

* $P < 0.05$ vs HW group. ** $P < 0.05$ vs OV group. HW, healthy weight; OV, overweight; OB, obese.

Table 1. Demographic, anthropometric characterization and food intake of adolescents, according to BMI.

Variable	BMI groups						<i>P value</i>
	HW (n=77)		OV (n=82)		OB (n=83)		
	Mean	SD	Mean	SD	Mean	SD	
Boys, n (%)	26 (35.7)		37 (52.6)		31 (44.4)		0.321
Girls, n (%)	51 (64.3)		45 (47.4)		52 (55.6)		
Age, year	14.4	2.2	13.3 [*]	1.9	14.2 ^{**}	2.6	0.006
Sexual Maturation							0.710
Stage 1	4.0	5.2	7.0	8.5	6.0	7.2	
Stage 2	73.0	94.8	75.0	91.5	77.0	92.8	
Anthropometry							
BMI, Kg/m ²	20.3	2.1	25.0 [*]	1.9	32.4 ^{*,**}	5.9	<0.001
WC, cm	69.0	5.8	80.7 [*]	5.7	98.0 ^{*,**}	16.5	<0.001
Fat mass, %	19.4	7.9	24.5 [*]	6.6	32.2 ^{*,**}	6.9	<0.001
Lean mass, %	76.1	13.9	71.3 [*]	9.8	67.0 ^{*,**}	7.5	<0.001
Ethnic							0.180
White	40.0	51.9	28.0	34.1	40.0	48.2	
Black	11.0	14.3	21.0	25.6	20.0	24.1	
Colored	25.0	32.5	32.0	39.0	21.0	25.3	
Native Brazilian	1.0	1.3	1.0	1.2	2.0	2.4	
Family history							
Diabetes	9.0	11.7	13.0	15.9	16.0	19.3	0.420
Hypertension	15.0	19.5	29.0	35.4	33.0 [*]	39.8	0.002
Obesity	13.0	16.9	11.0	13.4	26.0 ^{**}	31.3	0.010
Cardiovascular disease	7.0	9.1	10.0	12.2	6.0	7.2	0.320
Food Intake							
Carbohydrates, g	211.4	19.4	212.4	18.6	207.4	26.9	0.706
Proteins, g	64.3	11.4	64.2	10.0	65.9	14.5	0.719
Fat, g	50.4	7.8	51.1	6.2	51.3	6.7	0.313
Fiber, g	11.1	2.7	10.7	2.3	10.7	2.8	0.948
Vitamin A (UI)	2043.5	1071.1	1782.1	826.1	1795.5	866.7	0.223
Vitamin E, mg	3.6	0.9	3.8 [*]	0.7	3.6 ^{**}	0.8	0.019
β-Carotene, μg	17.7	3.1	17.4	2.8	17.7	3.3	0.599
Lycopene, μg	3.8	1.0	4.2 [*]	1.0	4.0	1.0	0.510

* $P < 0.05$ vs HW group. ** $P < 0.05$ vs OV group.

HW, healthy weight; OV, overweight; OB, obese; SD, standard deviation; BMI, Body Mass Index; WC, waist circumference.

Table 2. Biochemical profile of adolescents, according body mass index

Variables	BMI groups						<i>P value</i>
	HW (n=77)		OV (n=82)		OB (n=83)		
	Mean	SD	Mean	SD	Mean	SD	
TC, mg/dl	143	32	140	34	142	40	0.864
LDL-C, mg/dl	86	33	85	35	89	40	0.800
HDL-C, mg/dl	42	13	37 [*]	13	35 [*]	13	<0.001
TG, mg/dl	75	30	87	50	91 [*]	45	0.070
Apo AI, mg/dl	120.9	18.0	113.7	19.6	109.1 [*]	21.2	0.001
Apo B, mg/dl	62.8	10.6	65.2	15.8	65.2	17.5	0.533
Apo B/Apo AI	0.53	0.12	0.60	0.15	0.61 [*]	0.16	0.003
LDL-C/ApoB	1.38	0.40	1.33	0.48	1.37	0.53	0.810
HDL-C/ApoA	0.35	0.09	0.33	0.12	0.32	0.10	0.122
Glucose, mg/dl	79.3	12.8	78.0	12.5	82.0	13.2	0.130
Insulin, uU/mL	15.3	7.1	19.8 [*]	8.2	25.9 ^{*,**}	14.5	<0.001
HOMA-IR	3.0	1.5	3.9 [*]	2.0	5.3 ^{*,**}	3.1	<0.001
LDL (-), mg/mL	8.9	5.0	10.8 [*]	4.9	10.5 [*]	5.2	0.042
CETP, pmol/ul/hour	35.0	35.5	38.2	37.6	55.8 ^{*,**}	41.5	<0.001
NEFA, Eq/L	503.4	226.3	572.3	237.5	648.3	379.1	0.082
Leptin, ng/mL	9.0	8.5	25.6 [*]	15.1	40.0 ^{*,**}	21.9	0.034
Adiponectin, ng/mL	19.6	11.3	15.1 [*]	8.0	11.4 ^{*,**}	5.2	<0.001
Resistin, ng/mL	12.8	6.9	16.9 [*]	9.9	23.4 [*]	25.8	<0.001

* $p < 0.05$ vs HW group. ** $p < 0.05$ vs OV group.

HW, healthy weight; OV, overweight; OB, obese; SD, standard deviation; TC, Total Cholesterol, BMI, Body Mass Index.

Table 3: Correlations between antioxidants adjusted by total cholesterol and cardiometabolic parameters.

Variables	Sum of antioxidants		β -carotene		Lycopene		Retinol		α -Tocopherol	
	TC Adjusted r	LDL-C Adjusted r	TC Adjusted r	LDL-C Adjusted r	TC Adjusted r	LDL-C Adjusted R	TC Adjusted r	LDL-C Adjusted r	TC Adjusted r	LDL-C Adjusted r
Fat mass	-0.162*	-0.077	-0.227*	-0.090	-0.087	-0.206*	0.110	-0.077	-0.124	0.100
WC	-0.174*	-0.164*	-0.398*	-0.180*	-0.038	-0.369*	0.133*	-0.055	-0.199*	0.100
BMI	-0.232*	-0.200*	-0.405*	-0.215*	-0.077	-0.377*	0.104	-0.093	-0.236*	0.068
TG	-0.106	-0.043	-0.175*	-0.041	-0.061	-0.119	-0.072	-0.023	-0.107	-0.020
TC	-	-0.465**¶	-	-0.439**¶	-	-0.295**¶	-	-0.323**¶	-	-0.495**¶
HDL-C	0.205*	0.427**¶	0.156	0.427*	-0.050	0.352**¶	0.02	0.191**¶	0.159*	0.307**¶
LDL-C	-0.272**¶	-	-0.143*	-	-0.110	-	-0.294**¶	-	-0.226**¶	-
Apo AI	0.126	0.077	0.081	0.103	0.001	0.096	-0.111¶	0.009¶	0.161*	-0.091
Apo B	-0.055	-0.225*	-0.026	-0.208*	0.051	-0.124	-0.158**¶	-0.091¶	-0.004	-0.280*
LDL-C/ApoB	-0.278**¶	-	-0.124	-	-0.13¶	-	-0.285**¶	-	-0.249**¶	-
LDL (-)	-0.124	-0.144*	0.053	-0.137*	0.001	0.002	-0.24**¶	-0.047¶	-0.092	-0.203*
NEFA	-0.137*	-0.115	-0.140*	-0.121	-0.089	-0.109	-0.025	-0.087	-0.145*	-0.041
CETP	-0.157*	-0.205*	-0.114	-0.209*	0.002	-0.169*	-0.001	-0.070	-0.131*	-0.111
Insulin	-0.149*	-0.097	-0.231*	-0.094	-0.140*	-0.227*	0.072	-0.133*	-0.142*	0.072
Leptin	-0.189*	-0.150	-0.247*	-0.151	-0.178*	-0.242*	0.025	-0.172*	-0.180*	0.024
Resistin	-0.025	-0.038	-0.248*	-0.066	0.025	-0.250*	0.202**¶	-0.033	0.008	0.108
Adiponectin	0.146	0.170*	0.243*	0.183*	0.043	0.235*	-0.050	0.100	0.173*	0.010

* $P < 0.05$; ¶ Maintained after IMC adjustment. TC, Total cholesterol; WC, Waist circumference; BMI, Body Mass Index; TG, Triglycerids.

CONCLUSÕES

6. CONCLUSÕES

As conclusões obtidas nos artigos são apresentadas abaixo:

Artigo 1:

- A atividade da Lp-PLA₂ varia em função do estado nutricional de adolescentes, sendo a adiposidade central o fator antropométrico que mais influenciou a atividade da Lp-PLA₂.
- Correlações importantes foram observadas entre a atividade da Lp-PLA₂ e marcadores tradicionais de risco cardiovascular, especialmente com a razão entre Apo B/Apo AI, em adolescentes.
- Os resultados suportam a hipótese de que a enzima Lp-PLA₂ representa um importante biomarcador cardiovascular em adolescentes.

Artigo 2:

- A enzima Lp-PLA₂ se associa a eventos cardiovasculares e a vários marcadores cardiometabólicos.
- A hidrólise de fosfolípidos oxidados representa uma ação antioxidante da enzima Lp-PLA₂. Nesse sentido, sua ação minimiza a formação de LDLox, reduzindo sua aterogenicidade.
- A ação aterogênica da Lp-PLA₂ está associada à formação de lisofosfolípidos.
- Os efeitos da atividade da Lp-PLA₂ variam de acordo com a fração lipídica a que está associada. Quando ligada à HDL, demonstra efeitos antiaterogênicos e quando associada à LDL, mostra efeitos aterogênicos.
- Drogas e componentes dietéticos que atuam sobre o metabolismo lipídico, sobre a resistência à insulina e sobre a inflamação parecem afetar simultaneamente a concentração e a atividade da enzima Lp-PLA₂.
- A utilização da atividade da enzima Lp-PLA₂ em conjunto com os fatores de risco cardiovascular tradicionais parece favorecer a predição do risco cardiovascular.

Artigo 3:

- Antioxidantes plasmáticos variam em função do estado nutricional em adolescentes.

- Estes se correlacionam com marcadores de risco cardiometabólico de adolescentes.

- Não foram observadas correlações entre o consumo de antioxidantes e os fatores de risco.

- Os biomarcadores representam uma melhor ferramenta para a avaliação da influência de um padrão alimentar sobre o risco cardiometabólico em adolescentes.

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ANEXOS

8. ANEXOS

Anexo 1. Termo de consentimento livre e esclarecido

O estudo: **Papel dos Lipídios e Antioxidantes Dietéticos nas propriedades oxidativas e inflamatórias da PAF-AH em adolescentes obesos** tem como objetivo avaliar a influência dos lipídios e antioxidantes dietéticos na atividade da enzima PAF-AH e seu efeito sobre o estresse oxidativo e atividade inflamatória. Assim, pretende-se identificar possíveis hábitos alimentares e o estado nutricional que possam minimizar o estresse oxidativo. Para a realização do mesmo, será coletada uma amostra de sangue (20,0 mL), o adolescente responderá a um questionário alimentar e passará por aferições antropométricas. Todos os resultados obtidos estarão a sua disposição, sendo que sua identidade será mantida em total sigilo. O adolescente terá o direito de se retirar do projeto a qualquer momento. Sua participação ou não neste estudo não o comprometerá de qualquer forma.

Este projeto será desenvolvido pelo Departamento de Nutrição da Faculdade de Saúde Pública da Universidade São Paulo. A pesquisa tem caráter de diagnóstico e não de intervenção. Portanto, o risco é considerado mínimo. Contudo devido ao jejum para coleta de sangue ocasionalmente podem ocorrer tonturas ou enjoos. Os materiais de coleta de sangue são totalmente descartáveis, porém a coleta de sangue pode raramente gerar um pequeno hematoma (manchas roxas) no local de punção. Os benefícios deste estudo são a identificação da influência dos hábitos alimentares sobre a ação da enzima PAF-AH e sua influência no estresse oxidativo e na atividade inflamatória. Neste contexto, os benefícios para a população incluída no estudo ainda são experimentais, mas visualizam a identificação de comportamentos alimentares mais saudáveis para a população adolescente. Todos os resultados serão entregues aos participantes do estudo, sendo que orientações específicas (sugestão para procurar um profissional médico e/ou nutricionista) serão dadas quando estes resultados indicarem risco à saúde destes indivíduos.

Declaro que, após convenientemente esclarecido pelo pesquisador e ter entendido o que me foi explicado, autorizo _____
participar do presente Protocolo de Pesquisa:

São Paulo, ____ de _____ de 20____

Responsável:

Natureza (grau de parentesco, tutor, curador, etc.):

Sexo:

RG:

CPF:

Data de Nascimento:

Endereço:

Telefone:

Profa. Dra. Nágila Raquel Teixeira Damasceno – Coordenadora-Chefe

Faculdade de Saúde Pública / Departamento de Nutrição

Av. Dr. Arnaldo, 715 - Cerqueira César - CEP: 01246-904 – São Paulo – SP

Telefone para contato: (11) 3061-7701 ramal: 220 / Fax: (11) 3061-7701

Anexo 2. Protocolo de avaliação sócio-econômica cultural, clínica e antropométrica

AVALIAÇÃO SÓCIO-ECONÔMICA CULTURAL, CLÍNICA E ANTROPOMÉTRICA			
1. AVALIAÇÃO SÓCIO-ECONÔMICA			
1.1 Nome:		1.2 Idade:	1.3 Sexo: <u>1</u> () F <u>2</u> () M
Responsável:			
1.4 Endereço			
1.5 Telefone: Res -		Cel. -	Trab. Responsável:
1.6 Escolaridade: <u>1</u> () Ensino fundamental incompleto – 4ª série <u>2</u> () Ensino fundamental completo – 8ª série <u>3</u> () Ensino médio incompleto <u>4</u> () Ensino médio completo – 3º ano			
1.7 Membros na família: <u>1</u> () 2 <u>2</u> () 3-4 <u>3</u> () > 4			
2. AVALIAÇÃO CLÍNICA			
2.1 Usa algum medicamento e/ou vitaminas: <u>1</u> () Sim <u>2</u> () Não Qual? _____ Frequência? _____ Indicação? _____			
2.2 Tem algum tipo de doença? Qual?			
2.3 Existe algum familiar com: Diabetes () Sim () Não Quem? _____ Hipertensão () Sim () Não Quem? _____ Doença cardíaca () Sim () Não Quem? _____ Obesidade () Sim () Não Quem? _____ AVC () Sim () Não Quem? _____			
2.4 Pratica algum esporte? Qual? _____ Qual a frequência e duração? _____ Pratica esporte desde quando? _____			
3. AVALIAÇÃO ANTROPOMÉTRICA			
3.1 Peso atual (kg):		3.2 Peso habitual (kg):	
3.3 Altura (m):		3.4 IMC:	
3.5 Reactância (Xc):		3.6 Resistência (R):	
3.7 % água:	3.8 % gordura:	3.9 % massa magra:	

Anexo 3: Tabelas de desenvolvimento puberal

Desenvolvimento Puberal Feminino Tabelas de Tanner

Mamas

Pêlos Pubianos

Desenvolvimento Puberal Masculino Tabelas de Tanner

Genitália

Pêlos Pubianos

Anexo 4. Recordatório de 24 horas

NOME: _____ **TEL: ()** _____

DATA DE NASC: __/__/__ **ENTREVISTA** __/__/__ **DIA DA SEMANA:** _____

HORÁRIO E LOCAL	ALIMENTOS OU PREPARAÇÕES	QUANTIDADE	MARCA COMERCIAL	OBS

Entrevistador _____

Tempo da entrevista _____

Anexo 5: Manual do entrevistador

Para o sucesso da aplicação do método de inquérito alimentar escolhido, é preciso que o entrevistador atente-se a algumas questões, que serão descritas ao longo deste manual.

Apresentação inicial do entrevistador:

- É de extrema importância, antes da aplicação do inquérito alimentar, que o entrevistador se apresente, explicando os objetivos da entrevista e informando ao entrevistado a duração média da mesma.
- Esclarecer que todos os dados coletados são de ordem confidencial.
- Expor ao entrevistado o direito à não participação, sem qualquer prejuízo.

Considerações importantes:

- Os entrevistadores deverão estar treinados e possuir amplo conhecimento dos hábitos e costumes da comunidade em estudo, assim como dos alimentos e modo de prepará-los.
- Para auxiliar na quantificação do tamanho e volume das porções consumidas, será utilizado material de suporte com registros fotográficos e réplicas de alimentos.
- O entrevistador deverá estabelecer um canal de comunicação em que se obtenha a informação por meio de um diálogo agradável, uma vez que a qualidade da informação dependerá da memória e da cooperação do entrevistado.
- Respostas precisas e não tendenciosas exigem respeito e atitude neutra diante de hábitos e consumo de alimentos do entrevistado. Para tanto, o entrevistador deverá evitar expressar sentimentos de surpresa, tristeza ou decepção, o que poderia induzir o entrevistado a subestimar ou superestimar a quantidade de alimentos, dependendo da reação que ele observe.

RECORDATÓRIO DE 24 HORAS

O recordatório de 24 horas é um método de inquérito alimentar realizado mediante uma entrevista pessoal, na qual o entrevistado relata detalhadamente os alimentos consumidos no dia anterior, desde o primeiro alimento ingerido após acordar até a última refeição antes de dormir, incluindo os alimentos consumidos dentro e fora do domicílio.

Metodologia:

A aplicação do Recordatório de 24 horas deve obedecer a seguinte metodologia:

Passo 1: Pergunte ao indivíduo:

“A Sra pode, por favor, me dizer tudo o que comeu ou bebeu ontem, desde o momento que acordou até antes de dormir, começando pelo primeiro alimento ou bebida consumido?”.

Transcreva tudo o que for dito, sem preocupação com quantidade, por enquanto. Não interrompa a informante.

Passo 2: Pergunte o horário e local da refeição.

“A Sra pode lembrar o horário (mais ou menos) e em que lugar?”

Anote os horários e o local referido.

Passo 3: Volte à descrição dos alimentos e pergunte as quantidades em medidas caseiras consumidas, de cada alimento ou preparação.

a. No caso de alimentos como frutas, pães, biscoitos e ovos, pergunte quantas unidades foram consumidas. Exemplo: 1 fatia de pão de forma, 1 pão francês, 1 pão de queijo, 1 biscoito recheado, etc.

b. Se possível, registre a marca comercial e variedade dos alimentos. Exemplo: banana nanica, prata, maçã.

c. No caso de alimentos compostos, como por exemplo café com leite, mingaus, mamadeiras, vitaminas e sopas, pergunte os ingredientes, quantidades e medidas utilizadas na preparação.

d. Para alimentos como carnes (vaca, frango, porco, peixe) utilize unidades como: fatia (pequena, média, grande), pedaço (pequeno, médio, grande), posta (pequena, média, grande).

e. Registre se a preparação da carne foi frita, assada, à milanesa ou grelhada.

f. No caso de verduras e legumes, pergunte os ingredientes da salada. Legumes (cenoura, abobrinha, berinjela, milho, etc): registre em colheres de sopa ou de servir e pergunte o tipo de preparação (cozidos ou refogados).

g. Para preparações habituais como arroz, feijão e macarrão utilize as medidas caseiras de referência (colher de sopa, de servir, concha, pegador de macarrão).

h. Certifique-se da presença ou não de temperos nas saladas, açúcar/adoçante nas bebidas, manteiga/requeijão/outros no pão/biscoito/ou torrada e suas quantidades.

i. Na faça perguntas tendenciosas. Exemplo: “Você tomou café da manhã? Você come pouco?”

j. Ao longo da entrevista, lembre-se de perguntar se houve sobras e quantificá-las.

Revisão do recordatório:

Ao final da entrevista é importante que o entrevistador dê ao entrevistado a oportunidade para lembrar de algum alimento que porventura tenha consumido, mas esquecido de referir por ocasião da entrevista.

Por fim, é importante anotar o nome do entrevistador e quanto tempo durou a entrevista.

CURRÍCULO LATTES

Currículo do Sistema de Currículos Lattes (Isis Tande da Silva)

<http://buscatextual.cnpq.br/buscatextual/visualizacv.do?metodo=apresen...>

Isis Tande da Silva

Possui graduação em Nutrição pela Universidade Federal de Ouro Preto (2005). Atualmente é doutoranda em nutrição em saúde pública da Faculdade de Saúde Pública - USP e atua como professora convidada no curso de Especialização em Nutrição Clínica do Ganep Nutrição Humana. Tem experiência na área de Nutrição, com ênfase em adolescência, obesidade e doença cardiovascular. Atuou como Nutricionista no Instituto Central do Hospital das Clínicas de São Paulo de 2008 a 2010. Foi tutora da atuação prática dos alunos do curso teórico-prático e alunos da Especialização em Nutrição Clínica do Ganep (2010 a 2011). Atua como tutora de cursos virtuais no Ganep.

(Texto informado pelo autor)

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Dados pessoais

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Nome em citações bibliográficas SILVA, I. T.
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Formação acadêmica/Titulação

2007 Doutorado em andamento em Nutrição em Saúde Pública .
Faculdade de Saúde Pública - USP.
Título: Papel dos lipídeos e antioxidantes dietéticos nas propriedades oxidativas e inflamatórias da PAF-AH em adolescentes, *Orientador:* Nágila Raquel Teixeira Damasceno.
Palavras-chave: Adolescentes; Obesidade; Oxidação; PAF-AH; Perfil Lipídico; Inflamação.

2001 - 2005 Graduação em Nutrição .
Universidade Federal de Ouro Preto, UFOP, Brasil.

Atuação profissional

Ganep Nutrição Humana, GANEP, Brasil.

Vínculo institucional

2011 - Atual Vínculo: Colaborador, Enquadramento Funcional: Professora Convidada

Outras informações Professora convidada para lecionar as disciplinas Obesidade e Metodologia Científica para a Especialização em Nutrição Clínica

Vínculo institucional

2010 - Atual Vínculo: Colaborador, Enquadramento Funcional: Professora tutora

Outras informações Tutoria dos cursos à distância Obesidade, Envelhecimento, Síndrome Metabólica, Pediatria, Nutrição e Coração e Sistema Digestório

Vínculo institucional

2011 - 2011 Vínculo: Colaborador, Enquadramento Funcional: Professora Convidada

Outras informações Elaboração de material didático para os cursos à distância Nutrição e Coração, Envelhecimento e Gravidez.

Vínculo institucional

2010 - 2011 Vínculo: Colaborador, Enquadramento Funcional: Professora

Outras informações Professora responsável pela orientação dos alunos em trabalho de campo da Especialização em Nutrição Clínica e do curso teórico prático

Hospital das Clínicas, HC, Brasil.

Vínculo institucional

Nágila Raquel Teixeira Damasceno

Graduada em Nutrição pela Universidade Estadual do Ceará (1995). Fez Mestrado (1997) e Doutorado (2001) em Ciências dos Alimentos na Universidade de São Paulo. Realizou estágios de Pós-doutoramento em Imunologia (2005) na Universidade de São Paulo e em Nutrição e Endocrinologia na Universidade de Barcelona (2010). Recebeu prêmios nacionais e internacionais, além de ser co-autora de uma patente. Atualmente é professora doutora de Nutrição junto ao Depto. de Nutrição (FSP-USP). Nos últimos anos tem dedicado suas pesquisas aos efeitos dos componentes nutricionais sobre as doenças crônicas não transmissíveis (aterosclerose, obesidade, câncer, etc) com ênfase nas variáveis lipídicas, oxidativas, inflamatórias e imunológicas.

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Formação acadêmica/Titulação

- 2009 - 2010** Pós-Doutorado .
Universidade de Barcelona.
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico ,CNPq ,Brasil .
Grande área: Ciências da Saúde / *Área:* Nutrição .
Grande área: Ciências da Saúde / *Área:* Nutrição / *Subárea:* Bioquímica da Nutrição .
- 2003 - 2004** Pós-Doutorado .
Universidade de São Paulo, USP, Brasil.
Bolsista do(a): Instituto Danone .
Grande área: Ciências da Saúde / *Área:* Nutrição .
Grande área: Ciências da Saúde / *Área:* Nutrição / *Subárea:* Bioquímica da Nutrição .
- 1997 - 2001** Doutorado em Ciências dos Alimentos (Conceito CAPES 7) .
Universidade de São Paulo, USP, Brasil.
Título: Influência das Isoflavonas Extraídas da Soja na Hipercolesterolemia e na Aterosclerose Experimental Induzida pela Caseína, *Ano de Obtenção:* 2001.
Orientador: Dulcineia Saes Parra Abdalla.
Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo ,FAPESP ,Brasil .
Palavras-chave: Isoflavonas; Caseína; Oxidação; Lipoproteínas; Anticorpos; Aterosclerose.
Grande área: Ciências da Saúde / *Área:* Medicina / *Subárea:* Clínica Médica / *Especialidade:* Cardiologia.
Grande área: Ciências da Saúde / *Área:* Medicina / *Subárea:* Clínica Médica / *Especialidade:* Alergologia e Imunologia Clínica.
Grande área: Ciências da Saúde / *Área:* Medicina / *Subárea:* Clínica Médica / *Especialidade:* Endocrinologia.
Setores de atividade: Fabricação de Produtos Químicos; Fabricação de Produtos Farmacêuticos; Desenvolvimento de Produtos Tecnológicos Voltados Para A Saúde Humana.
- 1995 - 1997** Mestrado em Ciências dos Alimentos (Conceito CAPES 7) .
Universidade de São Paulo, USP, Brasil.
Título: Influência da Proteína Isolada da Soja e da Caseína no Desenvolvimento da Aterosclerose Experimental em Coelhos, Induzida pela Dieta Rica em Colesterol, *Ano de Obtenção:* 1997.
Orientador: Dulcineia Saes Parra Abdalla.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior ,CAPES ,Brasil .
Palavras-chave: Soja; Caseína; Aterosclerose; Colesterol; Anticorpos; hipercolesterolemia.
Grande área: Ciências da Saúde / *Área:* Medicina / *Subárea:* Clínica Médica / *Especialidade:* Cardiologia.
Grande área: Ciências da Saúde / *Área:* Medicina / *Subárea:* Clínica Médica / *Especialidade:* Alergologia e Imunologia Clínica.