Original/Obesidad

Macronutrient intake is correlated with dyslipidemia and low-grade inflammation in childhood obesity but mostly in male obese

Barbara de Moura Mello Antunes¹, Paula Alves Monteiro¹,², Loreana Sanches Silveira¹, Claudia de Carvalho Brunholi², Fábio Santos Lira² and Ismael Forte Freitas Júnior²

¹Exercise and Immunometabolism Research Group, Department of Physical Education, Universidade Estadual Paulista, Presidente Prudente, São Paulo. ²Study Center and Laboratory of Assessment and Prescription of motor activity, Department of Physical Education, Universidade Estadual Paulista, Presidente Prudente, São Paulo (Brazil).

Abstract

Background & Aims: condition of hypoxia caused by hypertrophy of adipose cells in obesity triggers macrophages recruitment and production of cytokines. Additionally, high consumption of saturated fatty acids (SFA) and high glycem index meals may contribute to oxidative stress and chronic low-grade inflammation by increases NF-κB activation. Thus, the aim of the study was to analyze the contribution of the macronutrients intake in the metabolic and inflammatory profile, by levels of lipoproteins, insulin resistance, anti and pro inflammatory cytokines, in obese adolescents according the gender.

Methods: sample was composed by 37 adolescents, both genders, identified as obese by body mass index (BMI). Body composition was assessed by Dual-energy X-ray absorptiometry (DEXA) and measures of intra-abdominal adiposity (IAAT) and subcutaneous adiposity tissue (SAT) were done by ultrasound. Biochemical analyses were done and the measurement of cytokitines; fatty acids and insulin were performed by the technique of immunoassay ELISA. The estimation of macronutrients consumption was made by 3 day food register regarding food intake. Statistical significance was set at p-value < 5% and the statistical software SPSS version 17.0 (SPSS Inc, Chicago, IL) performed all analyses.

Results: BMI (p = 0.316), FM (p = 0.416), IAAT (p = 0.505) and SAT (p = 0.935) presented similarities between genders. Cytokines and metabolable variables values were similar between the groups. Only in the male group, metabolic variables and cytokines were significant correlated with the consumption of total lipids or its fractions. Was observed that insulin concentration had significant interaction with MUFA(g) (β = -18.4; p = 0.004) and adiponectina with CHO(g) (β = -58.2; p = 0.032) in the group male and female, respectively.

Correspondence: Barbara de Moura Mello Antunes.
Exercise and Immunometabolism Research Group, Department of Physical Education, Universidade Estadual Paulista, UNESP, Rua Roberto Simonsen, 305, 19060-900 Presidente Prudente, SP, Brazil.
E-mail: ba.antonies2@gmail.com


La ingesta de macronutrientes se correlaciona con la dislipidemia y la inflamación de bajo grado en la obesidad infantil, pero sobre todo en los hombres obesos.

Resumen

Introducción y objetivo: la hipoxia causada por la hipertrofia de las células adiposas en la obesidad desencadena macrófagos de reclutamiento y la producción de citoquinas. Además, el alto consumo de ácidos grasos saturados (AGS) y las comidas con alto índice glucémico pueden contribuir al estrés oxidativo y la inflamación crónica de bajo grado por los aumentos de activación de NF-κB. Por lo tanto, el objetivo del estudio fue analizar la contribución de la ingesta de macronutrientes en el perfil metabólico e inflamatorio, por niveles de lipoproteínas, resistencia a la insulina, citoquinas anti y pro inflamatorias, en adolescentes obesos según el género.

Métodos: la muestra estaba compuesta por 37 adolescentes, de ambos géneros, identificados como obesos según el índice de masa corporal (IMC). La composición corporal se evaluó mediante absorciometría de energía dual de rayos X (DEXA) y medidas de adiposidad intratraabdominal (IAAT) y del tejido adiposo subcutáneo (SAT) se realizaron mediante ecografía. Los análisis bioquímicos se realizaron mediante la medición de citoquinas; los ácidos grasos y la insulina se realizaron por la técnica de ELISA. La estimación del consumo de macronutrientes fue llevado a cabo durante tres días mediante el registro de alimentos con respecto a la ingesta total. La significación estadística se estableció en el valor p <5% y todos los análisis se realizaron con el programa estadístico SPSS Inc, Chicago, IL, versión 17.0.

Resultados: IMC (p = 0.316), FM (p = 0.416), IAAT (p = 0.505) y SAT (p = 0.935) presentan similaridades entre géneros. Los valores de citoquinas y de variables metabólicas fueron similares entre los grupos. Solo en el grupo masculino, variables metabólicas y citoquinas fueron correlacionadas con el consumo de lípidos totales o sus fracciones. Se observó que la concentración de insulina tenía interacción significativa con MUFA (g) (β = -18.4; p = 0.004) y la adiponectina con CHO (g) (β = -58.2; p = 0.032) en el grupo masculino y femenino, respectivamente.
Conclusions: macronutrients intake is associated with low-grade inflammation in obesity, by production of inflammatory cytokines and alteration of the lipid profile, especially male obese adolescents which seem to be more responsive of this consumption when compared with female obese adolescents.

(Nutr Hosp. 2015;32:997-1003)
DOI:10.3305/nh.2015.32.3.9289

Key words: Carbohydrates. Lipids. MUFA. PUFA. Diet. Cytokine.

Introduction

In obesity, the weight gain, simultaneously with hypertrophy of adipose cells, exerts a constriction of the local blood vessels impeding the vascularization in the region and promoting the development of a local hypoxia with adipocyte death. Therefore, only the condition of hypoxia already stimulate chemotaxis of macrophages to the adipose tissue, by monocyte chemotactic protein-1 (MCP-1), and induce the expression of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), initiating a chronic low-grade inflammation condition and metabolic diseases.

The increase of fat mass and fat cell size are directly affected for dietary patterns, mainly lipids intake, and the low-grade inflammation may be maintained and enhanced by saturated fatty acids (SFA) intake because this lipids are able to activate an inflammatory pathway by toll-like receptor 4 (TLR4) and gene transcription of more inflammatory cytokines by nuclear factor kappa B (NF-kB). Studies have evidenced that inflammatory biomarkers, such as NF-kB, are responsive to meal type, mostly the type of lipids, favoring the oxidative stress and installation of inflammatory profile postprandial.

In the other hand, the excessive carbohydrate intake also can be considered a dangerous practice whereas high glycemic index meals may contribute to oxidative stress and chronic low-grade inflammation by increases NF-kB activation. The high carbohydrate consumption is deleterious not only for adipose tissue, excessive intake of this macronutrient promotes increase in the production and concentration of TNF-α in the liver evidencing that the high carbohydrate meal may contribute to obesity and comorbidities associated with this disease.

Thus, the aim of the present study was to analyze the contribution of the macronutrients intake in the metabolic and inflammatory profile, by levels of lipoproteins, insulin resistance, anti and pro inflammatory cytokines, in obese adolescents according the gender.

Methods

Subjects and setting

The study included 37 adolescents of both genders (23 male and 14 female), who met the inclusion criteria: a) body mass index (BMI) according age and gender; b) aged between 11 and 17 years until evaluation date; c) no engagement in regular physical activity within three months prior to the study; d) a consent form signed by the parents/guardians to participate in the study.

The present research was approved by the Ethical Research Expert Committee of the Universidade Estadual Paulista – Campus of Presidente Prudente (UNESP) (protocol number 07/2009).

Anthropometry

Body weight (BW) was measured with an electronic scale (precision 0.1 kg [Filizzola PL 150, Filizzola® Ltda]) and the height with a wall-mounted stadiometer (precision 0.1 cm [Sanny®, São Paulo, Brazil]), with the subjects wearing light clothing and no shoes. The BMI was the calculated from the equation: body mass, in kilograms, divided by height in meters squared, and classified according age and gender.

Waist circumference (WC) was measured with a metallic and inelastic tape (precision 0.1 cm [Sanny®, São Paulo, Brazil]), with the subjects in standing position, breathing normally and with arms relaxed beside the trunk. Anthropometric measurements were performed by trained researchers, according to standardized techniques.

Body composition

Estimative of body composition (whole-body and segmental) was made by Dual-energy X-ray absorptiometry (DEXA) (Lunar DPX-NT scanner [Lunar DPX-NT; General Electric Healthcare, Little Chalfont, UK]) and the fat mass and fat free mass were classified according age and gender.

Conclusions: la ingesta de macronutrientes se asocia con la inflamación de bajo grado en la obesidad, por la producción de citoquinas inflamatorias y la alteración del perfil lipídico, en los adolescentes obesos, especialmente masculinos, que aparecen ser más sensibles a este consumo en comparación con las adolescentes obesas femeninas.

(Nutr Hosp. 2015;32:997-1003)
DOI:10.3305/nh.2015.32.3.9289

Palabras clave: Carbohidratos. Lípidos. MUFA. PUFA. Dieta. Citoquinas.
Buckinghamshire, United Kingdom] software version 4.7). Fat-free mass (FFM), fat mass (FM), trunk fat mass (TFM) and percentage of fat mass (%FM) were estimated. All measurements were made at the laboratory of the University in a climate-controlled temperature room and the equipment was calibrated every day, in the morning, as described by the manufacturer.

**Intra-abdominal and subcutaneous adipose tissue**

The ultrasound examination of the upper abdomen was performed to measure intra-abdominal adiposity (IAAT) and subcutaneous adiposity tissue (SAT) and the examination was performed by only one qualified radiologist, using a TOSHIBA Eccocee with a 3.7 Mhz convex transducer. All adolescents followed the recommendation to fast for 4 hours prior to the evaluation according to medical literature.

**Blood Samples**

Blood samples were collected from subjects in tubes containing EDTA after a 10-12 hour fast. All collected blood samples (performed by nurses) and biochemical analyses were done in a private laboratory. In addition to the fasting overnight, it was requested that participants remain 72 hours (3 days) without performing physical exercises (exercise or unusual activities) for which there were no changes in circulating concentrations of cytokines. Fasting blood measurements were performed by the colorimetric method. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerols (TAG) and plasma glucose was analyzed.

The measurement of cytokines TNF-α, IL-6, IL-10, IL-1ra, adiponectin, fatty acids and insulin were performed in the laboratory of Laboratory of Cell Physiology (LAFICE) of the University, using serum obtained on the day of collection in a private laboratory that stored the tubes with content blood in a freezer at -80 °C after centrifugation.

Quantification of cytokines were performed by the technique of immunoassay ELISA (Enzyme-Linked Immunosorbent Assay) using a microplate reader of the brand Bio-tek model ELX 800 with a 450 nm filter to read the absorbance, and all analyzes performed in monoclata. The analysis of concentrations of circulating cytokines was used reagent kits of the brand Ray-Biotech Human (RayBiotech Inc, Norcross, GA, USA).

Sensibilities of each enzymatic kit are 6000-24.58 pg/ml to TNF-α, 1000-1.37 pg/ml to IL-6, 500-7.8 pg/ml to IL-10, 2000-31.2 pg/ml to IL-1ra, 18,000-24.69 pg/ml to adiponectin, 333-1.4 μM to fatty acids and 300-4.69 μU/ml to insulin. The intra-assay variability of the TNF-α, IL-6, adiponectin and insulin kits was <10% and its inter-assay variability was <12%; and intra-assay variability of the IL-10 and IL-1ra kits was 1.7-5.0% and 3.7-7.3% and its inter-assay variability was 5.9-7.3% and 6.7-11.0%, respectively.

**Dietary intake**

The estimation of caloric consumption was made by 3 day Food Register regarding food intake. Participants were properly instructed to fill out the document. The food register is divided according to the number of meals and amount of food were recorded in household measures. The analysis and interpretation of data was used the Nutrition Support Program (Nutwin) of the Federal University of São Paulo, which has resulted in the total energy and macronutrients.

**Statistical Analyses**

Data normality was verified using the Komogorov-Smirnov test. Numerical variables were presented as mean and standard-deviation. Analyses of independent sample test (Mann-Whitney) were used in order to verify the differences between the genders. Correlations analyses were made between macronutrients intake, body composition variables, blood sample and cytokines concentrations. Additionally, linear regression was applied to verify the interaction between the variables. Statistical significance was set at p-value <5% and the statistical software SPSS version 17.0 (SPSS Inc, Chicago, IL) performed all analyses.

**Results**

General characteristics and body composition, of adolescents are described by gender in the table I. There are similarities, according gender, between the variables BMI (p=0.316), FM (p=0.416), IAAT (p=0.505) and SAT (p=0.935). In the table II are described the mean and standard deviation of cytokines and metabolic variables according the gender, whose values are similar between the groups.

It was observed that the diet of 59.2% of adolescents is predominantly composed by carbohydrate. In general, all subjects showed some disequilibrium in macronutrients intake, verifying a higher consumption of carbohydrates compared to the recommended age range (approximately 48.5% of daily intake).

In the table III and table IV are showed the relationship between macronutrients intake, in grams (g), cytokines anti and pro inflammatory and variables of lipid profile according gender. In the male group was observed that the behavior of metabolic variables and cytokines are significant correlated with the consumption of total lipids, or their types, whereas in the female group the relations was lower when compared with male group.
In addition, the variables that showed correlations, in both groups, was included in a model of multiple linear regression to verify the interaction between variables and we observed that insulin concentration had significant interaction with MUFA(g) ($\beta = -18.4, p=0.004$) and adiponectin with CHO(g) ($\beta = -58.2, p=0.032$) in the group male and female, respectively. This dates suggest that nutrients has a close relationship with relevant and regulatory metabolic variables and, according the gender, the positive contribution of nutrient intake is distinct.

**Discussion**

The main result of the study was the relationship between CHO and LIP intake, mainly MUFA and PUFA, with cytokines and lipid profile in both genders. In the literature is well known that the excessive consumption of CHO and LIP is a trigger to activation of inflammatory pathway mediated by increase of TNF-α and IL-6.

In relation to CHO is very important to consider the glycemic index because high glycemic index postprandial is associated with hyperglycemia and an increase in the release of insulin may be a factor risk to cardiovascular diseases. Recent study of Gögebakan et al with several combinations of diets with the aim of weight loss and improvements in cardiovascular risk factors was observed that low glycemic index and, to a lesser extent, low protein intake may specifically reduce low-grade inflammation, by decrease of high-sensitivity C-reactive protein (hsPCR).

Currently some studies have shown that the excessive CHO intake is able to increase the expression of inflammatory genes as a result of changes in epigenetic mechanisms. Exposure of the body to high glucose concentrations, even transitory, can change histone methylation patterns at promoter expression of inflammatory genes related with generation of oxygen radicals. In addition, it is known that long term exposure to higher plasmatic concentrations of glucose, from diet, is correlated with high fasting insulin concentrations and insulin resistance.

**Table I**

| Body composition characteristic of adolescents obese stratified by gender. Presidente Prudente/SP-2013 |
|-------------------------------------------------|-------------------------------------------------|-----------------|
| **Male (n=23)** | **Female (n=14)** | **p-value** |
| Age (years) | 13.5 (1.6) | 12.4 (1.3) | 0.038 |
| Weight (Kg) | 92.3 (15.4) | 78.6 (18.2) | 0.017 |
| Height (m) | 1.66 (0.1) | 1.56 (0.1) | 0.012 |
| BMI (Kg/m²) | 33.3 (4.7) | 31.9 (5.0) | 0.316 |
| WC (cm) | 99.6 (10.6) | 91.7 (11.9) | 0.058 |
| FM (Kg) | 40.6 (8.3) | 39.2 (9.8) | 0.416 |
| FFM (Kg) | 47.3 (8.9) | 35.2 (8.3) | 0.001 |
| TFM (Kg) | 47.1 (4.5) | 54.7 (3.3) | 0.000 |
| %FM (%) | 44.8 (5.1) | 50.9 (3.2) | 0.001 |
| IAAT (cm) | 3.6 (1.3) | 3.9 (1.3) | 0.505 |
| SAT (cm) | 2.8 (0.6) | 2.8 (0.8) | 0.935 |

SD standard-deviation, BMI body mass index, WC waist circumference, FM fat mass, FFM fat free mass, TFM trunk fat mass, %FM percentage of fat mass, IAAT intra-abdominal adipose tissue, SAT subcutaneous adipose tissue. p<0,05.

**Table II**

| Cytokines and lipid profile of adolescents obese stratified by gender. Presidente Prudente/SP-2013 |
|-------------------------------------------------|-------------------------------------------------|-----------------|
| **Mean (SD)** | **Mean (SD)** | **p-value** |
| TAG (mg/dL) | 102.9 (48.9) | 117.9 (59.3) | 0.287 |
| TC (mg/dL) | 143.7 (22.5) | 149.5 (36.2) | 0.481 |
| HDL-c (mg/dL) | 40.5 (5.7) | 40.4 (6.9) | 0.975 |
| LDL-c (mg/dL) | 82.5 (20.6) | 85.6 (31.9) | 0.839 |
| TNF-α (pg/ml) | 563.8 (547.2) | 531.3 (265.3) | 0.627 |
| IL-6 (pg/ml) | 10.4 (7.1) | 13.3 (7.4) | 0.291 |
| IL-1ra (pg/ml) | 385.5 (331.7) | 583.1 (643.6) | 0.826 |
| IL-10 (pg/ml) | 36.6 (129.8) | 6.4 (6.1) | 0.595 |
| Adiponectin (pg/ml) | 3307.7 (3080.9) | 3925.9 (3876.7) | 0.730 |
| Insulin (µU/ml) | 62.3 (112.0) | 30.7 (26.1) | 0.814 |
| Fatty acids (µm) | 175.9 (127.2) | 209.5 (113.6) | 0.354 |

TAG triacylglycerol, TC total cholesterol, HDL-c high density lipoprotein cholesterol, LDL-c low density lipoprotein cholesterol, TNF-α tumor necrosis factor alpha, IL-6 interleukin 6, IL-1ra interleukin-1 receptor antagonist, IL-10 interleukin 10. p<0,05.
According to our results, the adolescents consume high amounts of CHO and this intake may be the reason of lower expression of anti-inflammatory cytokines, by inhibiting production or imbalance in the anti and pro-inflammatory cytokines ratio, resulting in low plasmatic concentrations of anti-inflammatory cytokines, such as IL-1ra and adiponectin respectively in boys and girls, and in the female group is a significant contribution to inflammatory pathway.

In this study, females had significant higher values of trunk fat and it is well known that adipose tissue from visceral area are prone to secrete TNF-α, furthermore, as a consequence of the increased levels of this pro-inflammatory cytokine, adiponectin concentration decreases\(^13\). Besides this, there is an insight regards to gender differences and adiponectin concentration, which suggest that male hormone may decrease adiponectin also explaining a higher cardiovascular risk in the gender\(^14\).

Though there is evidence about the amount and type of fat in the diet may have detrimental effects on metabolism, CHO are also able to influence the me-
In this sense, the digestion ofCHO appears to be related to some benefits on lipid metabolism, whereas carbohydrates with high glycemic index are associated with higher prevalence of immunometabolic diseases.

However, when is frequent the dietary predominant in CHO, according Seal et al., may contribute to exogenous glucose oxidation, thus producing low circulating of non-esterified free fatty acids (NEFA) and may contribute to the long term of an insulin sensitivity profile and, consequently, a low-grade inflammation condition with abnormal secretion of cytokines. Such a statement leads us to believe that the correlation between CHO and inflammatory cytokines, and not with NEFA, was due to the predominance of CHO intake.

Furthermore, the elevations of total cholesterol by CHO might be explained through hepatic metabolism that increases the process of lipogenesis de novo with significant elevation of triacylglycerol and lipoproteins. Our dates are contrary to the findings of Welsh et al. but both the studies found a relationship between CHO/sugar consumption and lipids profile alterations.

Excessive intake of lipids leads to a state of lipotoxicity, which is a key to development of chronic low-grade inflammation and metabolic diseases as insulin resistance. These chronic diseases are defined by changes in the quality and quantity of lipids used for metabolism and the inability of metabolic control mechanisms and manages the exhibits of lipid intake in the long term. This information corroborates with the present research, where the male obese group correlated with some inflammatory cytokines and the female group with HDL-c.

A constant excessive supply of lipids is able to activate an inflammatory cascade, mediated by TLRs and NF-kB, which have been proposed as a primary molecular mechanisms mediating inflammation of the adipose tissues. The TLR family in adipose tissue is cell surface receptors that are activated by SFA. The SFA connect naturally with TLR4, as well as endotoxin, and induces the activation of NF-kB in macrophages of adipose tissue inducing the gene transcription of IL-6 and TNF-α involved in low-grade inflammation, innate adaptive immunity and apoptosis.

Recent evidences also suggests that reduced activity of AMP protein kinase (AMPK) is associated with inflammation in adipose tissue and the peroxisome proliferator-activated receptor (PPAR), a nuclear transcription factor and a potent inducer of genes oxidizing lipids, capable of reducing inflammation in adipose tissue. Activation of PPAR increases the production of anti-inflammatory cytokines such as IL-1ra and adiponectin, explaining the possible cause of the negative correlation with cytokine these macronutrients in this research by inhibition of AMPK and PPAR pathway.

In literature is well established that the increase in consumption of unsaturated fats, such as MUFA and PUFA, is able to affect the total of HDL-c improving lipid profiles and, consequently, may be reduces metabolic factors associated with cardiovascular diseases. This dates support our findings when observed positive correlation between MUFA and PUFA with total cholesterol based on the premise that the unsaturated fats contributed to the increase in HDL-c alone and/or in the total/HDL-c ratio. In addition, we hypothesized that the positive association between total cholesterol and lipids intake is in response to increase of HDL-c and/or your ratio.

A recent review, including studies from 2000 to 2011, confirmed the benefits of MUFA intake compared to CHO or other type of lipids (PUFA) on insulin sensitivity. In animal models treated with MUFA, was demonstrated an improvement in peripheral insulin resistance induced by high-fat diet by increase glucose uptake and better response to insulin by mechanisms independents on PPAR-alpha. Not only MUFA intake but also PUFA presented negative correlations with an anti-inflammatory cytokine, IL-1ra, as also reported by Bjermo et al. confirming the hypothesis that PUFA, especially n-6 polyunsaturated fatty acids, has inflammatory effects according the type of lipid.

On the other hand, diet enriched with n-3 PUFA, may exerts a beneficial effect on inflammation via inhibition of NF-kB activation, furthermore, it is not clear which mechanism triggers the inactivation, if is the modified production of inflammatory lipid mediators, such as prostaglandin GE2 and it is also possible that n-3 PUFA changes the sensitivity of the TLR-4 receptor for LPS.

Limitations in this study are observed and other researchers should be performed filling out some gaps and better control of some variables such as glycemic index quantification of the foods. We believe that the sexual hormone influence the behaviour of inflammatory pathway mediated the difference between metabolism and more studies should be conducted with the control of these factors.

In summary, we conclude that the macronutrients intakes is associated with low-grade inflammation in obesity, by production of inflammatory cytokines and alteration of the lipid profile, but male obese adolescents is more responsive of this consumption when compared with female obese adolescents by inflammatory and lipid variables.

Acknowledgments

Study Center and Laboratory of Assessment and Prescription of motor activity (CELAPAM) and Cell Physiology Laboratory (LaFiCe).

Funding received

Capes – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.
Conflicts of interest

The authors have no conflicts of interest that are directly relevant to the content of this article.

References


