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"DIFFERENT EXPRESSION OF MICRO-RNAs IN VISCERAL AND SUBCUTANEOUS TISSUE OF OBESE PATIENTS UNDERGOING BARIATRIC SURGERY"

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Table of Contents

Table of Contents

Introduction

Obesity is a condition in which the accumulation of adipose tissue, particularly at the visceral level, is associated with a complex series of alterations involving multiple organs and systems. In this context, respiratory, cardiovascular, and metabolic complications represent the most frequent conditions that negatively affect quality of life and, more importantly, patient survival. To date, bariatric surgery remains the only proven treatment capable of inducing significant and sustained weight loss, with documented impact on the reduction of obesity-related comorbidities.

MicroRNAs (miRNAs), owing to their ability to modulate gene expression, play a key role in the regulation of numerous pathological processes. Several studies available in the literature, conducted on various biological specimens (such as blood or sputum), have demonstrated the involvement of miRNAs in the pathogenesis of respiratory and cardiovascular diseases. However, based on current knowledge, none of these studies has directly employed adipose tissue as the primary biological matrix.

In light of these considerations, it appears particularly relevant to investigate how the excess of visceral and subcutaneous adipose tissue—characteristic of obese patients—may influence the expression profile of the aforementioned microRNAs.

Chapter 1: Obesity

1.1 General aspects

The World Health Organization (WHO) defines obesity as a condition characterized by "an increase in the percentage of body fat (PBF) to a level that impairs an individual's health and well-being" [1]. Obesity results from a complex interplay of genetic, nutritional, and metabolic factors. Owing to its high prevalence, it represents a major global public health issue and is associated with multiple complications and reduced life expectancy [2].

Over time, several parameters have been proposed for patient classification. The most widely used at present is the Body Mass Index (BMI), calculated as the ratio between body weight and the square of height, and expressed in kg/m². Based on BMI values, the WHO identifies six categories within the general population:

	BMI (Kg/m²)
Underweight	< 18,5
Normal weight	18,5 – 24,9
Overweight	25 – 29,9
Obesity – Class I	30 – 34,9
Obesity – Class II	35 – 39,9
Obesity – Class III	≥ 40

It is important to clarify, however, that BMI represents only an approximate measure of body fat percentage (PBF), as it is derived from a simple mathematical formula that does not account for key individual factors such as muscle mass development, fluid retention, or physical disability. For these reasons, a more accurate assessment of the risk of obesity-related diseases also requires the measurement of waist circumference (WC) and hip circumference in adult subjects with a BMI > 35 kg/m² [2][3].

1.2 Epidemiology

The prevalence of overweight and obesity continues to rise across most world regions, assuming the characteristics of a global pandemic. Recent estimates indicate that approximately one in eight adults worldwide lives with obesity, with prevalence having more than doubled since the 1990s. If no effective interventions are implemented, projections suggest that more than 60% of adults may be overweight or obese by 2050

Within Europe, more than half of the adult population is currently classified as overweight (BMI ≥ 25 kg/m²), with significant variability in obesity prevalence across countries [2]. Italy occupies an intermediate position among European countries, with approximately 42–44% of adults being overweight or obese and an estimated adult obesity prevalence of around 11–12% [3,4].

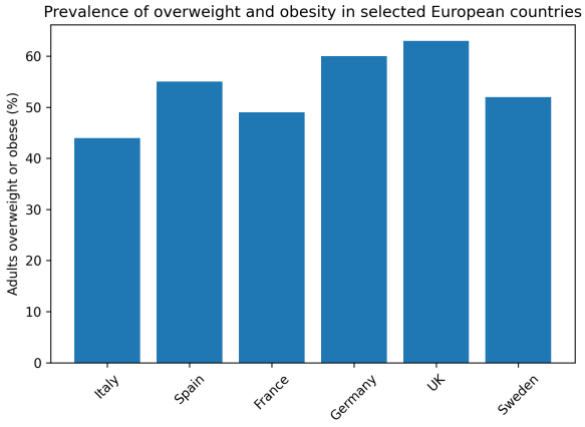


Figure 1.1. Prevalence of overweight and obesity in selected European countries (adults, illustrative estimates based on recent European data).

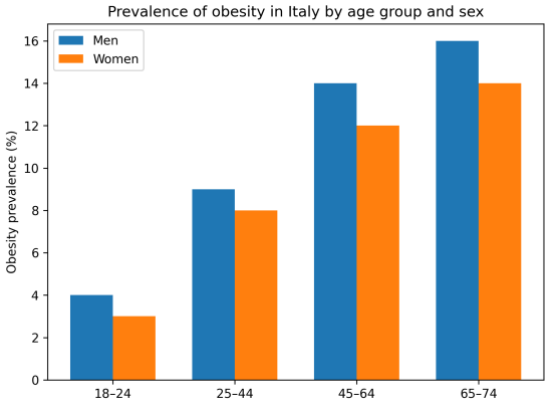


Figure 1.2. Prevalence of obesity in Italy by age group and sex (adults, illustrative pattern based on national surveys).

1.3 Classification

Obesity classification varies according to the parameters and criteria considered. In this section, particular attention will be given to aspects related to histology, morphology, and the distribution of body fat.

From a histological perspective [5], two main patterns can be distinguished:

- **Hypertrophic obesity:** characterized by an increase in adipocyte volume due to progressive lipid accumulation. It typically develops during adulthood and is often associated with metabolic disorders such as glucose intolerance, dyslipidemia, hypertension, and cardiovascular diseases.
- **Hyperplastic obesity:** characterized by an increase in the number of adipocytes. It generally develops during childhood and is frequently observed in individuals with a BMI < 40 kg/m².

In practice, this distinction is not always clearly defined; therefore, it is often more appropriate to refer to a mixed or hypertrophic–hyperplastic form, characterized by both an increase in adipocyte number and size. This pattern is also typical of younger individuals and, similar to pure hyperplastic obesity, demonstrates resistance to therapeutic interventions due to the inability to reduce the total number of adipose cells.

However, the distribution of body fat represents the most relevant aspect in determining the risk of obesity-related diseases, owing to the distinct physical and metabolic characteristics of adipose tissue depending on anatomical location. As previously noted, BMI—derived from a simple mathematical formula (weight/height²)—provides only an approximate estimation of body fat percentage and does not describe fat distribution [6]. A more accurate patient assessment therefore includes measurement of waist and hip circumference; the ratio between these parameters (WHR, Waist-to-Hip Ratio) allows the identification of three constitutional patterns:

- **Android obesity (WHR > 0.85):** found predominantly, though not exclusively, in males. Adipose tissue is primarily visceral and accumulates mainly in the abdominal region, but also at the thoracic, dorsal, and cervico-nuchal levels. This phenotype is associated with the highest risk of complications, particularly cardiovascular events.
- **Gynoid obesity (WHR < 0.78):** observed predominantly in females. Adipose tissue is mainly subcutaneous and tends to accumulate in the lower abdomen, thighs, and gluteal region.
- **Mixed obesity (WHR 0.78–0.85):** may occur in both sexes and presents intermediate characteristics between the two aforementioned phenotypes, with a more diffuse distribution of adipose tissue.

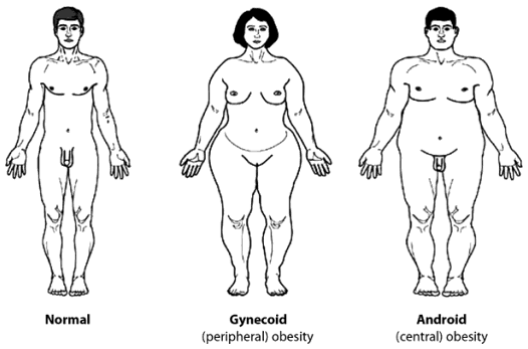


Figure 1.4: Body fat distribution according to obesity phenotype.

This subdivision, although useful in epidemiological contexts, is often insufficient for the evaluation of individual patients. To overcome these limitations and avoid classification errors, it is necessary to use direct measurement techniques for PBF such as skinfold thickness, hydrometry, bioimpedance analysis, DXA, CT, and MRI (Fig. 1.5) .

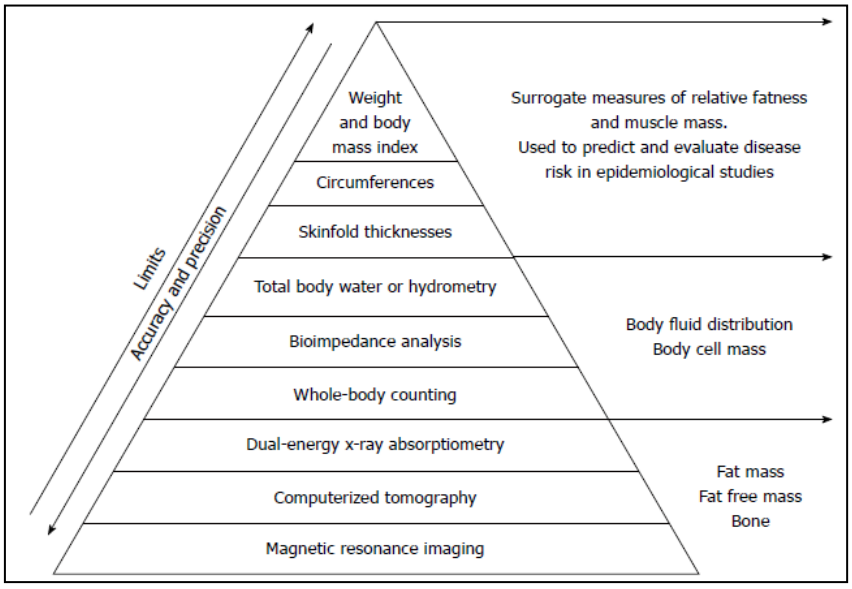


Figura 1.5: Schematic representation of the various methods required for body composition assessment, arranged according to progressively higher levels of accuracy and precision.

Based on these premises, a more recent classification identifies four different obesity phenotypes. They are distinguished not only on the basis of anthropometric parameters (BMI and WC), but also according to body fat percentage and certain laboratory indices (glycemia, total cholesterol, HDL, LDL, triglycerides, insulinemia, and CRP), which become fundamental for a correct metabolic assessment of the patient:

1) **Normal weight obese (NOW)**

Individuals with a normal BMI (18–24.9 kg/m²) but a high body fat percentage (PBF > 23.5% in men and > 29.3% in women). The prevalence of this phenotype worldwide is about 10%, with higher frequency in females and increasing with age. These patients, although not presenting with Metabolic Syndrome, are at increased cardiovascular risk, likely due to increased WC (indicative of increased visceral fat) and reduced subcutaneous adipose tissue (which generally plays a protective role for CVDs). BMI remains normal because the increase in fat mass is accompanied by a progressive reduction in lean mass, resulting in a certain degree of sarcopenia.

2) **Metabolically Obese Normal Weight (MONW)**

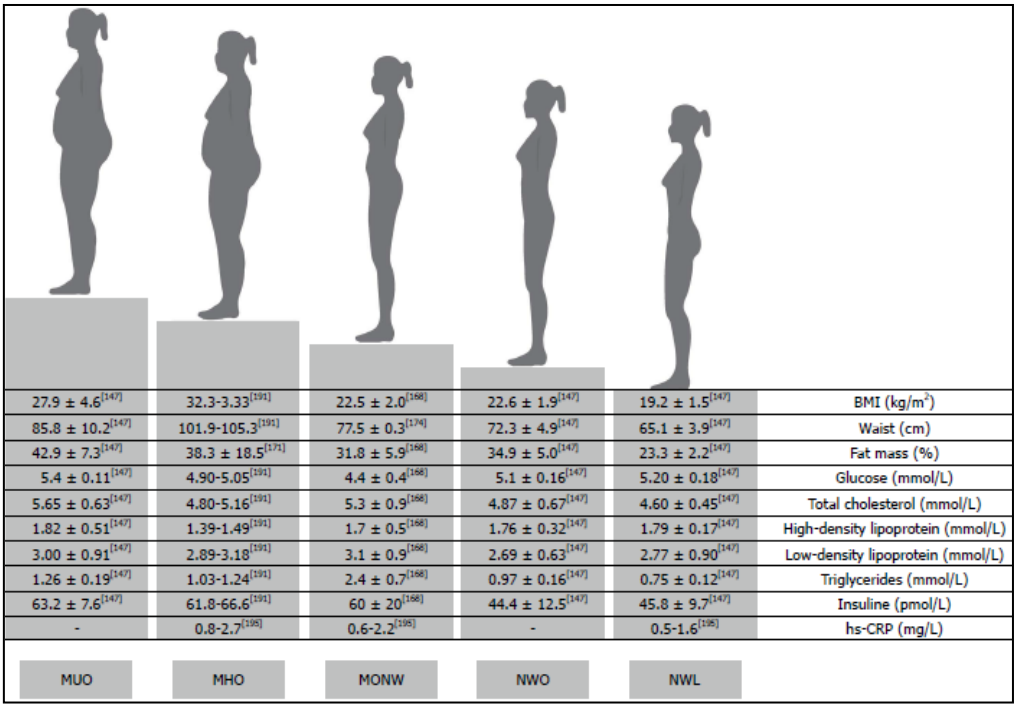
Individuals with a normal BMI but a cluster of metabolic characteristics that increase the risk of developing Metabolic Syndrome. These subjects often have increased WC associated with elevated blood levels of triglycerides, glucose, and CRP, along with reduced HDL and adiponectin.

3) **Metabolically Healthy Obese (MHO)**

Subjects with established obesity (BMI > 30 kg/m²) but without metabolic alterations. Despite excess fat mass, these patients have a metabolic profile characterized by high insulin sensitivity, favorable lipid profile, and normal systemic blood pressure. For diagnosis, at least 4 of the following criteria must be met: triglycerides < 1.7 mmol/L, total cholesterol < 5.2 mmol/L, HDL > 1.3 mmol/L, LDL < 2.6 mmol/L, and insulin sensitivity (HOMA < 1.95). However, MHO cannot be considered a stable condition; some studies have shown that at least 47.6% of patients initially classified as MHO progress to MUO within about 7–8 years [7].

4) **Metabolically Unhealthy Obese (MUO)**

Patients with BMI > 30 kg/m² and PBF > 30%, with fat distribution mainly at the visceral level. These factors are directly implicated in the development of Metabolic Syndrome, type 2 diabetes, atherosclerosis, and cardiovascular disease



	MUO	MHO	MONW	NWO	NWL
BMI (kg/m^2)	27.9 ± 4.6[147]	32.3-3.33[191]	22.5 ± 2.0[168]	22.6 ± 1.9[147]	19.2 ± 1.5[147]
Waist (cm)	85.8 ± 10.2[147]	101.9-105.3[191]	77.5 ± 0.3[174]	72.3 ± 4.9[147]	65.1 ± 3.9[147]
Fat mass (%)	42.9 ± 7.3[147]	38.3 ± 18.5[171]	31.8 ± 5.9[168]	34.9 ± 5.0[147]	23.3 ± 2.2[147]
Glucose (mmol/L)	5.4 ± 0.11[147]	4.90-5.05[191]	4.4 ± 0.4[168]	5.1 ± 0.16[147]	5.20 ± 0.18[147]
Total cholesterol (mmol/L)	5.65 ± 0.63[147]	4.80-5.16[191]	5.3 ± 0.9[168]	4.87 ± 0.67[147]	4.60 ± 0.45[147]
High-density lipoprotein (mmol/L)	1.82 ± 0.51[147]	1.39-1.49[191]	1.7 ± 0.5[168]	1.76 ± 0.32[147]	1.79 ± 0.17[147]
Low-density lipoprotein (mmol/L)	3.00 ± 0.91[147]	2.89-3.18[191]	3.1 ± 0.9[168]	2.69 ± 0.63[147]	2.77 ± 0.90[147]
Triglycerides (mmol/L)	1.26 ± 0.19[147]	1.03-1.24[191]	2.4 ± 0.7[168]	0.97 ± 0.16[147]	0.75 ± 0.12[147]
Insuline (pmol/L)	63.2 ± 7.6[147]	61.8-66.6[191]	60 ± 20[168]	44.4 ± 12.5[147]	45.8 ± 9.7[147]
hs-CRP (mg/L)	-	0.8-2.7[195]	0.6-2.2[195]	-	0.5-1.6[195]

	NWL	NWO	MONW	MHO	MUO
BMI (kg/m^2)	22.4-22.7[191]	24.1 ± 0.7[156]	22.87 ± 0.07[174]	32.3-33.3[191]	31.59 ± 0.74[203]
Waist (cm)	83.8-85.2[191]	89.4 ± 1.6[156]	82.5 ± 0.3[174]	108.3-112.1[191]	108 ± 7[172]
Fat mass (%)	19.4 ± 0.2[174]	26.4 ± 1.3[156]	22.3 ± 0.2[174]	31.1 ± 3.4[181]	26.44 ± 0.53[203]
Glucose (mmol/L)	5.1-5.2[191]	2.53 ± 0.19[156]	5.38 ± 0.02[174]	5.1-5.3[191]	5.44 ± 0.10[203]
Total cholesterol (mmol/L)	4.8-5.0[191]	5.09 ± 0.72[156]	5.08 ± 0.04[174]	4.8-5.2[191]	5.11 ± 0.99[203]
High-density lipoprotein (mmol/L)	1.3-1.4[191]	1.23 ± 0.24[156]	1.18 ± 0.01[174]	1.2-1.3[191]	1.23 ± 0.36[203]
Low-density lipoprotein (mmol/L)	4.8-5.0[191]	3.09 ± 0.62[156]	2.73 ± 0.04[174]	4.8-5.2[191]	3.20 ± 0.94[203]
Triglycerides (mmol/L)	1.1-1.3[191]	3.84 ± 1.77[156]	2.47-2.64[174]	1.2-1.4[191]	1.86 ± 0.19[203]
Insuline (pmol/L)	44.5-48.1[191]	42.3 ± 19.4[156]	52.8-55.8[174]	63.1-73.2[191]	16.37 ± 1.76[203]
hs-CRP (mg/L)	0.5-1.6[195]	-	0.6-2.2[195]	0.8-2.7[195]	-

Figura 1.6: Main characteristics of the four obesity phenotypes in females (top) and males (bottom) [2]. NWO: normal weight obese; MONW: metabolically obese normal weight; MHO: metabolically healthy obese; MUO: metabolically unhealthy obese.

Chapter 2: Obesity and Comorbidities

2.1 General aspects

Obesity is a condition associated with a range of diseases and complications that expose patients to increased morbidity and mortality compared to the general population. The main consequences are summarized in Table 2.1.

Respiratory disorders	Cardiovascular disorders	Metabolic complications	Neoplasms	Other
Obstructive Sleep Apnea Syndrome (OSAS)	Hypertension	Insulin resistance	Colorectal cancer	Hepatic steatosis
Obesity Hypoventilation Syndrome (OHS)	Atherosclerosis	Type 2 Diabetes (T2D)	Breast cancer	Osteoarthritis
Asthma	Myocardial infarction	Dyslipidemia	Prostate cancer	Cholelithiasis
Chronic Obstructive Pulmonary Disease (COPD)	Stroke	Hyperuricemia	Gallbladder disease	Abdominal hernias
Pulmonary embolism	Heart failure		Endometrial cancer	Infertility
Aspiration pneumonia				Psychosocial problems

Table 2.1: Main obesity-related diseases and complications.

L'origine di tali alterazioni può essere ricondotta a fattori meccanici (come ad esempio per l'OSAS o le artropatie), fattori umorali (per esempio nell'asma o nell'aterosclerosi) oppure ad una combinazione dei due.

The origin of these alterations can be attributed to mechanical factors (as in OSAS or osteoarticular disorders), humoral factors (for example in asthma or atherosclerosis), or to a combination of the two. Adipose tissue, in fact, is not merely a storage depot for fat, but is a true endocrine organ capable of influencing multiple physiological functions. These include appetite regulation, energy expenditure, insulin sensitivity, and the endocrine, reproductive, and immune systems. Moreover, several studies have shown that the increase in adipose tissue—particularly in the visceral

compartment—is accompanied by infiltration of immune cells (mainly macrophages and T lymphocytes) and is associated with an increased release of adipokines and pro-inflammatory cytokines (IL-1, IL-6, TNF-α). This pattern leads to a generalized inflammatory state which appears to be involved in the development and progression of numerous obesity-related chronic degenerative diseases [8-9].

2.2 Obesity and respiratory disease

The progressive increase in body weight negatively impacts the function of the respiratory system. In general, the accumulation of adipose tissue in the thoracic and abdominal regions markedly limits thoracic wall expansion and diaphragmatic excursion, reducing chest wall compliance and increasing the work of breathing. As a result, there is initially a reduction in the Expiratory Reserve Volume (ERV) and the Functional Residual Capacity (FRC = ERV + RV), and in some cases also a reduction in the Total Lung Capacity (TLC) and Vital Capacity (VC).
In severe obesity, a reduction in dynamic lung volumes such as FEV1 (forced expiratory volume in 1 second) and FVC (forced vital capacity) is also observed, while their ratio (Tiffenau Index) remains unchanged, framing the respiratory impairment of these patients within predominantly restrictive patterns.
Additional alterations include increased airway resistance, modification of the respiratory drive, and impairment of gas exchange.

The main obesity-related respiratory disorders will be described below.

2.2.1 Obstructive Sleep Apnea Syndrome (OSAS)

Obstructive Sleep Apnea Syndrome (OSAS) is an extremely common condition in obese patients. It can be defined as a sleep-related breathing disorder characterized by recurrent episodes of collapse and obstruction of the upper airways, leading to temporary cessation of airflow—either complete (apnea) or partial (hypopnea)—at the pulmonary level.
The risk in cases of severe obesity (BMI > 40 kg/m²) ranges from 55% to 90%. Other contributing factors include increased neck circumference (> 43 cm), a high Mallampati score (> 3), and the presence of craniofacial abnormalities.
The main pathogenetic mechanism consists of impaired control of the pharyngeal dilator muscles, which are therefore unable to effectively counteract the increase in extraluminal pressure that

develops during the inspiratory phase. In obese patients, this phenomenon is further promoted by the accumulation of adipose tissue surrounding the tongue and soft tissues of the neck, which mechanically contributes to increasing extraluminal pressure [10].

The consequences of nocturnal apnea/hypopnea include hypoxia, hemoglobin desaturation, hypercapnia, increased respiratory effort, arousal, and sleep fragmentation.

It is important to underline that obese patients with OSAS tend to desaturate much more rapidly during nocturnal apnea compared to non-obese patients with the same condition. [12]. This phenomenon is mainly related to the reduction in lung volumes (e.g., Functional Residual Capacity and Total Lung Capacity) that characterizes obese patients.

The combination of these factors results in a complex clinical picture. The main symptoms (Table 2.2) include nocturnal snoring and excessive daytime sleepiness (secondary to sleep fragmentation), which progressively lead to impaired work and/or academic performance [11].

Neuropsychiatric disturbances (also related to sleep deprivation) may additionally be present, along with a series of complications that include systemic hypertension, pulmonary hypertension, arrhythmias, coronary artery disease, congestive heart failure, polycythemia, and stroke.

Several studies have demonstrated that the recurrent hypoxic stress occurring in OSAS promotes an inflammatory state both at the airway level and systemically. This condition appears to be more pronounced in the presence of concomitant obesity and OSA and is likely responsible for the increased risk of cardiovascular disease observed in these patients[17][18]

Table 2.2: Symptoms that may be detected during the evaluation of patients with OSAS.

Witnessed apneas	Unrefreshing sleep	Difficulty concentrating
Nocturnal snoring	Sleep fragmentation	Memory loss
Nocturnal dyspnea/choking	Nocturia	Reduced libido
Unexplained daytime sleepiness	Headache	Irritability

According to the American Academy of Sleep Medicine guidelines, the diagnosis of OSAS requires an accurate clinical evaluation of the patient, with particular attention to the medical history (which should ideally be collected in the presence of the bed partner) in order to identify the symptoms previously described [12].

Polysomnography represents the instrumental *Gold Standard* for both diagnosis and staging. The Apnea–Hypopnea Index (AHI) is defined as the number of obstructive events (apnea, hypopnea, or respiratory effort–related arousals) recorded per hour of sleep.

An AHI > 5 in the presence of symptoms is diagnostic of OSAS. Since polysomnography values showing more than 15 obstructive events per hour of sleep are associated with a significant increase in morbidity—particularly of cardiovascular origin—an AHI > 15 is considered diagnostic even in the absence of symptoms.
Based on the Apnea–Hypopnea Index, OSAS can be classified into three grades: mild (AHI 5–14), moderate (AHI 15–29), or severe (AHI > 30) [13].
The main treatment consists of the use of continuous positive airway pressure (CPAP) during nighttime sleep. This device maintains a positive pressure during both inspiration and expiration, thereby preventing upper airway collapse. In selected cases, the use of oral appliances (bite splints) or surgical correction of craniofacial abnormalities may be beneficial.
Weight loss is strongly recommended, as it can significantly reduce the severity of the disease.

2.2.2 Obesity Hypoventilation Syndrome (OHS)

Formerly also known as Pickwickian syndrome, OHS is a respiratory disorder characterized by the association of obesity (BMI > 30 kg/m²), hypoxemia (PaO_2 < 70 mmHg), and daytime hypercapnia ($PaCO_2$ > 45 mmHg) in the absence of other evident causes of hypoventilation.
The pathogenesis is related to a set of factors summarized as follows:

1) **Alterations in respiratory mechanics:** Patients with OHS exhibit a reduction in lung volumes and capacities such as Total Lung Capacity (TLC), Vital Capacity (VC), and Functional Residual Capacity (FRC); an increase in Residual Volume (RV); reduced pulmonary compliance; and increased pulmonary resistance. The combination of these alterations results in increased work of breathing and consequently muscular fatigue.

2) **Alterations in respiratory drive:** Characterized by a blunted ventilatory response to hypercapnia and a reduced ventilatory response to hypoxemia.

3) **Neurohormonal factors:** Some studies have focused on the possible role of visceral adipose tissue in influencing the central control of ventilation. In particular, it has been shown that patients with OHS exhibit a condition of leptin resistance [14][15]. Leptin is a hormone produced by adipose tissue that acts at the hypothalamic level by stimulating appetite and at the central level by stimulating ventilation. Leptin resistance is therefore responsible, on one hand, for excessive food

intake (promoting weight gain) and, on the other, for a reduced ventilatory response to hypercapnia. [10].

4) **Presence of OSAS:** Among patients affected by OSAS, at least 10–38% also have OHS. This is related to the fact that the two conditions share several risk factors (obesity, increased waist circumference, high Mallampati score, and increased neck circumference). The concomitant presence of OSAS contributes to worsening hypoxia and hypercapnia, as nocturnal apnea/hypopnea events induce sleep fragmentation and a state of chronic hypoxemia, which in turn promote a progressive reduction in ventilatory response.

Clinical evaluation of the patient allows recognition of a series of symptoms and signs listed in Table 2.3.

Symptoms	Signs
Daytime sleepiness	Cyanosis
Snoring	Signs of chronic pulmonary heart disease
Nocturnal awakenings (with a sense of suffocation)	Secondary polycythaemia (consequence of chronic hypoxaemia)
Morning headache	Pulmonary hypertension
Personality disorders	
Depression	

Table 2.3: Main symptoms and signs that may be observed in a patient with OHS.

Instrumental investigations include arterial blood gas analysis ($PaO_2 < 70$ mmHg, $PaCO_2 > 45$ mmHg, and $HCO_3^- > 25$ mEq/L), spirometry (showing a global restrictive defect with reduced FEV_1 and VC and a normal Tiffeneau index), and echocardiography (evidence of pulmonary hypertension possibly associated with chronic cor pulmonale).

Polysomnography is, in this case as well, a fundamental test because it allows detection of the concomitant presence of OSAS (demonstrated by an AHI > 5). In a patient with OHS without OSAS, the study will be negative for apnea/hypopnea events, but hemoglobin saturation will remain consistently below 90%.

Weight loss represents the only truly effective treatment. In the event of failure of diet and physical exercise, bariatric surgery may be considered.
Patients with OHS and OSAS benefit from treatment with CPAP and supplemental oxygen. Persistent nocturnal desaturation despite CPAP may indicate the need for BPAP (Bi-level Positive Airway Pressure) ventilation until daytime hypoxemia and hypercapnia resolve.

2.2.3 Obesity and Asthma

Several epidemiological studies have demonstrated a clear association between asthma and obesity in both adults and children. In particular, Beuther and Sutherland showed an increase in asthma prevalence of 38% in overweight patients and 90% in obese patients [16–17]. Asthma is a chronic disease characterized by bronchial hyperreactivity, inflammation, remodeling, and reversible airway obstruction (spontaneously or after therapy). The pathogenetic mechanisms underlying this association are multiple and not yet fully clarified. However, the interaction between mechanical, genetic, immune, and endocrine–metabolic factors is well established.

Mechanical factors

As previously noted, obese patients exhibit a reduction in lung volumes and capacities related to decreased elasticity of the chest wall. In more severe cases, this may result in a true restrictive defect. Furthermore, the reduction in FRC, secondary to a decrease in Expiratory Reserve Volume, impairs the relaxation capacity of bronchial smooth muscle cells, especially at end-expiration. This leads to increased work of breathing (even at tidal volume) and consequently reduced exercise tolerance. Finally, excessive adipose tissue accumulation is responsible for increased abdominal pressure and a consequent reduction in chest wall compliance, thereby promoting distal airway closure and bronchial hyperreactivity.

Genetic factors

Linkage studies have identified the possible existence of genetic determinants common to both asthma and obesity. The most important candidate genes are those encoding the β_2-adrenergic receptor and TNF-α. In particular, some polymorphisms of the β_2-adrenergic receptor have been associated with specific asthma phenotypes and with response to therapy. Since catecholamines play an important role in regulating energy expenditure, it is plausible that the same polymorphisms involved in asthma may also be related to obesity [19-20].
Two additional candidate genes for obesity that may play a relevant role in asthma pathogenesis are the gene encoding the glucocorticoid receptor and the gene encoding IGF-1 (Insulin-like Growth

Factor 1). Polymorphisms in the glucocorticoid receptor gene have been associated with obesity; in asthma, increased glucocorticoid receptor expression has been linked to greater disease severity and fatal events. Regarding IGF-1, increased production by damaged or inflamed bronchial epithelial cells has been demonstrated; acting as a mitogenic agent, IGF-1 may stimulate bronchial myofibroblast proliferation and play a key role in the complex process of chronic airway remodeling characteristic of asthma [21].

Two additional candidate genes for obesity that may play a significant role in the pathogenesis of asthma are the gene encoding the glucocorticoid receptor and the gene encoding IGF-1 (Insulin-like Growth Factor 1). Polymorphisms in the glucocorticoid receptor gene have been associated with obesity; in asthma, increased glucocorticoid receptor expression has been linked to greater disease severity and fatal events.

Regarding IGF-1, increased production by damaged or inflamed bronchial epithelial cells has been demonstrated. Acting as a mitogenic agent, IGF-1 may stimulate the proliferation of bronchial myofibroblasts, playing a key role in the complex process of chronic airway remodeling characteristic of asthma [22].

Immune and endocrine–metabolic factors

As is well known, bronchial hyperreactivity and airway remodeling (basement membrane thickening, mucus hypersecretion, hypertrophy and hyperplasia of smooth muscle cells) observed in asthmatic patients are linked to an abnormal Th2-mediated inflammatory response. Once activated by dendritic cells, Th2 lymphocytes are responsible for releasing specific cytokines such as IL-4, IL-5, and IL-13. IL-4 and IL-13 drive B lymphocytes toward IgE production; IgE binds to the surface of mast cells, promoting their degranulation and histamine release. IL-5 induces eosinophil recruitment and activation.

In severe asthma, during viral exacerbations, in smoking asthmatic patients, and in some forms of occupational asthma, the inflammatory pattern becomes even more complex. In these cases, alongside the Th2-dependent response, there may be concomitant activation of Th1 and Th17 lymphocytes which, through the release of IL-6 and TNF-α, promote neutrophilic inflammation as well.

In obese patients, the presence of low-grade systemic inflammation may favor the mechanisms just described. Excess adipose tissue is associated with the production of so-called adipokines (IL-1, IL-6, TNF-α, IL-12, IL-17, and macrophage chemotactic proteins), which have also been detected in asthma and may play an important role in the shared inflammatory state [23].

Another likely link between obesity and asthma is leptin. Circulating leptin levels — involved in inflammation regulation by promoting the release of TNF-α, IL-6, and IL-12 — are known to be

elevated in obese patients, but increased leptin levels can also be found in asthmatic patients независимо of obesity status [24]. Furthermore, recent studies have shown that in sensitized animal models, allergen stimulation after leptin pretreatment enhances allergen-induced bronchial hyperreactivity without increasing eosinophils or Th2 cytokine expression [21][23]. This finding suggests that leptin contributes to the inflammatory cascade typical of asthma and may represent a product of the systemic inflammatory state present in the disease [20][24].

2.3 Obesity and cardiovascular risk

Cardiovascular risk is defined as the probability that cardiac and/or vascular events will occur. Risk factors are classified as modifiable and non-modifiable and are summarized in Table 2.4.

NON MODIFIABLE	MODIFIABLE
Age	Cigarette smoking
Sex (M > F)	Hypertension
Family history	Hypercholesterolaemia
	Low HDL levels
	Diabetes/Insulin resistence
	Obesity
	Physical inactivity

Table 2.4: Main cardiovascular risk factors.

Numerous studies in the literature have demonstrated a clear association between obesity and cardiovascular disease. Among the most important are the **Framingham Heart Study**, the **Manitoba Study**, and the **Harvard School of Public Health Nurses Study**, which showed an independent association between obesity and ischemic heart disease (angina pectoris and myocardial infarction) and also highlighted how obesity promotes the development of heart failure, atrial fibrillation, stroke, and sudden death [25].

In the Framingham study, for example, it was observed that the incidence of heart failure increases progressively with rising BMI, with a twofold higher risk in obese patients compared with normal-weight individuals. It was also found that among all cases of heart failure, 11% in men and 14% in women were a direct consequence of obesity, independently of other factors such as hypertension, hypercholesterolemia, and diabetes.

Similar findings were reported by the **Uppsala Longitudinal Study**, conducted in adult men, which showed that the increased risk of heart failure is associated both with higher BMI and with increased abdominal circumference (directly correlated with visceral fat), independently of diabetes, previous myocardial infarction, left ventricular hypertrophy, smoking, and cholesterol levels [26].

In any case, as already described in Chapter 1, BMI is not an optimal parameter for defining cardiovascular risk. In this regard, the **INTERHEART study** demonstrated a weak relationship between BMI and myocardial infarction, but a strong correlation between this condition and increased waist circumference (WC) and waist-to-hip ratio (WHR) [27–28]. This suggests that risk increases not only in relation to overall body weight, but especially as a function of greater visceral fat distribution.

Relative Risk*/Waist Circumference			
	BMI (Kg/m²)	**M ≤ 102 cm F ≤ 88 cm**	**M > 102 cm F > 88 cm**
Underweight	< 18,5	…	…
Normal weight	18,5 – 24,9	…	…
Overweight	25 – 29,9	Icreased	High
Obesity – 1° grade	30 – 34,9	High	Very High
Obesity – 2° grade	35- 39,9	Very High	Very High
Obesity – 3° grade	≥ 40	Extremely high	Extremely high

Table 2.5: Classification of obesity according to BMI and waist circumference (WC). *Relative risk of associated diseases (type 2 diabetes, hypertension, and cardiovascular diseases).

2.3.1 Cardiovascular adptation to obesity

Numerous studies in the literature have demonstrated a clear association between obesity and cardiovascular disease. Among the most important are the **Framingham Heart Study**, the **Manitoba Study**, and the **Harvard School of Public Health Nurses Study**, which showed an independent association between

obesity and ischemic heart disease (angina pectoris and myocardial infarction) and also highlighted how obesity promotes the development of heart failure, atrial fibrillation, stroke, and sudden death [29].

In the Framingham study, for example, it was observed that the incidence of heart failure increases progressively with rising BMI, with a twofold higher risk in obese patients compared with normal-weight individuals. It was also found that among all cases of heart failure, 11% in men and 14% in women were a direct consequence of obesity, independently of other factors such as hypertension, hypercholesterolemia, and diabetes.

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2.3.2 Role of adipokines in the pathogenesis of CVDs

Adipose tissue, as repeatedly emphasized, is in all respects an organ capable of synthesizing and secreting several molecules with endocrine function, the most important of which are adiponectin and leptin.

Adiponectin has an anti-inflammatory action, promotes insulin sensitivity, exerts an anti-atherogenic effect (by inhibiting the transformation of macrophages into foam cells within atherosclerotic plaques), and improves endothelial function by stimulating nitric oxide (NO) synthesis.

Leptin, on the other hand, has a pro-inflammatory action, promotes insulin resistance, and contributes to the development of atherosclerosis, likely through stimulation of plasminogen activator inhibitor-1 (PAI-1) synthesis [30].

In obese patients, the progressive expansion of adipose tissue shifts the balance between anti- and pro-inflammatory factors in favor of the latter. This results in reduced circulating adiponectin levels, increased leptin levels, and elevated cytokines such as TNF-α, IL-1, and IL-6. The consequence is a generalized inflammatory state that promotes vascular inflammation, oxidative stress, and atherothrombosis, with a consequent increase in the risk of myocardial infarction, stroke, and peripheral vascular disease.

2.3.3 Insulin resistance and type 2 diabetes mellitus

Obesity, particularly visceral obesity, is present in more than 80% of patients with type 2 diabetes mellitus.

The major initial contributors to the development of insulin resistance in obese patients are generalized inflammation (resulting from increased circulating adipokines) and an excess of circulating free fatty acids (FFA). FFAs, mainly released from triglyceride stores in adipose tissue, reduce insulin sensitivity both at the muscular level — by inhibiting glucose uptake — and at the hepatic level — by limiting glycogen synthesis. This leads to a state of hyperglycemia that is initially compensated by increased pancreatic insulin secretion, resulting in hyperinsulinemia. The persistent overactivity imposed on β-cells causes their progressive exhaustion, eventually leading to overt diabetes [31].

Under these conditions, insulin resistance and hyperinsulinemia promote increased circulating levels of lipids, lipoproteins, coagulation factors (particularly fibrinogen), and C-reactive protein, as well as reduced nitric oxide (NO) production by endothelial cells and proliferation of arterial smooth muscle cells. Together, these changes result in a marked increase in cardiovascular risk, especially ischemic events.

2.3.4 Hypertension

L'ipertensione è circa 6 volte più frequente nei soggetti obesi rispetto ai pazienti normopeso. L'incremento di 10 kg induce l'aumento di 3 mmHg della pressione sistolica e di 2-3 mmHg della pressione diastolica che corrisponde a un incremento del 12% del rischio di coronaropatia e del 24% del rischio di ictus.

Indipendentemente dal grado di obesità, la prevalenza dell'ipertensione aumenta con il progressivo incremento del tessuto adiposo in sede addominale.

Tutti i fattori che influenzano la portata cardiaca e le resistenze periferiche possono condizionare la relazione tra obesità e pressione arteriosa. I principali sono l'insulino-resistenza, il sistema nervoso simpatico, la disfunzione endoteliale, le sostanze rilasciate dal tessuto adiposo e l'OSAS.

Potenziali legami tra insulino-resistenza e ipertensione sono l'aumento del tono simpatico e lo squilibrio nella produzione endoteliale tra NO ed endotelina-1 a favore di quest'ultima. Nel complesso si genera uno stato di vasocostrizione tale da portare ad un aumento delle resistenze periferiche.

Inoltre, i pazienti obesi sono caratterizzati da una ridotta efficacia biologica del fattore natriuretico. Ciò comporta una maggiore ritenzione sodica che induce l'espansione del volume ematico.
Infine un altro possibile meccanismo alla base dell'ipertensione obesità-correlata è legato al ruolo vasoattivo che esercita il tessuto adiposo perivascolare. Infatti, le specie reattive dell'ossigeno e gli effetti paracrini mediati da altre molecole, quali la leptina o le citochine pro-infiammatorie (TNF-α), potrebbero contribuire alla compromissione della funzione endoteliale e causare, quindi, vasocostrizione locale [31].

2.3.5 Trombotic diathesis

Obesity, particularly visceral obesity, is present in more than 80% of patients with type 2 diabetes mellitus.
The major initial contributors to the development of insulin resistance in obese patients are generalized inflammation (resulting from increased circulating adipokines) and an excess of circulating free fatty acids (FFA). FFAs, mainly released from triglyceride stores in adipose tissue, reduce insulin sensitivity both at the muscular level — by inhibiting glucose uptake — and at the hepatic level — by limiting glycogen synthesis. This leads to a state of hyperglycemia that is initially compensated by increased pancreatic insulin secretion, resulting in hyperinsulinemia. The persistent overactivity imposed on β-cells causes their progressive exhaustion, eventually leading to overt diabetes [32].
Under these conditions, insulin resistance and hyperinsulinemia promote increased circulating levels of lipids, lipoproteins, coagulation factors (particularly fibrinogen), and C-reactive protein, as well as reduced nitric oxide (NO) production by endothelial cells and proliferation of arterial smooth muscle cells. Together, these changes result in a marked increase in cardiovascular risk, especially ischemic events.

Chapter 3: Treatment of Obesity

3.1 General aspects

The fundamental goals of obesity treatment include improving associated comorbidities and reducing the risk of their recurrence in the future. The decision regarding how intensive the treatment should be and which modalities to use is determined based on the patient's level of risk, expectations, and available resources.

Treatment	BMI Class				
	25-26,9	27-29,9	30-34,9	35-39,9	≥ 40
Diet, physical exercise, behavioral therapy	With comorbidity	With comorbidity	+	+	+
Pharmacotherapy		With comorbidity	+	+	+
Surgery				With comorbidity	+

Table 3.1: Indications for obesity treatment.

Obesity treatment always begins with lifestyle modification and may subsequently include pharmacologic and surgical therapy, depending on BMI category and the presence of comorbidities.

3.2 Diet and physical exercise

Non-pharmacologic treatment of overweight and obesity should be directed toward correcting unhealthy eating habits and resuming physical activity compatible with the patient's clinical condition.

In the absence of other specific therapeutic indications, the intervention should aim for a reduction of approximately 8–10% of initial body weight over a period of about 4–6 months.

Recent guidelines recommend starting treatment with a reduction of 500–1000 kcal/day compared with the habitual diet, consistent with the goal of losing approximately 0.5–1 kg per week. With regard to recommended macronutrient intake, total dietary calories should consist of 50–55% carbohydrates, 15% protein, and 20–30% fat (with cholesterol intake not exceeding 300 mg/day). A fiber intake of at least 30 g/day is also recommended. Fiber contributes to increased satiety and reduces the risk of conditions such as diabetes and CVDs, as well as certain gastrointestinal cancers. In some cases, very-low-calorie diets (VLCDs) are prescribed to promote rapid and significant weight loss (13–23 kg) over 4–6 months. This approach provides approximately 800 kcal/day and 50–80 g of protein and is indicated only for highly motivated individuals with moderate to severe obesity (BMI > 30 kg/m²) who have clinical conditions likely to improve rapidly with weight loss, such as uncontrolled type 2 diabetes mellitus, hypertriglyceridemia, OSAS, or symptomatic peripheral edema. Since this type of treatment is not free of complications (e.g., cholelithiasis), these patients require strict metabolic monitoring under the supervision of a physician specialized in obesity treatment [31–33].

Fasting, including intermittent fasting, is contraindicated in all cases, as caloric restriction below normal energy requirements does not appear to provide real benefit [33].

Appropriate management of the obese patient cannot overlook the importance of physical exercise, not only during the weight-loss phase (in combination with dietary modifications), but especially during maintenance.

Current guidelines recommend that adults perform at least 150 minutes per week of moderate-intensity aerobic exercise, combined with a calorie-restricted diet, to achieve meaningful weight loss, and at least 200 minutes of moderate-intensity activity to prevent weight regain during maintenance. Training programs should be individualized and proportional to the patient's clinical condition and often require the involvement of multiple professional figures [34].

Finally, the importance of adequate therapeutic education should not be underestimated. It enables patients to acquire and maintain the skills necessary for optimal long-term disease self-management. Cognitive-behavioral therapy can therefore represent a fundamental tool for facilitating change and reinforcing new behaviors related to diet and physical activity. Strategies include self-monitoring techniques (keeping a diary, weighing oneself, quantifying food intake and physical activity), problem-solving techniques, motivational strategies, and family support [35].

3.3 Pharmacological Therapy

Pharmacologic treatment of obesity is reserved only for adult patients when lifestyle modifications have not been successful and when the following criteria are met:

- BMI ≥ 30 kg/m²
- BMI ≥ 27 kg/m² in the presence of comorbidities

It is also essential to identify the possible presence of metabolic syndrome, since this high cardiovascular risk condition is in itself sufficient indication to initiate pharmacologic therapy immediately, regardless of BMI class.

The diagnosis of metabolic syndrome is based on the presence of at least 3 of the following criteria:

Table 3.2: Diagnostic criteria for metabolic syndrome.

Abdominal circumference	M > 102 cm F > 88 cm
HDL	M < 40 mg/dL F < 50mg/dL
Triglycerides	≥ 150 mg/dL
Blood glucose	≥ 100 mg/dL
Blood pressure	≥ 130 mmHg (systolic) ≥ 85 mmHg (diastolic)

Pharmacologic treatment may also be indicated to maintain weight loss or to induce further weight reduction initially achieved through lifestyle modifications, and it should be discontinued if at least a 5% reduction in body weight has not been achieved within 12 weeks.

1. **Appetite suppressants:**

- The **naltrexone/bupropion** combination acts synergistically to reduce appetite and increase energy expenditure. Naltrexone is an opioid antagonist, while bupropion is a selective dopamine and norepinephrine reuptake inhibitor. The resulting anorexigenic effect contributes to more effective control of caloric intake, although it may cause nausea (one of the most common side effects).
- Other agents, such as the phentermine–topiramate combination, are not currently marketed in Europe but are approved in other settings, such as the United States.

2. **Nutrient absorption inhibitors:**

- **Orlistat** works by inhibiting gastrointestinal lipases and reducing dietary fat absorption. This results in decreased caloric uptake and, in patients with diabetes or prediabetes, improved

glycemic control. Gastrointestinal adverse effects (steatorrhea, flatulence, fecal urgency) and the potential deficiency of fat-soluble vitamins limit its long-term use.

3. **Hormonal analogues:**

- GLP-1 receptor agonists such as **Liraglutide** and **Semaglutide** mimic the action of this incretin hormone, promoting satiety and slowing gastric emptying. Liraglutide requires daily administration, whereas semaglutide — which shares 94% structural homology with human GLP-1 — can be administered weekly and shows a dose-dependent effect on weight reduction.
- **Tirzepatide** acts as a dual GIP and GLP-1 receptor agonist, with greater affinity for GIP receptors. This combined mechanism allows for more effective reduction of both blood glucose and body weight.

These therapies are complemented by **Cagrilintide**, a long-acting amylin analogue, a peptide co-secreted with insulin. By acting on specific central and gastrointestinal receptors, cagrilintide regulates satiety, slows gastric emptying, and reduces glucagon secretion, contributing to significant and dose-dependent weight loss.

3.4 Bariatric Surgery

The use of bariatric surgery for the treatment of obesity is based on the fact that weight loss achieved with non-invasive methods (lifestyle modification and pharmacological therapy) is often associated with a particularly high incidence of weight regain within two years after maximal weight loss.
Surgical treatment can therefore be considered provided that all the criteria indicated in Table 3.3 are met.

Criteria	Controindications
• BMI > 40 Kg/m^2	• Inability to participate in a follow-up program
• BMI > 35 Kg/m^2 with comorbidities	• Schizophrenia or psychosis *
• Age between 18 and 65 years	• Mood disorders or eating disorders **
• Obesity lasting more than 5 years	• Alcoholism and drug addiction

• Documented failure of previous attempts to lose weight and/or maintain weight loss with non-surgical methods	• Presence of diseases associated with reduced life expectancy
• Full willingness to undergo prolonged postoperative follow-up	• Patient's inability to care for themselves or lack of adequate family and social support

Table 3.3: Eligibility criteria for surgical treatment and specific contraindications.

* The indication may be established exceptionally for serious medical reasons and always with the psychiatrist's consent.

** Unless otherwise specifically indicated by a psychologist and a psychiatrist.

In patients with a BMI > 35 kg/m², the comorbidities that may justify surgical treatment are those that significantly contribute to morbidity and mortality in the obese patient and are likely to improve following weight loss: respiratory diseases (OSAS, OHS), cardiovascular diseases (hypertension, ischemic heart disease, atherosclerosis), diabetes, hyperlipidemia, and joint diseases.
In patients with a BMI > 50 kg/m², surgical treatment may represent the first therapeutic intervention, regardless of previous attempts at weight loss.
The procedures currently available in bariatric surgery are numerous but, in general, can be divided into three categories:

• Restrictive procedures: these involve a reduction in gastric capacity, mechanically limiting food intake and consequently caloric intake. They include gastric banding, vertical gastroplasty, and sleeve gastrectomy;

• Malabsorptive procedures: these involve reduced fat absorption without compromising the absorption of water, electrolytes, vitamins, and proteins, which are essential to maintain a healthy nutritional status. They include biliopancreatic diversion and duodenal switch;

• Restrictive–malabsorptive procedures: these combine reduced gastric capacity with intestinal diversion. They include gastric bypass and mini gastric bypass.

For all types of procedures, laparoscopic feasibility has been demonstrated, and this approach should always be considered the first choice, as it offers advantages over open surgery in terms of improved postoperative recovery and reduced complications [36].

Currently, there are no specific guidelines that allow directing an individual patient toward a particular surgical procedure. Indeed, the various techniques act through different mechanisms, have specific advantages, and are associated with specific complications, resulting in different risk–benefit profiles. The choice of procedure is therefore based on a series of factors that largely depend on the patient, but also in part on the procedure itself and on the surgeon.

Patient-related factors	Procedure-related factors	Surgeon-related factors
Age and Sex	Technical difficulty	Technical skill
Grade of obesity	Mechanism of action	Experience
Comorbidities	Complications	Institutional level
Socioeconomic status		
Motivation		
Family support		

Table 3.4: Factors influencing the choice of the type of procedure.

In the following paragraphs, a brief description of the individual surgical procedures will be provided.

3.4.1 Gastric banding

It is a restrictive procedure that involves placing a band equipped with an inflatable chamber around the upper part of the stomach. The inflatable chamber is connected to a reservoir positioned subcutaneously in the abdominal wall, allowing adjustment of the band's diameter.

The mechanism of action is based on reducing gastric volume, which induces an early sense of satiety even after the intake of small amounts of food [33-38].

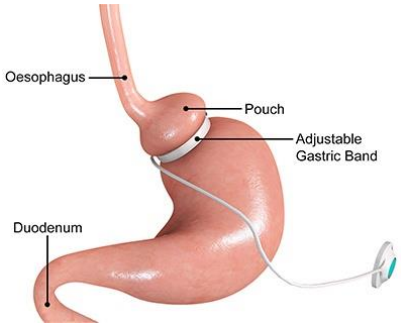

Figure 3.1: Gastric banding

The main advantage, in addition to its complete reversibility, is the ability to allow gradual patient adaptation through adjustment of the degree of restriction.

This procedure is associated with a weight loss equal to 40–50% of excess body weight, with long-term outcomes largely dependent on the patient's eating behavior. Poor adherence to dietary recommendations is, in fact, the main cause of therapeutic failure.

3.4.2 Sleeve gastrectomy

It consists of sectioning and removing approximately two-thirds of the stomach along a vertical line parallel to the lesser curvature, in order to create a tubular-shaped neo-stomach.

The main mechanism of action is the reduction of gastric volume, which leads to the intake of a smaller amount of food and an early sense of satiety. In addition, removal of part of the gastric body and fundus is associated with changes in gastric emptying and with modification of the secretion of entero-hormones involved in the regulation of energy balance and glucose metabolism. [33-38].

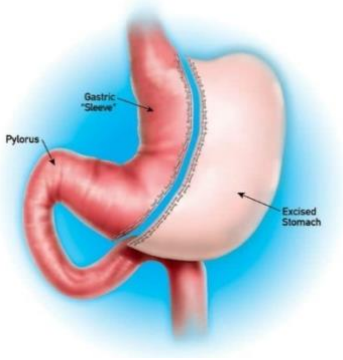

Figure 3.3: Sleeve gastrectomy.

Nel breve termine induce una riduzione del peso corporeo pari al 60% dell'eccesso, ma la scarsa aderenza del paziente al regime dietetico può determinare la progressiva dilatazione del residuo gastrico, con conseguente possibile recupero ponderale.

3.4.3 Biliopancreatic Diversion and Duodenal Switch

It is performed by carrying out a distal gastrectomy (resection of the distal two-thirds of the stomach including the pylorus), followed by construction of an internal intestinal diversion consisting of an intestinal limb of approximately 250 cm and a long blind biliodigestive limb, which is anastomosed to the former about 50 cm from the ileocecal valve.

A variant of the standard procedure is the so-called duodenal switch, which involves reducing gastric volume through sleeve gastrectomy while preserving the pylorus and the first 3–4 cm of the duodenum. As a result, the anastomosis with the alimentary limb is not gastrojejunal but duodenojejunal.

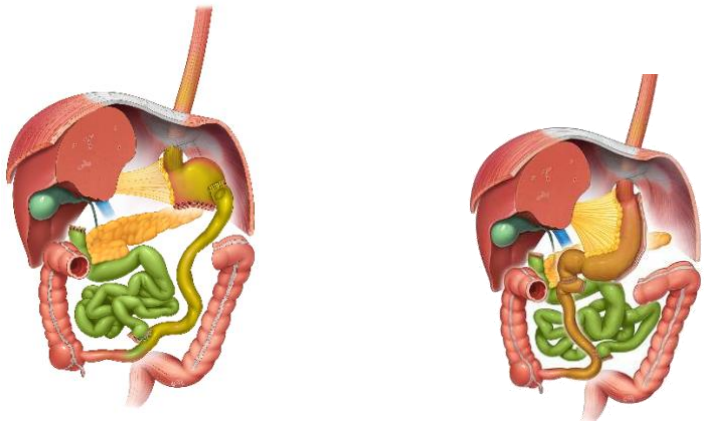

Figure 3.4: Biliopancreatic diversion (left) and duodenal switch (right).

In both cases, the mechanism of action is based on reduced digestion and therefore reduced absorption of fats and starches. The diet can remain essentially unrestricted, with the only limitation being foods high in simple sugars, since their absorption is not altered.

These techniques allow a weight reduction equal to about 70% of excess body weight, with stable long-term results and a marked improvement in obesity-related diseases. Specifically, disappearance of daytime somnolence and OHS is observed in 100% of patients one month after surgery, and in patients with type 2 diabetes or hypercholesterolemia there is normalization of serum glucose (98%) and cholesterol (100%) levels, respectively, within 12 months.

The main specific surgical complications include peptic ulcer (3.5% of cases), anastomotic stenosis, and obstruction of the biliodigestive limb.

Nutritional follow-up is essential to prevent nutritional complications (anemia, osteoporosis, fat-soluble vitamin deficiencies, and episodic or recurrent protein malnutrition) through adequate protein intake and lifelong supplementation with calcium, iron, vitamin D, and vitamin B12. [33-38].

3.4.4 Gastric bypass

It consists of creating a small gastric pouch (15–20 ml in volume) from the most proximal portion of the stomach, which is then directly anastomosed to the jejunum, thereby excluding the remaining portion of the stomach and the duodenum from food transit.

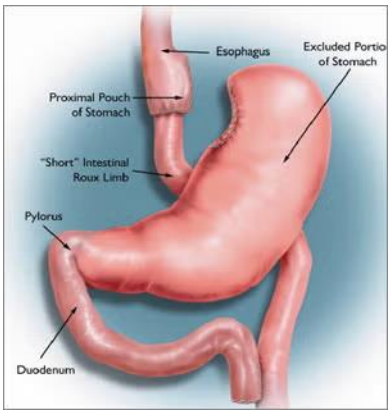

Figure 3.5: Gastric bypass.

It is a so-called mixed procedure, as weight loss is partly related to reduced food intake with early satiety (restrictive effect) and partly to reduced absorption of fats and carbohydrates (malabsorptive effect).

Long-term effectiveness is demonstrated by a stable loss of approximately 55–65% of excess body weight as early as two years after surgery, accompanied by resolution of diabetes in 84% of patients, hypertension in 83%, and obstructive sleep apnea syndrome in 95%. Weight loss is maintained over a long period, albeit with individual fluctuations [33-38].

Among the specific surgical complications, the most frequent are anastomotic leak (1%), anastomotic stenosis (1.5%), anastomotic ulcer (3%), and internal hernias (3%).

Nutritional complications (iron-deficiency anemia and osteopenia/osteoporosis) can be prevented through adequate supplementation with iron, calcium, and vitamins, as well as appropriate nutritional follow-up.

3.4.5 Minigastric bypass

It consists of a long, narrow gastric tube created along the lesser curvature of the stomach, which is anastomosed to a long jejunal loop brought into the supramesocolic compartment.

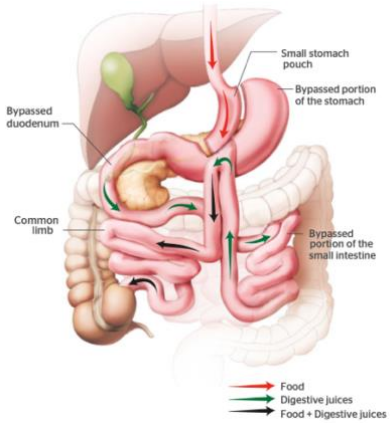

Figure 3.6: Minigastric bypass

This is also a mixed procedure, as weight loss results both from gastric tubulization (comparable to a sleeve gastrectomy) and from a malabsorptive mechanism due to the exclusion of 180–250 cm of small intestine from food transit.

The outcomes are comparable to those of gastric bypass, with the advantage of greater technical simplicity and a lower risk of postoperative complications. [33-38].

Chapter 4: Micro-RNAs

4.1 General aspects

MicroRNAs (miRNAs) are small non-coding RNA molecules (about 20–26 nucleotides) that regulate gene expression at the post-transcriptional level by interacting with a target mRNA, participating in numerous biological processes such as cell growth and differentiation, proliferation, apoptosis, and regulation of metabolic pathways [39].

The first identified miRNA, lin-4, was isolated in 1993 in the nematode C. elegans by Ambros et al., who demonstrated that this small RNA sequence was capable of modulating larval development [40].

Following these early studies, discoveries rapidly expanded, and it is now estimated that miRNAs account for approximately 1–5% of mammalian genes and are capable of regulating about 60% of protein-coding genes. [41].

4.2 Biogenesis of miRNA

miRNA genes can have different genomic locations, allowing their classification into two major groups: intergenic, when located between two protein-coding genes, and intragenic, when located within introns of coding and/or non-coding regions [42–43].

miRNA biogenesis is a multistep process that occurs partly in the nucleus and partly in the cytoplasm.

The first step involves synthesis of a precursor RNA known as pri-miRNA, which can be transcribed either as a polycistronic transcript or as an individual unit. In both cases, following complementary base pairing, pri-miRNAs assume a stem–loop tertiary structure and contain a 7-methylguanosine cap at the 5′ end and a poly-A tail at the 3′ end [44].

Within the nucleus, pri-miRNAs are processed by an enzymatic complex known as the Microprocessor, composed of Drosha (a type III RNase) and DGCR8 (an RNA-binding protein). This processing generates ~70-nucleotide stem–loop RNA sequences called pre-miRNAs, which are then transported to the cytoplasm by Exportin-5 [45–47].

The second step occurs in the cytoplasm and involves a second RNase, Dicer, which removes the loop of the pre-miRNA to produce a double-stranded RNA molecule of approximately 20–25 nucleotides, known as the miR–miR* duplex.

This duplex is loaded onto AGO 1–4 proteins, members of the Argonaute family, which together with Dicer and TRBP (Trans-activation Response RNA-binding Protein) form the RISC (RNA-Induced Silencing Complex). Through this mechanism, the miR* strand, being less stable, is degraded, while the miR strand is retained as the biologically active strand, thus constituting the mature miRNA. [39][44][48].

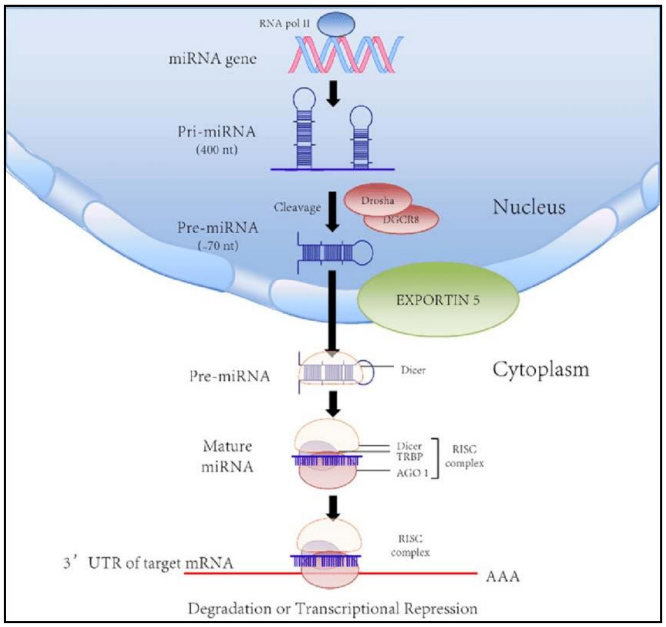

Figure 4.1: Synthesis and maturation of miRNAs.

4.3 Mechanism of action

An essential requirement for the regulatory action of miRNAs is recognition of the target mRNA. This occurs through base pairing between a sequence of approximately 6–8 nucleotides located at the 5′ end of the mature miRNA — known as the seed sequence — and the 3′UTR region of the target mRNA, to which it is complementary. [49].

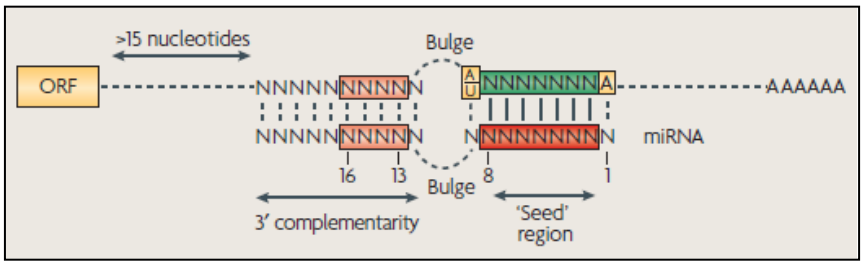

Figura 4.2: Interaction between mature miRNA and target mRNA occurs through base pairing. Perfect complementarity is achieved at the seed sequence, whereas in adjacent regions this may not occur due to the presence of bulges or non-canonical pairings..

In fact, some studies have shown that miRNA–mRNA interaction may also occur in other regions of the target mRNA, referred to as MREs (miRNA Responsive Elements), located along the entire transcript, including coding regions and the 5′UTR [50]. However, based on current knowledge, these sites are considered to have insufficient silencing capacity, and their functional role therefore appears to be largely marginal. [51].

Following recognition of the target mRNA, the RISC complex can inhibit protein synthesis through two main mechanisms: translational repression or mRNA degradation.
Translational inhibition may affect different phases of protein synthesis and can be summarized as follows:
The RISC complex, particularly AGO proteins, interferes with the initiation phase of protein synthesis and competes with the 60S ribosomal subunit, preventing its proper assembly on the mRNA;
The RISC complex acts during the post-initiation phase, slowing elongation or causing ribosomal dissociation;
The RISC complex can act after translation has already begun by inducing the activity of specific proteases responsible for cleavage of the nascent polypeptide.
Mechanisms leading to mRNA degradation involve a deadenylation process mediated by multiple factors. After target recognition, AGO proteins within the RISC complex activate members of the GW182 protein family, which in turn interact with PABP (Poly-A Binding Protein) and recruit the deadenylases CCR4 and CAF1. Deadenylation destabilizes the transcript, which is subsequently degraded [52]. This process is thought to begin in the cytoplasm and continue within specialized subcellular structures known as P-bodies, which function as sites for transcript storage and decay.[52][54].

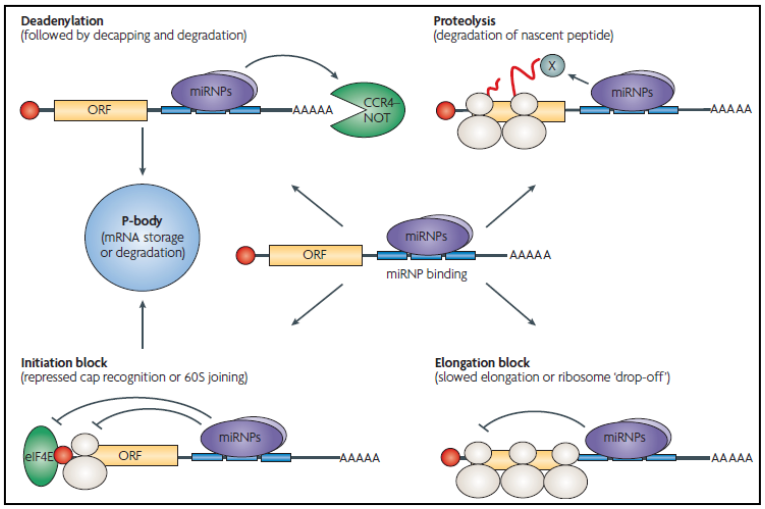

Figura 4.3: Schematic representation of the silencing mechanisms induced by the miRNP (miRNA-RISC).

Chapter 5: Experimental Design

5.1 Introduction and Aim of the study

Obesity is defined by the World Health Organization (WHO) as a chronic disease characterized by excessive body fat accumulation that adversely affects health and overall well-being. Its pathogenesis reflects a complex interaction between genetic susceptibility, nutritional factors, and metabolic dysregulation. The worldwide prevalence of overweight and obesity continues to rise across both pediatric and adult populations, representing a major public health burden associated with increased morbidity, impaired quality of life, and reduced life expectancy.

Various anthropometric indices have been proposed to assess obesity at the population level. Among these, body mass index (BMI) remains the most commonly used clinical measure. BMI is calculated as body weight in kilograms divided by height in meters squared (kg/m^2). According to WHO criteria, obesity is defined by a BMI ≥ 30 kg/m^2, while values between 25 and 29.9 kg/m^2 indicate overweight. Nonetheless, BMI provides only an indirect estimate of adiposity and fails to account for important individual factors such as lean body mass, fluid retention, or functional limitations. Consequently, additional parameters—including waist circumference and waist-to-hip ratio—are recommended, particularly in individuals with BMI > 35 kg/m^2, to more accurately evaluate cardiometabolic risk.

Obesity is strongly associated with a broad spectrum of comorbid conditions that significantly increase morbidity and mortality compared with normal-weight individuals. These include respiratory disorders, cardiovascular disease, metabolic complications, and certain malignancies. Adipose tissue is now recognized not merely as an energy storage depot but as an active endocrine organ that influences appetite regulation, insulin sensitivity, immune function, reproductive physiology, and systemic metabolism. Expansion of adipose tissue—especially within the visceral compartment—is associated with immune cell infiltration and increased production of adipokines and pro-inflammatory cytokines. This chronic low-grade inflammatory state plays a pivotal role in the pathogenesis of obesity-related degenerative diseases.

The association between obesity and respiratory disease has been extensively explored. Epidemiological evidence consistently demonstrates a higher prevalence of asthma among overweight and obese individuals. Genetic linkage studies suggest that shared molecular mechanisms may underlie both asthma and obesity. In particular, polymorphisms of the β_2-adrenergic receptor have been linked to distinct asthma phenotypes. Given the role of catecholamines in regulating energy expenditure, these genetic variants may represent a common biological pathway connecting airway dysfunction and adiposity.

Obesity also constitutes a major risk factor for other respiratory disorders, including obstructive sleep apnea and obesity hypoventilation syndrome. Concurrently, substantial evidence supports a strong relationship between excess adiposity and cardiovascular disease. Large population-based studies—such as the Framingham Heart Study, the Manitoba Study, and the Harvard Nurses' Health Study—have demonstrated a clear association between obesity and ischemic heart disease, as well as elevated risks of heart failure, atrial fibrillation, stroke, and sudden cardiac death. The INTERHEART study further emphasized that cardiovascular risk increases proportionally with abdominal adiposity, particularly visceral fat accumulation, as reflected by increased waist circumference and waist-to-hip ratio.

Adipose tissue is anatomically and functionally divided into subcutaneous and visceral depots, which differ in cellular composition, metabolic activity, and molecular signaling pathways. Emerging data indicate that these fat compartments also display distinct microRNA (miRNA) expression profiles. miRNAs are small non-coding RNA molecules that regulate gene expression at the post-transcriptional level and play a crucial role in cellular proliferation, differentiation, apoptosis, and metabolic homeostasis. While miRNAs have been widely implicated in the pathophysiology of respiratory and cardiovascular diseases, direct analyses of miRNA expression within adipose tissue remain relatively limited.

Based on these considerations, the aim of the present study was to characterize the expression profiles of two specific miRNA panels. The first panel is associated with chronic and intermittent hypoxemia, while the second is involved in adipogenesis and the regulation of cardiovascular risk. Specifically, we sought to investigate how excess visceral and subcutaneous adipose tissue in obese patients influences miRNA expression, thereby exploring their potential role as molecular biomarkers for advancing the understanding of obesity-related cardiovascular and pulmonary diseases.(Table 5.1) [55–57].

Hypoxemia	Cardiovascular Risk
miR-16	miR-27b
miR-26	miR-223
miR-145	miR-483
miR-338	

Table 5.1: Main miRNAs considered in the study.

5.2 Materials and methods

Population and tissue sampling

A total of 39 obese patients (15 males) with a body mass index (BMI) greater than 39 kg/m² who underwent bariatric surgery were consecutively enrolled at the Department of Medical and Surgical Sciences, General Surgery Unit, University Hospital of Foggia, Italy. The control group consisted of 11 non-obese subjects (five males) with a BMI below 24 kg/m², undergoing elective surgical procedures for non-neoplastic conditions.

All participants provided written informed consent prior to enrollment. The study protocol was approved by the Institutional Ethics Committee of the University of Foggia (Institutional Review Board approval no. 17/CE/2014) and was conducted in compliance with the principles of the Declaration of Helsinki and its subsequent revisions.

During the surgical procedures, paired samples of visceral and subcutaneous adipose tissue were collected, immediately stabilized in RNAlater® (Thermo Fisher Scientific), and stored at −80 °C until further processing.

RNA isolation

Adipose tissue samples were mechanically homogenized, and total RNA, including the miRNA fraction, was extracted using TRIzol® reagent (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions. RNA concentration and integrity were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). RNA purity was evaluated by measuring the absorbance ratio at 260 and 280 nm (OD260/OD280).

RNA reverse transcription and miRNA expression analysis

Total RNA was reverse transcribed using the TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. miRNA expression was subsequently quantified by real-time polymerase chain reaction (qRT-PCR) using TaqMan™ MicroRNA Assays (Thermo Fisher Scientific) on an ABI PRISM 7300 sequence detection system (Applied Biosystems).

Relative expression levels of target miRNAs were normalized to the endogenous control RNU6B and calculated using the comparative $2^{-}\Delta\Delta Ct$ method. All PCR reactions were performed in triplicate, and a no-template control was included in each experiment to exclude contamination.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). The Shapiro–Wilk test was used to assess data normality. Differences in miRNA expression between visceral and subcutaneous adipose tissue were evaluated using the Mann–Whitney U test.

Receiver operating characteristic (ROC) curve analysis was performed to calculate the area under the curve (AUC), as well as sensitivity and specificity, for each miRNA in discriminating obese from non-obese subjects. Spearman's rank correlation coefficient was used to assess the relationships between relative miRNA expression levels and clinical or biochemical parameters.

Hierarchical clustering analysis was conducted based on miRNA expression values across the available samples. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software, Inc.).

5.3 Results

miRNA expression was evaluated in visceral and subcutaneous adipose tissue samples obtained from **39 obese subjects**and **11 non-obese controls**. The main demographic, clinical, and functional characteristics of the study population are summarized in **Table 5.2**.

Variable	Obese (n = 39)	Non-obese (n = 11)	p value
Sex (male), n (%)	15 (38.5%)	5 (45.5%)	>0.05
Age, median (years)	40.4	55.1	>0.05
Weight (kg), mean (SD)	125.36 (15.2)	68.8 (12.9)	>0.05
Height (cm), mean (SD)	167.9 (8.7)	168.7 (6.8)	0.001
BMI (kg/m²), mean (SD)	44.5 (4.8)	24.0 (3.55)	<0.0001
Smoking, n (%)	11 (28.2%)	1 (9.0%)	>0.05
Hypertension, n (%)	10 (25.6%)	4 (36.4%)	>0.05
Diabetes, n (%)	9 (23.1%)	2 (18.2%)	>0.05
Total cholesterol (mg/dL)	198.88 (33.7)	—	—
HDL cholesterol (mg/dL)	48.15 (8.6)	—	—
LDL cholesterol (mg/dL)	137.31 (26.3)	—	—
Triglycerides (mg/dL)	131.62 (78.8)	—	—
Hemoglobin (g/L)	14.28 (2.39)	—	—
Serum creatinine (mg/dL)	5.91 (20.1)	1.36 (0.8)	0.03
FEV1 (% predicted)	88	—	—
FVC (% predicted)	90	—	—
FEV1/FVC (%)	99	—	—

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 5.2. Demographic and Clinical Characteristics of the Study Population

In subcutaneous adipose tissue, the expression levels of **miR-338, miR-27b, miR-223, and miR-483** were significantly lower in obese subjects compared with non-obese controls. In visceral adipose tissue, a similar significant reduction was observed **only for miR-223 and miR-27b** (Fig. 5.1A–D and Table 5.3).

miRNA	Obese Average	Obese SD	Non-obese Average	Non-obese SD	p value
Visceral adipose tissue					
hsa-miR-16-5p	2.09	2.88	1.00	1.08	ns
hsa-miR-26a-5p	1.15	0.95	1.00	1.16	ns
hsa-miR-145-5p	1.37	0.99	1.27	1.16	ns
hsa-miR-338-5p	0.22	0.19	1.00	1.51	ns
hsa-miR-27b-5p	0.42	0.35	1.00	0.78	0.01
hsa-miR-223-5p	0.24	0.28	1.00	1.14	0.03
hsa-miR-483-5p	0.36	0.45	1.00	1.67	ns
Subcutaneous adipose tissue					
hsa-miR-16-5p	1.52	2.26	0.40	0.24	ns
hsa-miR-26a-5p	0.20	0.20	0.50	0.99	ns
hsa-miR-145-5p	1.18	0.08	1.00	0.58	ns
hsa-miR-338-5p	0.13	0.21	0.20	0.20	ns
hsa-miR-27b-5p	0.05	0.05	0.15	0.21	0.01
hsa-miR-223-5p	0.20	0.25	0.62	0.85	ns
hsa-miR-483-5p	0.05	0.06	0.13	0.12	0.02

Values are expressed as mean ± SD. Significant results ($p < 0.05$) are indicated in bold in the original dataset. Relative microRNA levels were calculated using the $2^{-}\Delta\Delta Ct$ method.

Table 5.3. Visceral and Subcutaneous Adipose Tissue MicroRNA Expression Profiles in Obese and Non-Obese Subjects

Among all miRNAs analyzed, **miR-338, miR-27b, and miR-483** exhibited significantly different fold changes when comparing visceral and subcutaneous adipose tissue within the obese group (Fig. 5.1A–D and Table 5.3). In contrast, no significant differences between adipose tissue compartments were detected for the same miRNAs in the non-obese group.

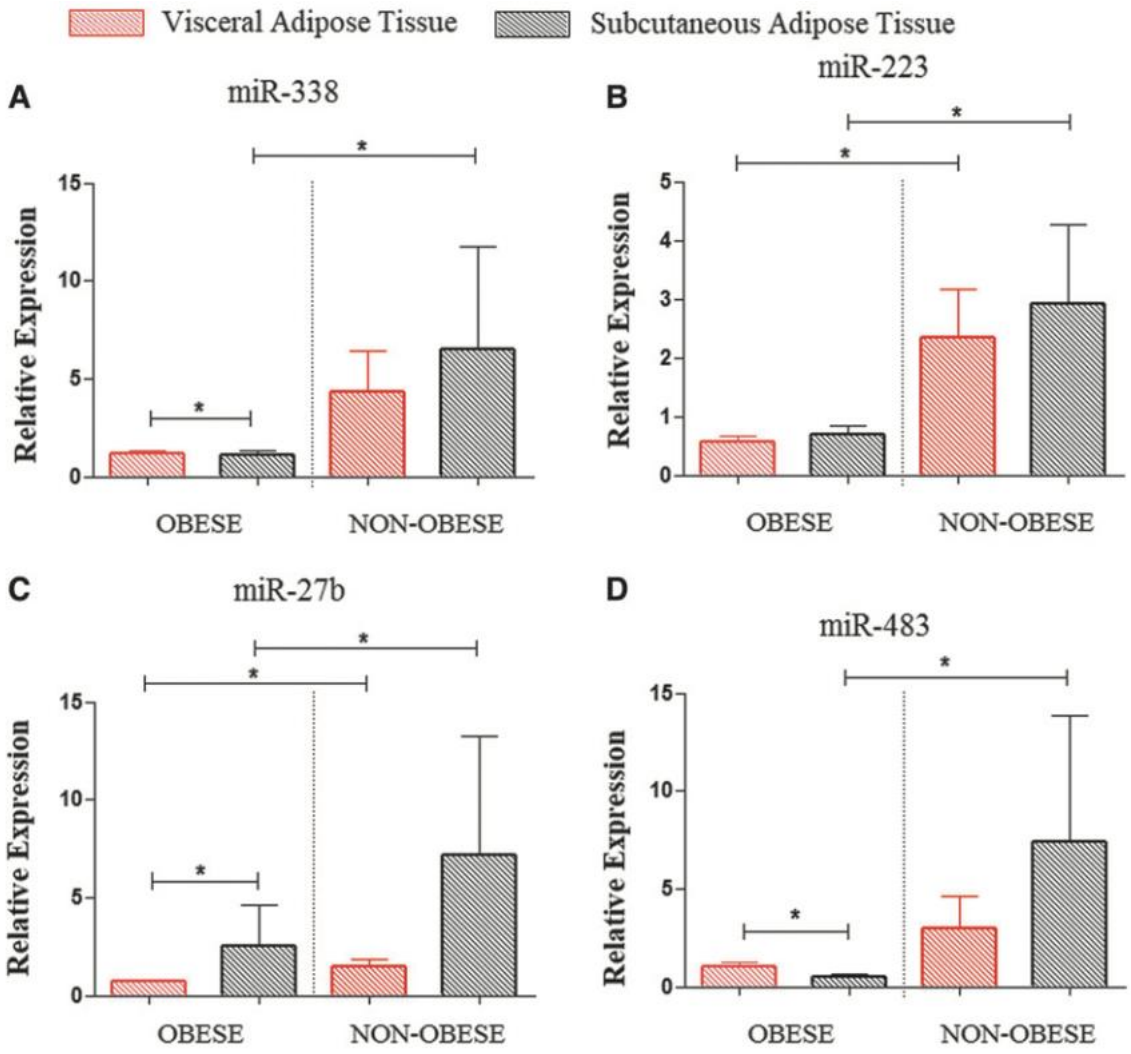

Figure 5.1: FIG. 1. Fat miRNAs expression. Quantitative real-time PCR analysis of differentially expressed miRNAs in visceral and subcutaneous adipose tissue from obese subjects versus nonobese controls. (A) miR-338; (B) miR-223; (C) miR-27b; and (D) miR-483. The relative expression levels of miRNAs were calculated using the comparative 2-DDCt method. *$p < 0.05$. miRNAs, micro-RNAs; PCR, polymerase chain reaction.

Correlation analysis between miRNA expression levels and clinical or respiratory function parameters revealed a **significant inverse relationship between BMI and the expression of miR-16, miR-26a, miR-27b, miR-223, and miR-483** in subcutaneous adipose tissue. Additionally, **miR-145 and miR-338** showed a similar inverse correlation with BMI in visceral adipose tissue from obese subjects (data not shown).

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminative ability of the analyzed miRNAs (Table 5.4). In subcutaneous adipose tissue, the area under the curve (AUC) values for **miR-27b, miR-223, and miR-483** were **0.7500 (95% CI: 0.5977–0.9023)**, **0.7079**

(95% CI: 0.5162–0.8996), and **0.7654 (95% CI: 0.6090–0.9217)**, respectively, in distinguishing obese from non-obese subjects. In visceral adipose tissue, **miR-27b** and **miR-223** also demonstrated good discriminative performance, with AUC values of **0.7273 (95% CI: 0.5355–0.9190)** and **0.7081 (95% CI: 0.5199–0.8963)**, respectively.

Comparison / miRNA	AUC	95% CI	p value	Cutoff	Sensitivity (%)	Specificity (%)
Obese vs non-obese – visceral adipose tissue						
miR-27b	0.7273	0.5355–0.9190	0.023	>1.007	63.64	78.95
miR-223	0.7081	0.5199–0.8963	0.045	>0.6722	60.00	67.57
Obese vs non-obese – subcutaneous adipose tissue						
miR-27b	0.7500	0.5977–0.9023	0.016	>0.4131	70.00	69.44
miR-223	0.7079	0.5162–0.8996	0.0450	>1.061	60.00	81.58
miR-483	0.7654	0.6090–0.9217	0.0103	>0.2968	70.00	58.97
Visceral vs subcutaneous adipose tissue in obese subjects						
miR-26a	0.8526	0.7660–0.9391	<0.0001	<0.2490	65.79	82.05
miR-27b	0.6769	0.5548–0.7990	0.009	<0.3565	63.89	60.53
miR-338	0.7180	0.5971–0.8389	0.001	<0.0915	61.11	75.00
miR-483	0.6483	0.5262–0.7703	0.0243	<0.1895	48.72	71.79

AUC, area under the curve.

Table 5.4. ROC Curve Analysis

Finally, in obese subjects, ROC analysis comparing visceral and subcutaneous adipose tissue revealed AUC values of **0.8526 (95% CI: 0.7660–0.9391)** for miR-26a, **0.7180 (95% CI: 0.5971–0.8389)** for miR-338, **0.6769 (95% CI: 0.5548–0.7990)** for miR-27b, and **0.6483 (95% CI: 0.5262–0.7703)** for miR-483.

Hierarchical clustering analysis of miRNA expression profiles identified **four distinct clusters (C1–C4)**, clearly demonstrating differential miRNA expression patterns between visceral and subcutaneous adipose tissue. The average silhouette coefficient of the global clustering was approximately **0.30**, supporting the reliability of the separation among the four miRNA clusters (Fig.

5.2).

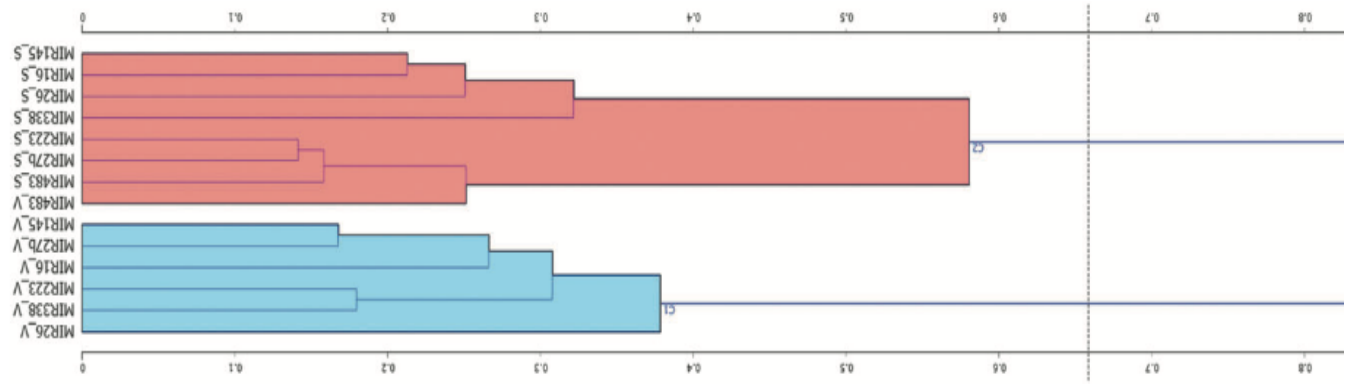

Figura 5.2: Cluster analysis of miRNAs expression.

5.4 Discussion

The main finding of the present study is the identification of a distinct miRNA expression profile in the adipose tissue of obese patients compared with non-obese subjects, particularly involving miRNAs associated with increased cardiovascular risk. Specifically, **miR-27b and miR-483** were significantly downregulated in subcutaneous adipose tissue, whereas **miR-27b and miR-223** showed reduced expression in visceral adipose tissue. In addition, **miR-26a and miR-338** displayed significant differences in expression between visceral and subcutaneous adipose tissue within the same obese individuals. No comparable differences were observed in the non-obese group[58]..

miRNAs are known to play a critical role in respiratory and cardiovascular biology, regulating key processes such as smooth muscle cell maturation and proliferation, epithelial cell growth, endothelial function, and the expression of genes involved in cardiac development. Dysregulation of miRNA expression has been implicated in several pathological conditions, including asthma, chronic obstructive pulmonary disease (COPD), atherosclerosis, heart failure, cardiomyopathies, and cardiac fibrosis.

Previous studies have demonstrated that, under physiological conditions, **miR-27b and miR-483** negatively regulate the expression of peroxisome proliferator-activated receptor gamma (PPARγ), thereby promoting an “anti-obesity” state. Reduced expression of these miRNAs leads to increased PPARγ levels, resulting in enhanced adipocyte proliferation and lipid accumulation. Although not definitive, our findings are consistent with these observations and support the hypothesis that miR-27b and miR-483 may contribute to the molecular mechanisms underlying adipose tissue expansion in obesity [59].

Moreover, accumulating evidence indicates that **miR-223** plays a pivotal role in modulating macrophage activation within adipose tissue. Through interactions with transcription factors such as

NFAT5, RASA1, and PKNOX1, miR-223 promotes macrophage polarization toward the anti-inflammatory M2 phenotype. Reduced levels of miR-223 therefore favor a shift from M2 to pro-inflammatory M1 macrophages, contributing to chronic low-grade inflammation. In the present study, the reduced expression of miR-223 in visceral adipose tissue supports its involvement in the inflammatory milieu characteristic of obesity.

Given that visceral adipose tissue is metabolically more active and closely linked to the development of cardiovascular diseases—including hypertension, atherosclerosis, and heart failure—it is reasonable to hypothesize that alterations in miR-27b and miR-223 expression within this compartment may play a role in increasing cardiovascular risk in obese patients. Furthermore, visceral fat may influence miRNA expression in other tissues, thereby contributing to systemic metabolic and cardiovascular dysfunction [60].

Regarding **miR-26a and miR-338**, our study demonstrated differential expression between visceral and subcutaneous adipose tissue in obese subjects. Several investigations have identified **miR-338** as a key regulator in respiratory diseases. In particular, Zhuang et al. reported that decreased miR-338 expression in fibroblasts is associated with the progression of idiopathic pulmonary fibrosis. Additionally, miR-338 has been found to be downregulated in various malignancies, including non-small cell lung cancer, where it plays a role in cell differentiation and lymph node metastasis. **miR-26a**, while traditionally associated with tumorigenesis, has more recently been recognized as an important regulator of hepatic metabolism [61-63].

Despite these findings, the present study did not assess circulating levels of the analyzed miRNAs. Therefore, further investigations are required to determine whether these miRNAs may serve as easily measurable circulating biomarkers in obese patients.

5.5 Conclusions

This study confirms that visceral and subcutaneous adipose tissues exhibit distinct miRNA expression signatures, supporting the concept that miRNAs play a meaningful role in the pathophysiology of obesity-related diseases. Although further research is warranted, our findings provide a valuable foundation for future investigations aimed at elucidating the molecular mechanisms linking adipose tissue dysfunction to metabolic, cardiovascular, and respiratory complications associated with obesity.

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