

UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE AND TECHNOLOGY „GEORGE EMIL PALADE”
TÂRGU MUREŞ

DOCTORAL SCHOOL

SUMMARY OF THE DOCTORAL THESIS

PhD THESIS

Study of Metabolic, Immunological, and Hematological Changes Associated with Overweight and Obesity in Healthy Young Adults

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INTRODUCTION

Excess adipose tissue is associated with chronic low-grade systemic inflammatory syndrome (CLGSIS) and metabolic alterations that lead to the development of non-communicable chronic diseases and neoplasms. Additionally, aging is associated with immunosenescence and inflammaging, further contributing to maintaining a pro-inflammatory environment.

OBJECTIVES

This study aims to accurately assess the impact of excess adipose tissue on inflammatory, metabolic, and immunological parameters in clinically healthy young adults aged between 20-35 years. *Study 1* aims to: evaluate the prevalence of CLGSIS and determine the serum concentrations of pro-inflammatory cytokines, as well as their correlations with adipometric indices; identify the interrelationship between adiponectin, leptin, and excess adipose tissue by analyzing correlations with adipometric indices. *Study 2* aims to: assess the prevalence of metabolic syndrome (MetS) and the correlations between metabolic indices derived from fasting serum concentrations of glucose, insulin, and C-peptide with the defining criteria; evaluate the inflammation associated with MetS by determining the correlations between hsCRP and metabolic indices; determine threshold values for the early definition of MetS using the most frequently employed indices (HOMA-IR, HOMA-BETA, and QUICKI). *Study 3* aims to: analyze the expression of leukocyte populations in peripheral blood, the phenotypic expression of lymphocyte subtypes, and their correlations with anthropometric indices; analyze the correlations between T lymphocytes (TL) and NK cell subsets with the serum concentration of IFN- γ and TNF- α ; investigate the effect of age on the expression of NK subsets.

GENERAL METHODOLOGY

A total of 167 voluntary subjects participated, of which 128 (divided by BMI into normal weight-NW, overweight-OW, and obese-OB) met the eligibility criteria: inclusion criteria (age between 20-35 years, without acute or chronic pathologies, in an apparently healthy clinical state) and exclusion criteria (pregnant or postpartum women, use of anti-inflammatory medication in the last 30 days, recent test results indicating acute inflammation). Participants completed an Inclusion Questionnaire to identify personal and family medical history, followed by a set of mechanical anthropometric measurements (circumference of arm-AC, waist-WC, hip-HC, height, waist-to-hip ratio-WHR, waist-to-height ratio-WHtR) and electronic measurements (bioelectrical impedance: visceral fat level-VFL, total body fat mass-TBFM, BMI), as well as blood pressure. From venous blood samples, the following parameters were measured: glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, AST, ALT, uric acid, albumin, hsCRP, insulin, C-peptide, adiponectin, IFN- γ , IL-6, leptin, TNF- α , IL-1 β , complete blood count, and lymphocyte subsets (CD3, CD4, CD8, CD16, CD19, CD45, CD56, CD69).

RESULTS

Study 1: The serum concentrations of all cytokines were higher in the OW/OB group, with significant differences for IL-6, hsCRP, TNF- α , and IFN- γ . Compared to IL-6, hsCRP showed a stronger positive correlation with all adipometric indices. Although total leukocytes were within the reference range, they were significantly higher in the OW/OB group. IL-1 β was the only marker that did not show a significant difference between the study groups. Except for IL-1 β , cytokine concentrations increased in parallel with the values of all adipometric indices. hsCRP, TNF- α , and total leukocytes showed significant positive correlations with all adipometric indices. IL-6 demonstrated a significant positive correlation with WHR, WHtR, BMI, TBFM, and VFL, while IFN- γ showed a significant positive correlation with HC, BMI, and TBFM. Although IL-1 β did not show any significant correlations, two trends were identified: a positive correlation with WHR and VFL, and a negative correlation with AC, WC, HC, WHtR, BMI, and TBFM. Serum adiponectin concentration was significantly lower, and leptin concentration was significantly higher in the OW/OB group. All adipometric indices showed significant negative correlations with serum adiponectin concentration. With the exception of the correlation with WHR, leptin had significant positive correlations with all adipometric indices. The adiponectin/leptin ratio was negatively and

significantly correlated with all adipometric indices, and showed a significantly lower value in the OW/OB group.

Study 2: HOMA-IR INS, HOMA-BETA, and QUICKI showed statistically significant differences between the MetS(+) and MetS(-) groups. The accuracy of these tests and their ability to differentiate between subjects with and without MetS were statistically significant only in males. The optimal threshold values for early detection of MetS were as follows: for HOMA-IR INS: females = 1.805, males = 2.115, both sexes = 1.855; for HOMA-BETA: females = 71.305, males = 106.370, both sexes = 82.250; for QUICKI: females = 0.355, males = 0.345, both sexes = 0.355. Except for DI and 20/C-peptide*glucose, BMI showed significant correlations with all metabolic indices. In both sexes, WC was significantly positively correlated with HOMA-IR INS and HOMA-BETA, and negatively correlated with QUICKI. Fasting serum glucose was significantly positively correlated with HOMA-IR INS, HOMA-IR CP1, HOMA-IR CP2, and negatively correlated with HOMA-BETA CP, DI, and QUICKI. In females, HDL-cholesterol was significantly positively correlated with QUICKI, while in males, it was significantly negatively correlated with HOMA-IR INS and HOMA-BETA, and positively with QUICKI. Triglycerides were significantly positively correlated with HOMA-IR INS and HOMA-BETA, and negatively correlated with QUICKI. Systolic blood pressure was significantly positively correlated with HOMA-IR INS and HOMA-BETA, while diastolic blood pressure was positively correlated with DI. In the mixed group (both sexes), hsCRP was positively correlated with all metabolic indices except for QUICKI and 20/C-peptide*glucose, with all correlations being significant except for DI. In males, hsCRP was significantly correlated positively with all metabolic indices except for DI and 20/C-peptide*glucose. In females, the only significant positive correlation identified was between hsCRP and HOMA-IR CP2.

Study 3: The OB group reported a significantly higher population of B lymphocytes (BL), which positively correlated with all adipometric indices. Total T lymphocytes (TTL) showed a insignificant positive correlation with IFN- γ and TNF- α . Early activated T lymphocytes (EATL) negatively correlated with IFN- γ and TNF- α , with a significant correlation only with IFN- γ . Total NK lymphocytes (TNKL) and NK^{bright} cells showed a insignificant negative correlation with TNF- α , while NK^{dim} cells had a significant negative correlation with TNF- α . All NK cell subpopulations (TNK, NK^{bright}, NK^{dim}) showed insignificant positive correlations with IFN- γ . As adipose tissue increased, the expression of TNKL decreased, showing insignificant negative correlations between TNKL and all adipometric indices, with the lowest average expression observed in the OB group. NK^{dim} cells made up 80-90% of TNKL in each group, while NK^{bright} cells represented 4-5%. The average expression of NK^{dim} showed a insignificant successive decrease from the NW to the OB group, while NK^{bright} expression remained constant across all three groups. Age was insignificantly negatively correlated with TNKL and NK^{dim}, and positively correlated with NK^{bright}. The frequency of helper T lymphocytes (ThL) was insignificantly lower in the NW group, while EATL showed significantly higher expression. EATL had a significant negative correlation with all adipometric indices. ThL frequency gradually decreased insignificantly in groups with excess adipose tissue and negatively correlated with all adiposity indices. Cytotoxic T lymphocytes (TcL) showed minimal differences between groups, with the highest value observed in the OW group. Serum concentrations of IFN- γ and TNF- α insignificantly increased as the TTL population grew. EATL had a significant negative correlation with serum IFN- γ concentration and a non-significant negative correlation with TNF- α .

ORIGINALITY OF THE THESIS

The state of "clinically apparent health" allowed for the investigation of the baseline status of the control group (NW) and comparison with the changes primarily associated with excess adipose tissue in OW and OB individuals. The age range of 20-35 years is hormonally stable and early enough for immunosenescence not to significantly affect the immune system. These criteria exclude the effects of other comorbidities and provide a basis for personalized prevention strategies in Public Health. Since the entire cohort is apparently healthy, the data can serve as a control group for other pathologies, contributing to mapping out key pathophysiological mechanisms. Such studies support global health data collection and raise awareness of MetS. This is a pilot study in Romania investigating subclinical chronic inflammation associated with obesity, analyzing NK lymphocyte subsets in young adults, and identifying cut-off values for metabolic indices for early detection of

MetS. It was highlighted that metabolic indices, including C-peptide, do not provide additional clinical value for the detection of MetS. The study demonstrates that excess adipose tissue influences the expression of inflammatory markers even when the values remain within normal ranges.