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Obesity as a risk factor: evaluation of inflammatory markers in a population of for healthcare workers (HCWs)

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Introduction

Definition of Obesity

Obesity represents a multifactorial pathological condition characterized by an excessive accumulation of adipose tissue, which entails significant health risks. The World Health Organization (WHO) defines obesity through the body mass index (BMI), calculated using the formula: BMI=weight (kg)/height² (m^2) [1].

According to the WHO classification, the following weight categories are identified: normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI=25–29.9 kg/m^2), class I obesity (BMI=30–34.9 kg/m^2), class II obesity (BMI=35–39.9 kg/m^2), and class III obesity (BMI=≥40 kg/m^2).

However, it is important to note that BMI, although a practical and widely used tool, has significant limitations as it does not distinguish between fat mass and lean mass, nor does it consider the distribution of adipose tissue an element crucial in stratifying cardiometabolic risk [2-7].

Obesity is now recognized not only as a simple risk factor but as a chronic, relapsing, and complex disease that requires a personalized and multidisciplinary therapeutic approach. The recent Lancet Commission introduced an important distinction between preclinical obesity, characterized by excess fat in the absence of clinically detectable damage and clinical obesity, marked by the presence of biological, metabolic or psychological dysfunctions associated with adiposity [8-9].

Epidemiology of obesity globally and in Italy

Obesity has become a true global pandemic, with an alarming rise in its prevalence in recent decades. Worldwide, from 1990 to 2021, the prevalence of obesity increased by 155% in men and by 105% in women, reaching over 2 billion adults over the age of 25 in 2021. According to the most recent data published, in 2022 more than one billion people globally suffered from obesity, including 159 million children and adolescents and 879 million adults [10-12].

Future projections are particularly worrisome: estimates indicate that by 2050, about 60% of adults worldwide (3.8 billion people) will be living with overweight or obesity, representing an unprecedented threat in terms of disease and premature death. Among young people, obesity prevalence has tripled from 1990 to 2021, rising from 2.0% to 6.8%, and a further increase of 121% is projected by 2050, with around 360 million youths aged 5 to 24 expected to be obese [13].

In Italy, the epidemiological situation reflects the global trend but with specific characteristics. According to the latest ISTAT data for 2021, 12% of the adult population is obese, while about 46.2% is overweight or obese combined. In 2023, obesity reached 11.8% among adults, up 38% since 2003. Overall, in Italy, there are an estimated 23.3 million adults over 18 years with excess weight [14-18].

Obesity in Italy presents significant geographic and demographic differences. Southern regions show higher obesity rates than the national average, with Puglia (15%), Molise (14.9%), and Basilicata (14%) leading. By contrast, northern regions like Lombardy and Trentino-Alto Adige record prevalences around 10-11%. As for age groups, obesity increases significantly with aging, reaching nearly 15% of the population over 65. Of particular concern is the rise of obesity among younger generations: the rate of excess weight at ages 20-24 is much higher among those born in the early 2000s (21.6%) compared to those born in the 1960s (13.4%) [14, 15, 17].

Obesity as a risk factor for diseases

Obesity is one of the main risk factors for the development of numerous chronic non-communicable diseases, with a significant impact on morbidity, mortality, and quality of life. The close association between obesity and cardiovascular diseases (CVD) is well documented: overweight and obesity account for about 55% of hypertension cases and 35% of heart attack cases. Obesity increases the risk of coronary artery disease, heart failure, atrial fibrillation, and sudden cardiac death, with a risk proportional to BMI and worsened by comorbidities such as hypertension and dyslipidemia [19-22].

Type 2 diabetes mellitus represents one of the most frequent complications of obesity, with 80% of type 2 diabetes cases attributable to overweight and obesity. The insulin resistance typical of obese patients is the fundamental pathogenetic mechanism, with obesity increasing the risk of developing diabetes by more than fourteen times when associated with an unfavorable lifestyle and high genetic predisposition [23].

Obesity is also associated with an increased risk of developing numerous cancers. According to previous epidemiological studies 5–6% of all cancers diagnosed in 2012 were attributable to the combined effects of diabetes and high BMI as independent risk factors. The cancers most frequently associated with obesity include those of the esophagus, liver, pancreas, gallbladder, uterus, kidney, colon, and breast [24-27].

Other significant complications of obesity include non-alcoholic fatty liver disease (which can evolve to cirrhosis and hepatocellular carcinoma), obstructive sleep apnea syndrome (OSAS), gallstones, osteoarthritis, reproductive system disturbances (male and female), and psychological disorders. Obesity is also associated with greater susceptibility to infections, including respiratory, postoperative and nosocomial infections [28, 29].

Description of obesity as a disease

Obesity is a chronic multifactorial disease that results from a complex interaction between genetic, metabolic, behavioral, environmental, and sociocultural factors. From a pathogenetic point of view, obesity develops when energy balance is chronically positive, with caloric intake exceeding energy expenditure over long periods [30, 31].

Genetic factors play a significant role, regulating not only basal energy expenditure but also diet-induced thermogenesis and the distribution of body fat, particularly abdominal fat, which increases the risk of metabolic syndrome.

However, the most common forms of obesity have a multifactorial etiology resulting from a complex interaction between the biology of energy metabolism and the environment [32].

The modern obesogenic environment, characterized by easy access to highly caloric foods and sedentary lifestyles, contributes significantly to the obesity epidemic.

Environmental factors such as nutrition, sleep patterns, and alcohol consumption can epigenetically alter gene expression in various metabolic pathways, suggesting the possible reversibility of some environmental factors [33].

From a clinical perspective, obesity is characterized by a pathological expansion of adipose tissue that can occur through two main mechanisms: hypertrophy (increase in the size of existing adipocytes) and hyperplasia (increase in the number of adipocytes).

When the expansion of adipose tissue exceeds its vascularization capacity, cellular hypoxia occurs, triggering a cascade of pathological events including endoplasmic reticulum stress, mitochondrial dysfunction, apoptosis, macrophage infiltration and chronic inflammation [34-36].

Pathophysiology of the obese subject

The pathophysiology of obesity is characterized by profound changes in the function of adipose tissue, which shifts from a functional endocrine organ to dysfunctional and inflammatory tissue. In obese individuals, progressive adipocyte hypertrophy leads to an insufficient vascular supply with consequent cellular hypoxia, which represents one of the main triggers of adipose tissue inflammation [37-40].

Hypoxia induces the activation of pro-inflammatory mediators in adipocytes and macrophages, including TNF-α, IL-1, IL-6, CCL2 and inducible nitric oxide synthase (iNOS). This state of chronic hypoxia causes structural and functional changes in adipose tissue, compromising its ability to store energy as lipids and exposing other tissues to an excessive flow of lipids [40].

Adipose tissue dysfunction also manifests itself through alterations of the endoplasmic reticulum (ER stress). In obese adipose tissue, ER stress is markedly increased, with a 3–4-fold upregulation of proteins related to the unfolded protein response (UPR), such as glucose-regulated protein 78

(GRP78/BiP). Prolonged UPR activation progressively impairs the biosynthetic capacity of insulin and triggers CHOP-dependent apoptotic programs via calcium-dependent mechanisms and mitochondrial dysfunction [36].

Mitochondrial dysfunction is another crucial aspect of the pathophysiology of obesity. In obesity, adipocytes display fragmented mitochondrial networks and accelerated generation of reactive oxygen species (ROS). ROS concentrations in the cells typically range from physiological levels (100nM H_2O_2) to pathological ones (>500nM H_2O_2). Reduced activity of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a key regulator of mitochondrial biogenesis, correlates with elevated inflammatory markers and oxidative damage in adipose tissue [41].

The accumulation of circulating free fatty acids (FFA), derived from visceral adipose tissue, plays a key role in the pathogenesis of the metabolic complications of obesity. Increased FFAs stimulate hepatic synthesis of triglyceride-rich lipoproteins (VLDL), promoting the atherogenic process and contributing to insulin resistance through ectopic lipid accumulation in skeletal muscle, liver, pancreas and heart [42, 43].

Bio-humoral changes in the obese

Obese individuals present significant alterations in their bio-humoral profile that characterize a state of metabolic dysfunction. Insulin resistance represents the main metabolic alteration, characterized by an inadequate response of insulin target organs (skeletal muscle, liver, and adipose tissue) to the hormone's effects. In an effort to compensate for this resistance, the pancreas increases its insulin production, resulting in a state of chronic hyperinsulinemia [44-49].

Alterations in glucose metabolism include fasting hyperglycemia, impaired glucose tolerance, and, in more advanced cases, manifest type 2 diabetes mellitus. Insulin resistance manifests through a complex scenario that includes alteration in translocation of the glucose transporter GLUT4, with about a 70–80% reduction in the cellular uptake capacity for glucose compared to healthy adipocytes [50, 51].

The characteristic dyslipidemia of obesity, especially visceral obesity, features: 1) increased plasma triglyceride concentration; 2) small, dense, highly atherogenic low-density lipoproteins (LDL); 3) low levels of HDL cholesterol. Hypertriglyceridemia is a consequence of the high flow of free fatty acids to the liver, which stimulates increased VLDL synthesis [52, 53].

Other altered biohumoral markers in obesity include high levels of C-reactive protein (CRP), indicative of a chronic inflammatory state. Serum uric acid is typically elevated, increasing the risk of gout, while a prothrombotic state develops (with increased levels of fibrinogen and type I plasminogen activator inhibitor), as well as a systemic inflammatory state [54, 55].

Modifications of the immune system in obesity

Obesity induces profound alterations in the immune system, characterized by chronic activation of both the innate and adaptive immune responses. In obese individuals, adipose tissue is infiltrated by numerous immune cell populations, with macrophages accounting for up to 50% of the immune cells present in obese adipose tissue [56, 60].

Adipose tissue macrophages (ATMs) undergo a crucial phenotypic change in obesity: under normal conditions, macrophages predominantly display an anti-inflammatory M2 phenotype, but in obesity there is a shift toward the pro-inflammatory M1 phenotype. This transition is accompanied by increased secretion of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β [58].

Macrophage infiltration in adipose tissue is promoted by the release of chemokines, particularly monocyte chemoattractant protein-1 (MCP-1/CCL2), which recruits monocytes from peripheral blood via the CCR2 receptor. Once in the adipose tissue, these macrophages form characteristic crown-like structures around dead or dying adipocytes, amplifying the inflammatory response [58, 61, 62].

Lymphocytes also play a significant role in the immune dysfunction associated with obesity. In obese adipose tissue, there is a reduction in regulatory T lymphocytes (Treg), which normally perform anti-inflammatory and immunotolerance functions. This reduction, associated with an increase in pro-inflammatory T lymphocytes (Th1 and Th17), contributes to the amplification of the inflammatory state [59, 63, 64].

Regulatory B lymphocytes, constitutively expressed in white adipose tissue and characterized by the production of IL-10 and TGF-β with anti-inflammatory effects, are significantly reduced in the obese population. Obesity is also associated with an increased presence of innate immune cells in peripheral blood, with higher levels of monocytes, lymphocytes, immature granulocytes, and neutrophils compared to normal-weight individuals [65, 66].

Metabolic overload in obesity can suppress the regulation of the immune system and induce inflammatory responses, increasing the risk of developing autoimmune diseases. Overnutrition promotes chronic activation of immune cells through engagement of intracellular pathways that sense nutrients and energy, resulting in systemic low-grade inflammation [63].

Modifications of the inflammatory profile in obesity

Obesity is characterized by a state of chronic low-grade inflammation, which represents the connecting link between excess body weight and the development of metabolic and cardiovascular complications. This inflammatory state is mainly mediated by visceral adipose tissue, which takes on the characteristics of a truly dysfunctional endocrine organ, secreting numerous bioactive pro-inflammatory molecules [67-70].

Adipokines represent the main mediators of inflammation in obesity. Leptin, produced proportionally to fat mass, is a pro-inflammatory adipokine that increases the production of TNF-α and IL-6 by monocytes and stimulates the production of chemokines (CCL3, CCL4). In obesity, elevated leptin levels are associated with a state of central leptin resistance, perpetuating the vicious cycle of excess weight [70-72].

By contrast, adiponectin, the only adipokine with anti-inflammatory properties, is significantly reduced in obesity. Adiponectin modulates insulin sensitivity and has vasoprotective and anti-inflammatory actions; its levels are inversely correlated with the amount of fat mass. The reduction in adiponectin in obesity is induced by inflammatory stimuli such as IL-6 and TNF-α, as well as by hypoxia and oxidative stress [70, 73, 74].

The pro-inflammatory cytokines TNF-α and IL-6 play a central role in obesity-associated inflammation. TNF-α, produced mainly by infiltrating macrophages in adipose tissue, impairs insulin-mediated phosphorylation of IRS-1 in muscle and adipose tissue, promoting insulin resistance. TNF-α levels are increased in the adipose tissue and plasma of obese individuals, and weight loss results in a reduction of TNF-α expression [70, 75-77].

IL-6 is produced both by adipocytes and by infiltrating macrophages and is increased 5-10 times in obese adipose tissue compared to lean controls. IL-6 is involved in activating NF-κB-dependent pro-inflammatory signaling pathways and contributes to insulin resistance through multiple mechanisms. Clinical studies have shown that high levels of C-reactive protein (CRP) and IL-6 predict the development of diabetes [70, 78, 79].

Oxidative stress represents another key element of the inflammatory state in obesity. In obese adipose tissue, excessive generation of ROS by dysfunctional mitochondria and NADPH oxidase complexes induces oxidative damage to DNA, proteins and lipids. This oxidative stress activates pro-inflammatory signaling pathways, triggering positive feedback cycles that perpetuate inflammation [68, 80].

Chronic inflammation in obesity is also characterized by the activation of specific intracellular signaling pathways, including JNK (Jun N-terminal kinase) and IKK (IκB kinase), which converge in the activation of the NF-κB transcription factor. NF-κB activation results in increased expression

of genes encoding pro-inflammatory cytokines, chemokines, adhesion molecules, and enzymes involved in the production of inflammatory mediators [70].

Obesity as an occupational risk factor in healthcare workers (HCWs)

The work environment plays a significant role in the epidemiology of obesity, with healthcare workers (HCWs) representing a professional category that is particularly exposed. The relationship between obesity and occupational risk must be understood in two ways: on the one hand, obesity acts as a cause or contributing cause in exposing the worker to the risk of injuries or work-related illnesses; on the other, specific occupational risk factors (shift work, occupational stress) favor the development of obesity itself [81-84].

Among HCWs, shift work, especially night shifts, represents an important risk factor for obesity. A systematic review found that the risk of obesity increases by 12% in nurses working night shifts compared to those working two daytime shifts. Night work alters circadian rhythms and cortisol production, favoring the accumulation of adipose tissue through mechanisms that involve the differentiation of adipocyte precursors into mature adipocytes [81, 83, 85, 86].

Chronic work-related stress is another significant risk factor for the development of obesity among HCWs. Studies conducted in the United Kingdom have shown that exposure to chronic workplace stress increases the likelihood of becoming obese by 50%, and in men doubles the probability of developing metabolic syndrome. HCWs subjected to excessive workloads, grueling shifts, and chronic psychophysical stress display high levels of burnout, which is associated with compensatory eating behaviors and reduced physical activity [82, 87, 88].

From an occupational health and safety point of view, obese or overweight workers have a higher risk of developing occupational diseases, especially musculoskeletal disorders. Obese workers are twice as likely as those of normal weight to develop upper limb tendinopathies and four times as likely to develop carpal tunnel syndrome. Furthermore, obesity limits physical functionality, including mobility and flexibility, increasing the risk of workplace injuries by 62% compared to normal-weight workers [82, 84].

Obese healthcare workers also experience an increased risk of absenteeism from work. According to studies conducted in various countries, obese employees are 1.5 times more likely to be absent compared to colleagues with optimal weight. In aggregate, overweight or obese workers are absent from work 450 million more days per year compared to their normal-weight colleagues in the United States, with an estimated productivity loss of $153 billion per year [87].

The assessment of inflammatory markers in HCWs is particularly relevant, considering that this professional population is exposed to multiple risk factors for obesity and its metabolic complications.

Early identification of alterations in inflammatory biomarkers can enable targeted preventive interventions, contributing not only to the individual worker's health but also to reducing healthcare and social costs associated with obesity and its complications [82, 84, 89-91].

Aim of the study

The aim of this study was to investigate, in a cohort of healthcare workers (HCWs), the impact of obesity and hypercholesterolemia on HDL metabolism and inflammatory markers, and to determine whether these associations are modified in a subgroup of workers undergoing a hypocaloric diet.

Materials and Methods

Population

Participation in this study was offered to the HCWs at an Emergency Hospital, who had undergone mandatory health surveillance, pursuant to Law Decree (DL) 81/08, from January to December 2024 (12 months). All HCWs invited to take part in the project were informed about the objectives and procedures of the study. It was not necessary to receive confirmation from the ethical committee as the activity is ruled by the Law Decree (DL) 81/08 article 25 within the health promotion actions. Participation in the study was on a voluntary basis and each participant gave informed written consent. The workers' council was involved in the study design.

The workers were divided into 5 different groups: 1) HCWs with high BMI>30 and high total cholesterol>200 mg/dl (group HBHC); 2) HCWs with high BMI>30 and total cholesterol within the limits<200 mg/dl (group HBLC); 3) HCWs with normal BMI<25 and elevated total cholesterol values>200 mg/dl (group LBHC); 4) HCWs with normal BMI<25 and total cholesterol within the limits<200 mg/dl (group LBLC); 5) HCWs with high BMI>30 and high total cholesterol>200 mg/dl subjected to a low-calorie diet for 12 months (group HBLCD).

Therefore, the inclusion criteria were those of each group to which they belonged. Assignment to the 5 groups was voluntary, based on inclusion criteria.

The exclusion criteria were refusal of written informed consent; diabetes mellitus under pharmacological treatment; dyslipidemia under pharmacological treatment; recent cancer diagnosis; pregnancy; or chronic diseases such as kidney failure, heart disease, uncompensated endocrine disorders, severe cognitive deficits, history of digestive tract diseases, history of gastrointestinal surgery, acute or chronic illnesses or infections, alcohol or drug use, smoking habits. No age limits were applied.

Clinical Parameters

Each person of the five groups underwent a medical examination, including history of eating habits, objective examination, weight, and height measurement for the calculation of BMI, and other anthropometric parameters for a waist/hip ratio (WHR) index assessment.

Subjects in the first 4 groups (group HBHC, group HBLC, group LBHC and group LBLC) underwent health checks only once at enrollment. Subjects of HBLCD group (HCWs with high BMI and high total cholesterol subjected to a low-calorie diet for 12 months) were tested at time zero (T0), after 6 months (T6) and after 12 months (T12).

For each subject the following parameters were measured during the medical examination: (i) Physical activity was measured in terms of type, frequency (days/week), and duration (in minutes) through a self-questionnaire. To ascertain the energy consumption produced by physical activity, the data were converted into their metabolic equivalent (MET) [92]. The total weekly caloric consumption was calculated using the formula of the International Physical Activity Questionnaire (IPAQ) [93]. Each subject was also required to say whether the activity performed really coincided with the experimental period (Σ (MET × frequency × duration)). (ii) For blood pressure, systolic and diastolic blood pressures (mmHg) were measured three times using the left arm, with the subject seated and at rest for 5 min. The averages of the second and third readings were recorded. (iii) Waist circumference was measured to the nearest centimeter, using a flexible meter, at the end of exhalation, positioning the meter at navel level [94]. (iv) For the BMI, height and weight were measured, with the subject barefoot and lightly dressed and their body mass index was calculated according to the formula weight (kg) divided by height (m) squared. (v) The routine laboratory tests that were performed included liver and kidney function, cholesterol levels (total, LDL, and HDL), triglycerides, blood sugar, and Serum protein electrophoresis (SPEP). (vi) The skinfold caliper measurement (GIMA Fit. Comp Pli Pli Fitness & Computer, Gessate Milano) and impedance analysis (body fat analyzer, Model BT905, Skylark Tokyo, Japan) were performed to evaluate percentage of fat and lean mass and calculate basal metabolism.

The IPAQ (2004) was used to assess the physical activity of each participant. Each participant was asked to indicate the type, frequency (days per week), and duration (hours or minutes per day) of each physical activity performed during the last seven days. The assessment was based on the intensity of the physical activities classified as vigorous (e.g., aerobic walking, jogging, and running), moderate (e.g., brisk walking, general exercises at home, and recreational swimming), or normal walking. The level of physical activity was classified as low, moderate, or high, based on metabolic energy (MET). Further data were collected using a standardized questionnaire aimed at assessing the degree of adherence to the Mediterranean diet, as validated by Martínez-González et al. [95]. The responses to

the questions regarded daily and weekly intake of nutrients such as fruits, vegetables, seasonings (oil), meat, and fat generated a total score. The higher the score, the closer the respondent's eating habits were to the Mediterranean model, i.e., poor adherence ≤5, average adherence 6–9, and good adherence ≥10.

The following checks were performed only for the HCWs of the HBLCD group. A week after the medical checkup, with a specific software (Nutrigeo8, Progeo, Ascoli Piceno, Italy) an individualized qualitative/quantitative diet was designed for each subject which established the Kcal/days. The software performed calculations related to food chemistry (qualitative and quantitative characterization), basal metabolism, energy requirements, and food portions. The software draws on an official database containing over 2200 foods and recipes. For 14 days (7 days before diet delivery and 7 days from the start of the diet) all subjects were asked to send via mobile phone/email a photographic diary of all the foods consumed during the day; the first 7 days before the start of the diet were to study the eating habits and the 7 days after the start of the diet were to assess adherence to the prescribed diet.

Four weeks after recruitment, the HCWs were called back for a further individual interview with a nutritionist, who offered personalized advice on dietary habits and physical activity level, during which they discussed the results of the dietary and physical activity diary and their adherence to the Mediterranean diet. At the return interview, the nutritionist, who was also trained in motivational techniques, encouraged participants to identify and set goals for their physical activity and proper nutrition. Using a motivational counselling approach, the HCWs were helped to identify strategies for achieving these goals, by providing them with useful material, such as the food pyramid [36] and brochures on physical exercise and reducing the use of salt in food, published by the Italian Ministry of Health.

The check-in meetings with the nutritionist were scheduled once a month; at each meeting, each participant received additional motivational support that reminded them of their goals and how to change their life habits. After 6 months (T6) from T0, the HCWs met again with the occupational physician involved in the project, who measured anthropometric parameters (weight, height, and waist), requested blood tests, and collected a diary on dietary and physical activity. For the 12-month follow-up (T12), the same procedure as the T6 follow-up was followed.

Plasma Lipids

Plasma lipids of each subject were determined on 10 ml samples of peripheral venous blood, collected in the morning after an 8-hour fast. Blood tests included a complete blood count (CBC), liver and kidney function tests, blood glucose, and protein analysis. Total cholesterol, high-density

lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TRIG) were also determined. A complete urinalysis was also performed. Lipid profile was measured by enzymatic photometric transmission measurement (Cobas Pro, Roche Diagnostics, Mannheim, Germany).

Determination of Apolipoprotein by Enzyme-Linked Immunosorbent Assay (ELISA)

In order to determine Apolipoprotein A4, Apolipoprotein B, Apolipoprotein E, protein content, 20 µL of plasma samples, were assayed by enzyme-linked immunosorbent assay (ELISA) (antibodies.com LLC, St Louis, MO, USA), according to the manufacturer's guidelines. Results are expressed as ng/mg and ng/mL.

Adipokines and Inflammatory marker

Leptin, adiponectin and interleukin-6 (IL-6) were determined in serum samples by specific ELISA in accordance with the user manual (antibodies.com LLC, St Louis, MO, USA). In brief, samples were incubated in 96-well microtiter plate pre-coated with specific anti-human antibody. The standards and test samples are added into the wells. Following incubation, the wells are washed and then incubated with Biotinylated Antibody, which binds the captured the marker present in each well. Following incubation, unbound biotinylated detection antibody is removed by washing, and an HRP-Streptavidin conjugate is added to the wells and the microtiter plate is incubated. Following incubation and washing, TMB substrate solution is then used to visualize the HRP enzymatic reaction by catalysis to produce a blue-coloured product that changes to yellow after addition of acidic stop solution. The density of yellow is proportional to the amount of protein marker captured in each well. The concentration of protein marker can then be calculated by reading the O.D. absorbance at 450nm in a microplate reader and referring to the standard curve.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics (version 28). Data are summarized as mean and standard deviation according to the result of the normality test. Differences in apolipoproteins, adipokines and inflammatory markers were analyzed across five categories of BMI and cholesterol levels using the Wilcoxon rank-sum test for pairwise comparisons. Pearson correlation test was applied to analyze relationships between continuous parameters. P-values were adjusted for multiple testing using the Bonferroni correction. Two-tailed tests were used, with a nominal significance level of $p < 0.05$.

Results

A total of 100 (100%) HCWs were enrolled during the mandatory occupational health surveillance and divided into 5 groups: 1) 20 (20%) HCWs group HBHC (with high BMI and high total cholesterol); 2) 20 (20%) HCWs group HBLC (high BMI and total cholesterol within the limits); 3) 20 (20%) HCWs group LBHC (normal BMI and elevated total cholesterol values); 4) 20 (20%) HCWs (group LBLC (normal BMI and total cholesterol within the limits); 5) 20 (20%) HCWs group HBLCD (high BMI and high total cholesterol subjected to a low-calorie diet for 12 months). Of the 20 (100%) HCWs in the HBLCD group (i.e., workers followed for 12 months on a low-calorie diet), 6 (30%) did not continue the study: 2 (10%) due to lack of time; 3 (15%) because they did not want to continue the low-calorie diet; and 1 (5%) no longer attended the periodic check-ups.

The study sample of 94 HCWs (100%) included 30 (32%) female and 64 (68%) male HCWs. All workers were divided into five groups as previously explained. Analysis of the five groups revealed homogeneity (no statistically significant differences) in terms of gender, age, seniority, and work shifts. Table 1 reports the main characteristics of the sample divided into 5 groups.

Table 1. Main characteristics of study HCWs divided into 5 groups.

	HBHC	HBLC	LBHC	LBLC	HBLCD^	*p*-Value
Subjects 94 (100%)	20 (21%)	20 (21%)	20 (21%)	20 (21%)	14 (16%)	-
Mean age yrs	54.1 ± 7.8	55.7 ± 5.5	53.3 ± 6.5	51.2 ± 5.3	53.3 ± 8.2	n.s
Sex	13 Male (65%)	15 Male (75%)	14 Male (70%)	13 Male (65%)	9 Male (65%)	n.s
BMI (cm2/kg)	31.6 ± 2.2	32.6 ± 2.4	23.2 ± 1.4	22.5 ± 1.4	32.1 ± 2.3	*p<0.01
Waist circumference (cm)	96.1±6.2	97.4±5.8	91.7 ± 3.4	90.4 ± 4.4	95.8 ± 5.1	*p<0.01
WHR index	0.96 ± 0.18	0.98 ± 0.94	0.92 ± 0.78	0.91 ± 0.62	0.94 ± 0.88	*p<0.01
Seniority work (yrs)	14.3 ± 2.8	15.6 ± 2.5	14.5 ± 3.5	15.2 ± 2.1	13.9 ± 1.4	n.s.
n. night shift work	20 (21%)	20 (21%)	20 (21%)	20 (21%)	14 (16%)	n.s.
Physical activity (n. sub.)	3 (14%)	6 (28%)	5 (23%)	15 (71%)	11 (78%)	*p<0.01
Physical activity (MET)	305.4 ±98.6	357.7 ±120.9	386.4 ±219.4	518.6 ±254.3	405.1 ±245.9	*p<0.01
Adherence to med. diet	4.2 ± 2.5	6.8 ± 1.9	4.4 ± 2.1	7.5 ± 1.6	7.9 ± 1.4	*p<0.01
SBP (mmHg)	135.4 ± 14.7	134.1 ± 12.3	128.9 ± 12.6	123.6 ± 9.4	131.6 ± 10.4	*p<0.01
DBP (mmHg)	88.6 ± 7.1	86.6 ± 5.8	83.6 ± 6.2	80.2 ± 5.3	86.5 ± 7.4	*p<0.01

n.c.=not calculable; n.s.=not significant; * $p<0.05$, statistically significant; ^data collected at T12 (after 12 months of diet).

Regarding BMI, waist circumference, and WHR index, significantly higher values were found in subjects with a high BMI compared to workers with an average BMI within the normal range. The mean values of diastolic (DBP) and systolic (SBP) blood pressure were within the normal range (60 > DBP < 90 mmHg and 100 < SBP < 140 mmHg), for both female and male HCWs. However, significantly higher mean blood pressure values (systolic and diastolic) were found in workers with a high BMI (groups HBHC and HBLC) compared to those with a normal BMI (groups LBHC and LBLC). Adherence to the Mediterranean diet, calorie consumption, and physical activity were significantly lower in subjects with a high BMI (groups HBHC and HBLC) compared to workers with a low BMI (groups LBHC and LBLC).

In group HBLCD (diet subjects) a significant improvement in BMI, waist circumference, WHR index, DBP, and SBP was observed from T0, T6 (after 6 months), and T12 (after 12 months) (data not shown). Similarly, in this group, adherence to the Mediterranean diet, calorie consumption, and physical activity performed also significantly improved from T0 to T12 (data not shown).

The HBHC group had significantly higher mean values than the other groups and similar to those of the LBHC group. Comparison of adherence to the Mediterranean diet shows that both the HBHC and LBHC groups exhibit poor adherence; conversely, the groups with lower total cholesterol levels exhibit higher adherence. Analysis of total cholesterol values with adherence to the Mediterranean diet shows a significant inverse correlation (r=-0.72).

Regarding HDL cholesterol, mean values were always within the normal range (normal range 27-67 mg/dL). Regarding LDL cholesterol, however, mean values were elevated above the normal range (<130 mg/dL) in the HBHC and LBHC groups, which exhibit higher total cholesterol values.

Mean triglyceride values were significantly elevated, above the normal range (<150 mg/dL) in the first HBHC group compared to the other four groups.

No statistically significant changes were observed in mean liver transaminase values across the five groups. Mean gamma-GT values were significantly elevated in the HBHC and LBHC groups, which were the two groups of workers with hypercholesterolemia.

Table 2 shows the results distributed across the five worker categories.

Table 2. Results of plasma lipid and pro-inflammatory parameter of HCWs divided into 5 groups.

	HBHC	HBLC	LBHC	LBLC	HBLCD^	p-Value
Subjects 94 (100%)	20 (21%)	20 (21%)	20 (21%)	20 (21%)	14 (16%)	-
Total cholesterol (mg/dL)	236,6 ±25,2	163,4 ±27,2	232,3 ±27,9	172.8 ± 22.3	183.6 ± 30.8	*p<0.01
HDL cholesterol (mg/dL)	53,07 ±8,6	49,8 ±9,9	60,8 ±11,6	60.2 ± 12.3	53.2 ± 8.6	n.s
LDL cholesterol (mg/dL)	151,7 ±22,1	92,7 ±25,1	148,3 ±28,1	96.9 ± 23.5	112.3 ± 28.6	*p<0.01
Triglycerides (mg/dL)	168,7 ±83,2	131,5 ±46,2	119,1 ±103,5	92.5 ± 41.6	106.7 ± 37.9	*p<0.01
GOT (U/L)	22,8 ±6,1	23,9 ±4,6	24,2 ±6,1	22,7 ±6,0	23,8 ±5,3	n.s
GPT (U/L)	31,1 ±13,1	26,3 ±7,8	27,2 ±11,9	24,5 ±11,6	30,9 ±13,6	n.s
gamma-GT (U/L)	45,2 ±50,7	28 ±12,8	35,9 ±29,7	24,7 ±36,9	22,4 ±9,9	*p<0.01
Adiponectin (ng/mL)	19,8±2,2	22,3±1,6	27,6±1,9	38,5±2,3	26,9±2,1	*p<0.01
Apolipoprotein B (ng/mL)	1178,3±58,7	551,2±22,6	932,6±43,5	485,9±34,2	519,1±33,8	*p<0.01
Apolipoprotein E (µg/dL)	5,6±1,2	8,9±0,8	6,3±0,9	15±1,4	11±0,7	*p<0.01
APOA-4 (ng/mL)	6,2±0,4	12,7±1,1	7,8±1,2	15,9±0,7	14,3±0,9	*p<0.01
Leptin (pg/dL)	778,4±12,4	704,9±18,6	539±21,5	347±11,4	572±21,6	*p<0.01
IL-6 (pg/mL)	180±14,7	154±11,3	163±18,6	114±15,9	132±10,4	*p<0.01
CRP (mg/L)	27,6±2,1	21,8±1,8	18,3±1,4	4,2±0,7	14,7±1,7	*p<0.01

n.c.=not calculable; n.s.=not significant; * $p<0.05$, statistically significant; ^data collected at T12 (after 12 months of diet).

Mean Adiponectin concentrations were significantly elevated in subjects of normal weight and normal cholesterol (LBLC group).

Mean Apolipoprotein B, leptin, IL-6, and CRP were significantly elevated in subjects with high BMI and hypercholesterolemia (HBHC group). Apolipoprotein B was also elevated in the normal-weight group with hypercholesterolemia (LBHC group). Leptin, IL-6, and CRP were also significantly elevated in subjects with high BMI and normal cholesterol (HBLC group).

Apolipoprotein E and APOA-4 were significantly elevated in the groups of subjects of normal weight with normal cholesterol (LBLC group) and in those on a hypocaloric diet (HBLCD group). Therefore, these values were higher in subjects with greater adherence to the Mediterranean diet.

Discussion

In this study, we evaluated how, in an occupational cohort of HCWs, obesity and hypercholesterolemia are associated with changes in HDL-related parameters and inflammatory markers, and whether a structured hypocaloric dietary intervention can modulate these alterations. Workers were divided into five groups according to BMI, total cholesterol levels, and participation in a 12-month low-calorie diet, allowing us to distinguish the relative contributions of excess adiposity, hypercholesterolemia and lifestyle modification on cardiometabolic and inflammatory profiles.

A first relevant finding is that obese HCWs, regardless of cholesterol category (HBHC and HBLC), displayed a clustering of adverse anthropometric and hemodynamic traits compared with normal-weight colleagues (LBHC and LBLC): higher BMI, larger waist circumference and WHR, and higher systolic and diastolic blood pressure. These differences emerged in a sample that was otherwise homogeneous with respect to age, sex, seniority and work shifts, suggesting that adiposity per se is a major driver of the unfavorable clinical profile. This pattern is consistent with epidemiological data showing that obesity markedly increases the risk of hypertension, metabolic syndrome and CVD in the general population and in working adults [96-98].

From a behavioral point of view, obese HCWs reported lower levels of physical activity and poorer adherence to the Mediterranean diet than normal-weight workers, while the hypocaloric-diet group (HBLCD) showed the most pronounced improvements over time. The strong inverse correlation between adherence to the Mediterranean diet and total cholesterol ($r = –0.72$) underscores the importance of dietary quality in modulating lipid profile even in a relatively young and working population [99]. These findings are in line with previous evidence that adherence to Mediterranean-style dietary patterns, together with regular physical activity, improves lipid metabolism and reduces cardiometabolic risk in individuals with excess weight [100].

Interestingly, despite clear differences in adiposity, blood pressure and lifestyle, mean HDL-cholesterol values remained within the normal range across all groups. In contrast, total cholesterol, LDL-cholesterol and triglycerides were significantly higher in groups HBHC and LBHC, with particularly high triglyceride levels in obese subjects with hypercholesterolemia.

Indeed, an increase in LDL and triglycerides is observed in relation to hypercholesterolemia.

This HDL-cholesterol concentration might be confusing if it is interpreted in isolation, but emerging literature indicates that obesity and chronic low-grade inflammation can induce qualitative changes in HDL particles—often referred to as HDL dysfunction—without necessarily reducing HDL-cholesterol levels [101].

Our data on apolipoproteins and adipokines support this concept. Obese and hypercholesterolemic HCWs showed higher concentrations of apolipoprotein B, reflecting an increased burden of atherogenic lipoproteins, and altered levels of ApoE and ApoA-IV, which are involved in HDL remodeling and reverse cholesterol transport. These changes may contribute to impairing HDL's anti-atherogenic functions, including its antioxidant, anti-inflammatory and cholesterol efflux capacities, as suggested by experimental and clinical studies that have described structural and functional modifications of HDL in obesity and metabolic syndrome [101;102].

The inflammatory and adipokine profile of our cohort further highlights the role of dysfunctional adipose tissue as an active endocrine and immune-metabolic organ. Obese HCWs, especially those with concomitant hypercholesterolemia (HBHC), exhibited higher levels of leptin, IL-6 and CRP and lower adiponectin compared with normal-weight and normocholesterolemic workers (LBLC), indicating a chronic low-grade inflammation and impaired adipokine balance. These observations are consistent with the pathogenic model whereby expansion of adipose tissue leads to macrophage infiltration, increased production of pro-inflammatory cytokines and adipokines, activation of NF-κB and JNK signaling and enhanced oxidative stress, all of which converge in promoting insulin resistance, endothelial dysfunction and atherosclerosis [103]. Clinical studies have shown that elevated IL-6 and CRP predict incident diabetes and cardiovascular events, and that weight loss and lifestyle interventions can reduce these markers [102; 104; 105; 106].

The subgroup of obese, hypercholesterolemic HCWs who underwent a 12-month hypocaloric diet (HBLCD) provides useful insights into the potential reversibility of these alterations in a real-world occupational setting. In this group we observed a progressive and significant improvement in BMI, waist circumference, WHR and blood pressure from baseline to 6 and 12 months, accompanied by increased physical activity and better adherence to the Mediterranean diet. At 12 months (T12), their lipid and inflammatory profiles were generally intermediate between those of persistently obese hypercholesterolemic subjects (HBHC) and normal-weight normocholesterolemic workers (LBLC), suggesting that even a non-pharmacological intervention based on dietary counselling and lifestyle support can partially correct the pro-atherogenic and pro-inflammatory milieu associated with obesity.

These results are in line with previous trials showing that hypocaloric diets and combined diet-plus-exercise interventions reduce leptin levels, increase adiponectin and decrease IL-6 and TNF-α in

obese individuals, with corresponding improvements in insulin sensitivity and cardiovascular risk markers [107-108]. Notably, in our study the intervention was integrated into the routine framework of occupational health surveillance and delivered through repeated contacts with a nutritionist trained in motivational counselling, suggesting that such programs are feasible and potentially scalable in hospital settings.

From an occupational health perspective, the present findings have several implications. First, they confirm that HCWs—a category exposed to shift work, irregular schedules and psychosocial stress—are at substantial risk of obesity and its metabolic consequences, with downstream effects on musculoskeletal disorders, absenteeism and productivity. Second, they support the integration of cardiometabolic risk assessment, including simple anthropometric measures and selected inflammatory markers, into routine mandatory health surveillance. Early identification of workers with obesity, hypercholesterolemia and subclinical inflammation could allow timely, targeted interventions that might reduce long-term morbidity, healthcare costs and work disability. Third, our experience shows that workplace-based lifestyle interventions, even when relatively low-intensity and non-pharmacological, can induce measurable improvements in weight, diet, physical activity and cardiometabolic biomarkers over 12 months, supporting international calls to view obesity as a disease requiring structured, multidisciplinary management rather than solely an individual responsibility.

Strengths and limitations of the study

This study presents several strengths. It investigates a well-defined, homogeneous occupational population drawn from a single hospital, with standardized procedures for anthropometric evaluation, blood pressure measurement and biochemical assessment, including a detailed lipid profile, apolipoprotein concentrations and adipokine and inflammatory markers measured with validated ELISA methods. The combination of objective clinical data with information on physical activity (expressed in METs) and adherence to the Mediterranean diet allows a comprehensive characterization of workers' cardiometabolic risk beyond simple BMI or cholesterol values. Another important strength is the pragmatic design of the hypocaloric-diet program, which was embedded in routine occupational health surveillance and delivered through repeated motivational counselling sessions, reflecting conditions that could realistically be implemented on a larger scale in similar healthcare settings.

Nonetheless, some limitations must be acknowledged when interpreting the results. First, the overall sample size was limited, and the number of participants who completed the 12-month dietary intervention was particularly modest, reducing statistical power and potentially increasing the risk of

type II error. Second, assignment to the groups—especially participation in the hypocaloric-diet arm—was voluntary and not randomized; therefore, self-selection bias cannot be excluded. Workers who chose to adhere to the diet program may have been more motivated or health-conscious than those who did not, and this difference in motivation could partially explain the favorable changes observed.

Third, lifestyle variables such as diet and physical activity were assessed through self-report questionnaires and food diaries, which are subject to recall bias and social desirability bias, potentially leading to under- or over-estimation of true behaviors. Finally, HDL metabolism was assessed indirectly through HDL-cholesterol and HDL-related apolipoproteins; we did not perform functional assays of HDL (for example, cholesterol efflux capacity or measures of anti-oxidant/anti-inflammatory activity), which would have provided more direct evidence of HDL dysfunction in obese and hypercholesterolemic HCWs.

Despite these limitations, the study adds novel data on the interplay between obesity, hypercholesterolemia, HDL-related parameters and inflammatory markers in HCWs, and illustrates how occupational health services can be leveraged to implement preventive interventions aimed at weight reduction and lifestyle improvement.

Conclusions

Obese and hypercholesterolemic HCWs showed higher concentrations of apolipoprotein B, reflecting an increased burden of atherogenic lipoproteins, and altered levels of ApoE and ApoA-IV, which are involved in HDL remodeling and reverse cholesterol transport. These changes may contribute to impairing HDL's anti-atherogenic functions, including its antioxidant, anti-inflammatory and cholesterol efflux capacities. The inflammatory and adipokine profile observed in the HCWs further highlights the role of dysfunctional adipose tissue as an active endocrine and immune-metabolic organ. Obese HCWs, especially those with concomitant hypercholesterolemia (HBHC), exhibited higher levels of leptin, IL-6 and CRP and lower adiponectin compared with normal-weight and normocholesterolemic workers (LBLC), indicating a chronic low-grade inflammation and impaired adipokine balance. Elevated IL-6 and CRP predict incident diabetes and cardiovascular events.

The hypocaloric diet combined with Mediterranean diet, accompanied by increased physical activity provides useful insights into the potential reversibility of these alterations in a real-world occupational setting. Indeed, the combination of Mediterranean diet and physical exercise resulted in progressive and significant improvement in BMI, waist circumference, WHR and blood pressure from baseline to 6 and 12 months and improvement in lipid and inflammatory profiles, suggesting that even a non-

pharmacological intervention based on dietary counseling and lifestyle support can partially correct the pro-atherogenic and pro-inflammatory milieu associated with obesity.

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