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Pulmonary gas exchange and severe obesity: bariatric surgery effects

Eva Rivas Ferreira

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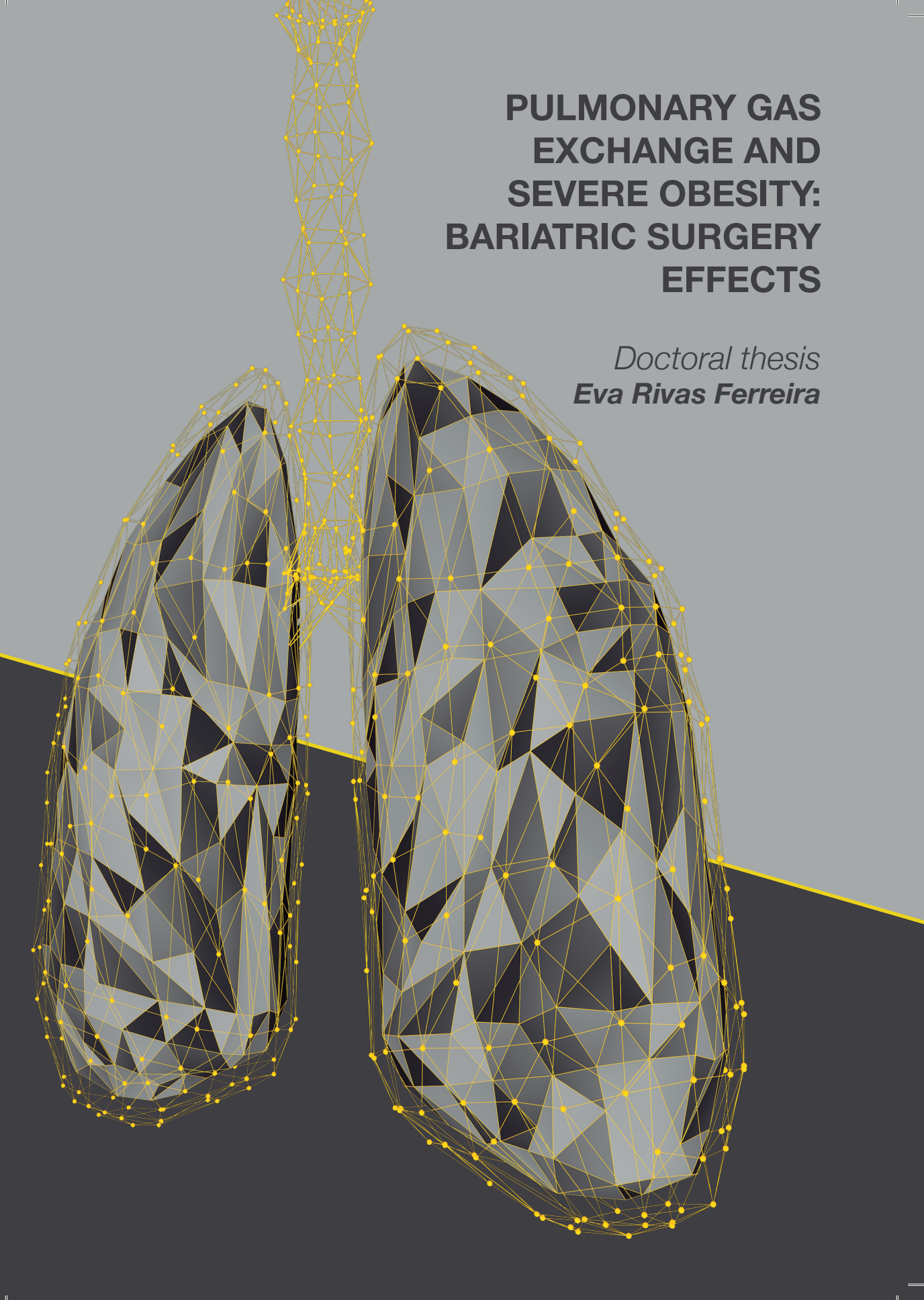

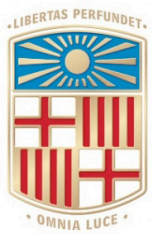

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Facultat de Medicina

DISSERTATION

PULMONARY GAS EXCHANGE AND SEVERE OBESITY:

BARIATRIC SURGERY EFFECTS

by

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PREFACE

…What was realized in San Diego in the early 1970s was that the relationship between retention (R), solubility (λ), and ventilation-perfusion (V_A/Q) ratio was a very smooth function over the whole range: calculus can be used to show that R as a function of λ ($R = \lambda / (\lambda + V_A/Q)$) is monotonic with no zero derivatives, or, in other words, very smooth…

…The six partition coefficients were chosen to cover a very wide range—from 0.005 (sulfurhexafluoride) to 300 (acetone). That is a range of 60,000, and allows a corresponding five-decade range of V_A/Q from about 0.005 to 100 to be resolved. The basic data set required for the multiple inert gas elimination technique (MIGET) was, therefore, the retention–solubility curve (as estimated from the retention values of each of six gases)…

…Much of the clinical application of the MIGET has been accomplished in Barcelona, Spain. This reveals an interesting and possibly unique way in which the National Institutes of Health (NIH) has been involved in scientific development beyond the United States. In 1980, one of his senior members came as a postdoctoral fellow to work with the authors using the MIGET. On his return to Spain he took the technique with him and began clinical research. In 1984 he noticed that the NIH was offering a unique opportunity for research development in Spain via a special grant mechanism that brought researchers from the United States and Spain…

...Together we applied for these funds (CCA 8309185), and the rest is, as they say, history. In difficult circumstances, the Spanish group built a strong clinical research program, initially based mostly on the MIGET… Their senior members have since become leaders in the European respiratory community, and now their program, of course expanded well beyond gas exchange, is attracting fellows, especially from the Spanish-speaking world of South America...

From ***West JB and Wagner PD. Pulmonary Gas Exchange. AJRCCM 1998; 157: S82–S87***

The MIGET is a very complicated research tool, based on complex algorithms, that requires solid, hard work and motivated technical skills along with experienced personnel and solid scientists. Suffice to say that not more than a dozen of research centers worldwide have developed MIGET and used with regularity.

This **MD Thesis** is centered on a very thorough investigation of pulmonary gas exchange using MIGET in women morbidly obese, an area never explored so far. The first study **(*Rivas E et al. Chest 2015; 147: 1127-1134)*** focuses on the baseline findings of pulmonary gas exchange using MIGET just before bariatric surgery. This study is also the original number 81st paper using MIGET (all published in 1st quartile of international journals by the group of Barcelona since 1984). The MIGET study was used while breathing ambient air and also during 100% oxygen. This is the common approach in most of the MIGET studies in order to extend and complement the knowledge of pulmonary gas exchange to eventually expand our understanding of oxygen-induced pulmonary vascular responses. The second study (number 82nd of Barcelona's MIGET papers) **(*Rivas E et al. Minerva Anestesiologica 2015 Jun 9 [Epub ahead of print]. PMID: 26054299***) refers to the postural effects on pulmonary gas exchange in the same obese population during ambient air alone, an area with important clinical implications in the management of obese subjects in anesthesiology and critical care medicine. Finally, the third study (***Santos A and Rivas E et al. Obesity Surgery 2016 Mar21 [Epub ahead of print]. doi: 10.1007/s11695-016-2137-9*)**, based on the use of thoracic computed tomography (CT) scanning with a special software, also during ambient air, assessed the likelihood of imaging outcomes, more specifically lung tissue volume, in a smaller subgroup of the original sample of the first study.

In summary, the findings of these three studies complement each other and provide a full comprehensive understanding of the likelihood of pulmonary gas exchange from the perspective of the inert gas methodology, including pulmonary and systemic hemodynamics, within the context of a low-grade systemic and pulmonary inflammation status, the underlying vital mechanism. Last but not least, the effects a common very effective intervention nowadays, *i.e.* bariatric surgery, after a follow-up of one year, were also completed hence providing the actual therapeutic dimensions and advantages on pulmonary gas exchange in this very specific obese population. The latter components contextualize the unique findings of pulmonary gas exchange and the different pathophysiologic mechanisms involved, all debated and discussed in the present **MD Thesis**.

Eva Rivas, Barcelona, April 2016

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List of abbreviations

$AaPO_2$	alveolar to arterial oxygen pressure difference
ABGs	arterial blood gases
ALI	acute lung injury
ARDS	acute respiratory distress syndrome
BMI	body mass index (kg/m^2)
BS	bariatric surgery
CI	cardiac index
CHD	coronary heart disease
COPD	chronic obstructive pulmonary disease
CRP	*C*-reactive protein
CT	computed tomography
CV:	closing volume
CVD:	cardiovascular disease
Dead space	alveolar units with V_A/Q ratios >100, expressed as % of alveolar volume (V_A)
EELV	end-expiratory lung volume
ERV	expiratory reserve volume
EWL	excess weight loss
FEV_1	forced expiratory volume in 1 s
FFA	free fatty acids
FRC	functional residual capacity
FVC	forced vital capacity
HPV	hypoxic pulmonary vasoconstriction
HU	hounsfield unit
IL	interleukin
Log SDQ	dispersion of blood flow distribution
Log SDV	dispersion of ventilation distribution
MIGET	multiple inert gas elimination technique
MS	metabolic syndrome
NALV	non-aerated lung volume
NIDDM	no insulin dependent diabetes mellitus
NO	nitric oxide
O_2	oxygen
OSAS	obstructive sleep apnea syndrome
PaO_2	arterial oxygen pressure
PvO_2	mixed venous oxygen pressure
Q	perfusion
Q_T	cardiac output (L/min)
PEEP	positive end-expiratory pressure
PVAT	perivascular adipose tissue
RYGB	*Roux-en-Y* gastric by-pass
RV	residual volume
Shunt	non-ventilated alveolar units (V_A/Q ratios <0.005, expressed as % of Q_T)
SG	sleeve gastrectomy
TLC	total lung capacity
TNF-α	tumor necrosis factor-α
V_A	alveolar ventilation (L/min)
V_A/Q	ventilation-perfusion

V_E	minute ventilation
VO_2	Oxygen consumption
Vtiss	lung tissue volume
WC	waist circumference
WHO	World Health Organization
WHR	waist-to-hip ratio

1. INTRODUCTION

1.1. State-of-the art

Obesity is defined as abnormal or excessive fat accumulation that may have deleterious effects on health. Body mass index (BMI) is the most useful, albeit crude, population-measure to classify overweight and obesity. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2). Classification of overweight and obesity according to BMI (**Table 1**) allows the identification of individuals at increased risk of morbidity and mortality.[1] As shown in **Table 1**, obesity is defined as a BMI ≥ 30 kg/m^2.

Table 1. World Health Organization obesity classification

Classification	BMI (kg/m^2)	Risk of Comorbidity
Underweight	< 18.5	Low
Normal Range	18.5 – 24.9	Average
Overweight	25.0 – 29.9	Increased
Obese class I	30.0 – 34.9	Moderate
Obese class II	35.0 – 39.9	Severe
Obese class III	≥ 40.0	Very Severe

BMI, body mass index. BMI values are age-independent and the same for both sexes. This table shows the relationship between BMI and the risk of obese-related multi-morbidity (taken, in part, from ref. 1).

However, obese individuals differ not only in the amount of excess fat that they store, but also in the regional distribution of that fat within the body. This observation was first described by *Vague*[2] in 1947,who noted that upper body adiposity (android or male-type obesity) was the type most often associated with the metabolic abnormalities (diabetes and cardiovascular disease (CVD).[2] Nowadays, it is well established that the greater excess of abdominal fat (visceral intra-abdominal fat) the greater is the risk factor for obesity related illness.[3,4] Therefore, measurement of waist circumference (WC) or waist-to-hip ratio (WHR) correlates to intra-abdominal and subcutaneous adipose tissue and provides a simple and practical method to identifying obese subjects at highest risk.[1,3,5] Although, WHR is an index of the relative accumulation of abdominal fat, WC, which is a crude but

relevant index of the absolute amount of abdominal fat, has been found to correlate better with visceral fat deposits as measured by computed tomography (CT).[3]

Table 2. Waist circumference and risk complications associated with obesity

Risk of Metabolic Complications	Waist Circumference (cm)	
	Women	Men
Increased	≥ 80	≥ 94
Substantially increased	≥ 88	≥ 102

This table shows enhance relative risk associate to sex-specific waist circumference levels in a Caucasian population (taken, in part, from ref. 5).

1.1.1. Epidemiology

The worldwide rising prevalence of overweight and obesity has been described as a global pandemic.[1] The number of overweight and obese individuals increased from 857 million in 1980, to 1.9 billion in 2014 (increased by 28%), currently representing a global prevalence of 39% (**Table 3**).[6,7] When obesity (BMI ≥ 30 kg/m^2) has been considered alone, its prevalence in 2014 was 13%, being 11% in men and 15% in women, which is nearly twice the prevalence in 1980 (5% for men and 8% for women) (**Table 3)**.[1,7,8]

Table 3. Worldwide, Europe and Spain prevalence of overweight and obesity in 2014

	Prevalence of Overweight (BMI ≥25 kg/m^2)			Prevalence of Obesity (BMI ≥30 kg/m^2)		
	Both Sexes	**Female**	**Male**	**Both Sexes**	**Female**	**Male**
Global	39.0% (37.3 - 40.7)	39.6% (37.2 - 42.1)	38.5% (36.1 - 41.2)	12.9% (12.0 - 13.9)	15.2% (13.8 - 16.8)	10.7% (9.4 - 12.0)
Europe	58.6% (56.3 - 60.8)	54.9% (51.6 - 58.4)	62.6% (59.4 - 65.8)	23.0% (20.7 - 25.2)	24.5% (21.5 - 27.7)	21.5% (18. 7- 24.3)
Spain	62.9% (59.2 - 66.6)	58.2% (52.6 - 63.6%)	67.8% (62.7 - 72.6)	24.2% (20.7 - 27.7)	25.7% (20.8 - 30.9)	22.6% (18.1 - 27.4)

Data expressed as mean (uncertain interval 95%, 2.5 – 97.5 percentiles of the distributions) (taken, in part, from refs. 6 and 7).

This increase represents a mean BMI increase of the order of 0.4–0.5 kg/m² per decade in men and women, respectively.[8] There are marked variations in obesity prevalence across countries. It is over four times higher in high-income countries compared to low-income countries and in the former countries the peak prevalence of obesity is moving to younger ages. Moreover, women's obesity prevalence is markedly higher than men's, with the exception of high-income countries where it is close. In low- and lower-middle-income countries obesity among women is more than double that observed in men.[1]

Understanding the effects of adiposity on major health outcomes have never been more urgent, given the rapid rise in obesity worldwide in recent years and the deleterious effect of overweight and obesity in health and life expectancy.[9] In obese subjects there is a U-shaped relation between current BMI and the risk of death, with the highest risk in the lowest and the highest categories of BMI. The stratified analyses according to smoking status observed stronger associations between obesity and an increased risk of death among those obese subjects who had never smoked than among ex-smokers and current smokers. This relationship between obesity and the risk of death was consistently stronger among participants without pre-existing chronic disease than among those with it.[10] Moreover, it has been demonstrated that above 25 kg/m² overall mortality is about 30% higher for every 5 kg/m² higher BMI. This BMI increases is associated with about 40% higher ischemic heart disease, stroke and other causes of cardiovascular diseases (heart failure, hypertensive disease) mortality; 60 - 120% for diabetic, renal, and hepatic mortality; 10% for neoplastic (uterus, gallbladder, kidney, cervix, thyroid, leukemia, liver, colon, ovarian and postmenopausal breast cancers); and, 20% for respiratory and for all other mortality. In terms of life expectancy, at 30–35 kg/m², median survival is reduced by 2–4 years and at 40–45 kg/m², it is reduced by 8–10 years which is comparable with the effects of smoking.[9,11] For these reasons, concerns about the health risks associated with rising obesity have become nearly universal so that member states of the World Heath Organization (WHO) introduced a voluntary target to stop the rise in obesity by 2025.[1,7]

1.1.2. Obesity related multi-morbidities

Pathophysiological processes that could plausibly mediate the connection between BMI and the risk of death include insulin resistance, lipid abnormalities, hormonal alterations and chronic inflammation.[11] Therefore, the main burden of obesity lies in its interconnection with a number of metabolic and non-metabolic diseases including non-insulin-dependent diabetes mellitus (NIDDM), dyslipidemia, arterial hypertension and atherosclerosis, leading by and large to a substantially increase in cardiovascular and cerebrovascular morbidity and mortality.[12,13] The WHO in 2000 has reported that the relative risks of particular diseases in obese individuals, compared to lean subjects, are fairly similar throughout the world and have classified them into three broad categories: greatly, moderately and slightly increased risk (**Table 4**).[5]

Table 4. Relative risk of health problems associated with obesity

Greatly Increased Relative Risk >3	**Moderately Increased Relative Risk 2-3**	**Slightly Increased Relative Risk 1-2**
NIDDM	CHD	Cancer (colon and breast)
Gallbladder disease	Hypertension	Reproductive hormone abnormalities
Dyslipidemia	Osteoarthritis	Polycystic ovary syndrome
Insulin resistance	Hyperuricemia	Impaired fertility
Breathlessness		Low back pain
OSAS		Anesthesia complications
Metabolic Syndrome		

NIDDM, non-insulin-dependent diabetes mellitus; CHD, coronary heart disease; OSAS, obstructive sleep apnea syndrome (taken, in part, from ref. 5).

The more life-threatening, chronic, health problems associated with obesity fall into 5 main areas:

- *Cardiovascular disease* (CVD), including coronary heart disease (CHD), stroke and peripheral vascular disease. The association between systemic arterial hyperten-

sion and obesity is well documented. Arterial hypertension is approximately 6 times more frequent in obese than in lean subjects and this risk increases with the duration of obesity. The factors that link obesity to an increase in blood pressure include: (1) direct effects of obesity on systemic hemodynamics: increase in blood volume, stroke volume, and cardiac output; and, (2) mechanisms linking obesity and an increase in peripheral vascular resistance, such as endothelial dysfunction, insulin resistance, substances released by adipocytes (interleukin [IL]-6, tumor necrosis factor-α [TNF-α]), and sleep apnea.[14] It is well known that obesity is an independent predictor of CHD. Post-mortem coronary artery examinations reveal that the extent of the deposits of cholesterol at the intima of arteries and advanced lesions in the right coronary artery and in the abdominal aorta are associated with obesity.[14,15] However, in patients with known CVD or clinical CHD, obesity has a protective effect and it is inversely related to mortality. Each 1-unit increase in BMI was associated with an increase of 4% in the risk of ischemic stroke and 6% for hemorrhagic stroke. The increase of stroke in obesity may be predicted by the pro-thrombotic/pro-inflammatory state present in obesity.[14]

- *Higher incidence of insulin resistance and non-insulin-dependent diabetes mellitus* (NIDDM). Obesity is significantly associated with diabetes, so that 46% of adults with type 2 diabetes are overweight or obese. Insulin resistance has been defined as a defect in insulin action that results in fasting hyperinsulinemia to maintain euglycemia. Insulin resistance is the first sign of NIDDM. In obese subjects hyperinsulinemia is secondary to a decrease in glucose uptake and metabolism in sensitive tissues (*i.e.*, muscle and adipose tissue). Moreover, a major contributor to the development of insulin resistance is an overabundance of circulating free fatty acids (FFA). Hyperglycemia stimulates insulin secretion in pancreatic islet β-cells. Consequently, if β-cells are unable to produce additional insulin to maintain euglycemia, both glucose intolerance and NIDDM could appear.

- *Obstructive sleep apnea syndrome* (OSAS). Obesity is the most important predisposing factor to OSAS and it is estimated that 50% to 60% of subjects who are obese have OSAS. The former entity promotes enlargement of soft tissue structures within and around the airways and, reduction in traction forces and pharyngeal wall tension, thereby contributing significantly to pharyngeal airway narrowing.[16] This instability of upper airways during sleep induces an absence (apnea) or severe reduction (hypopnea) of airflow.[17] These episodes result in arterial oxyhemoglobin desaturation and frequent arousals. Subjects and patients with OSAS are classified according to the apnea/hypopnea index (AHI) as mild (5-14 AHI events/h), moderate (15-29 AHI events/h) and severe (≥ 30 AHI events/h). Likewise, OSAS is a risk factor mostly associated to systemic arterial hypertension, but also to coronary heart disease, stroke and heart failure.[15,16] In addition, markers of cardiovascular risk, including sympathetic activation, systemic inflammation (C-reactive protein [CRP]) and endothelial dysfunction are significantly increased in obese patients with OSAS as compared with those without it, suggesting that the connections between obesity and OSAS are complex and bidirectional.[16,18]

- *Metabolic syndrome* (MS) was first described by *Kylin* in the 1920s as the clustering of hypertension, hyperglycemia and gout.[19] Although there has been considerable disagreement over the terminology and diagnostic criteria related to MS, there appears to be a consensus that the term MS is acceptable for the condition of the presence of multiple metabolic risk factors for CVD and diabetes. These factors include abdominal obesity (which is highly correlated with insulin resistance), elevated triglyceride levels, reduced high-density lipoprotein cholesterol levels, elevated blood pressure, and elevated fasting glucose (**Table 5**).[4] Metabolic syndrome definition has evolved in order to facilitate a better understanding of the syndrome and targeting of care to patients who would benefit from cardiovascular risk reduction. In 1998, the WHO required the presence of insulin resistance (or impaired glucose regula-

tion) for the diagnosis of MS. After that, the *International Diabetes Federation and National Cholesterol Education Program Adult Treatment Panel III* criteria did not require demonstration of insulin resistance *per se*, as it is difficult to measure in day-to-day clinical practice, but they included at least 3 of the 5 factors (**Table 5**).[20] However, there was no agreement on the definition of abdominal obesity measured by waist circumference with cut-points that are sex- and ethnic-group specific.[4] The MS is not an absolute risk CVD indicator, because it does not contain the absolute risk factors (age, sex, cigarette smoking, and low-density lipoprotein cholesterol levels). Nonetheless, patients with MS are at twice the risk of developing CVD over the next 5 to 10 years as individuals without the syndrome. The risk over a lifetime undoubtedly is even higher.[3,4,21]

Table 5. Criteria of metabolic syndrome definition

Measurement	Categorical cut-offs
Elevated Waist Circumference	≥ 102 cm in men ≥ 88 cm in women
Elevated triglycerides or drug treatment for hypertriglyceridemia	≥150 mg/dL
HDL cholesterol Reduction or drug treatment for reduced HDL-c	<40 mg/dL in men <50 mg/dL in women
Elevated BP or drug treatment for reduced BP	Systolic BP (≥130 mmHg) and/or diastolic BP (≥ 85 mm Hg)
Elevated fasting glucose or drug treatment for reduced glucose	≥100 mg/dL

HDL-c, HDL cholesterol; BP, blood pressure (taken, in part, from ref. 20).

- *Several types of cancers*. There is a positive association between overweight and the incidence of some cancers, especially those hormonally related and also those with large-bowel cancer. Thus, each 5 kg/m² increase in BMI was associated with a large increase in risk of cancer of the uterus, gallbladder, kidney and liver, and small increases in risk for colon, cervical, thyroid, ovarian, and postmenopausal breast cancers and leukemia.[9]

1.2. Low-grade chronic systemic inflammation associated to obesity

Nowadays obesity is regarded and recognized as a state of low-grade chronic inflammation or "meta-inflammation'' (metabolically triggered inflammation) characterized by an increase in circulating pro-inflammatory factors and the absence of clinical signs of inflammation (hence the term subclinical inflammation).[22,23]

In the 1990s, the classical view of adipose tissue as an inert reservoir of energy storage was abandoned because it was discovered that: (*1*) there is a clear link between inflammation, obesity and insulin resistance [22,24] so that the exogenous administration of TNF-α, which is over-expressed in the adipose and muscle tissues of obese humans, leads to insulin resistance; and, (*2*) adipose tissue is able to produce adipo-cytokines (adipocyte-derived hormones that are structurally similar to cytokines), such as leptin and adiponectin. In the setting of obesity the ability of the adipose tissue to elaborate adipocytokines, which possess pro-inflammatory properties, such as leptin, resistin, and visfatin increases whereas the synthesis of an anti-inflammatory adipocytokine (adiponectin) declines. Moreover, substantial changes in the amount and function of immune cells have been observed, hence increasing the number and activity of some of them (most notably macrophages, mast cells, neutrophils, and T- and B lymphocytes) while simultaneously reducing others, such as eosinophils and several subsets of T lymphocytes (T helper 2 , regulatory T and invariant natural killer T cells).[12] These adipose tissue products and reactions are suitable to be involved in numerous metabolic, hormonal, and immune processes and to act not only locally but also influence other organs and systems, playing a crucial role in the whole-body homeostasis.[23,25]

From an histological viewpoint, adipose tissue is composed of adipocytes (mature fat cells) and the stroma formed by extracellular matrix with dispersed fibroblasts, pre-adipocytes, endothelial, and immune cells.[12] During development of obesity, the adipocyte is overloaded with triacylglycerol and its ability to store more lipids declines. Conse-

quently, circulating levels of triacylglycerol and non-esterified fatty acids increased leading to ectopic storage of lipids in skeletal muscle, the pancreatic islets and the liver. Moreover, individual adipocytes undergo hypertrophy, and the vasculature fails to adequately perfuse the expansion of adipose tissue, resulting in tissue hypoxia and apoptotic cell death. The cellular debris left behind these cells induces the elaboration of monocyte chemoattractant protein-1, which recruits macrophages and T cells from the peripheral circulation. The recruited macrophages produce TNF-α, IL-6, and other cytokines, which all inhibit adipocyte differentiation.[21,25] The pro-inflammatory mediators produced in adipose tissue spill over into the peripheral circulation, hence increasing the liver production of acute phase reactants as CRP, and contribute to a low-grade state of chronic systemic inflammation.[23,26]

Until recently, the primary consequences of this inflammation were thought to be a reduction in insulin sensitivity and an increase in non-esterified fatty acids. As a consequence, higher risk of arterial hypertension, insulin resistance, and cardiovascular disease are described.[25] However, given the profound effects of cytokines on arterial function, it has been suggested that the changes seen in adipose tissue in obesity are likely to have detrimental effects on vasoactive properties of perivascular adipose tissue (PVAT).[27]

Thus, the aberrant secretion of vasoactive molecules from adipose tissues is described as a key link between obesity and endothelial dysfunction, which is characterized by impaired vasodilatation and augmented vasoconstriction.[28] On the one hand, leptin induces a balanced vasoconstriction via sympathetic system and peripheral endothelium-dependent vasorelaxation, resulting in no changes in the vascular tone and blood pressure under normal conditions. In severe obesity and coexisting MS, the peripheral stimulatory effects of leptin on endothelial nitric oxide (NO) synthase activation and production are impaired whereas its central effect on activation of sympathetic system remains intact. On the other hand, it is accepted that in healthy individuals PVAT has anti-contractile

effects. Recently, *Greenstein et al.*[27] have demonstrated that obesity-related changes in adipose tissue have direct effects on the vasoactive properties of PVAT. The predominant physiological mechanism by which perivascular fat modulates contraction in human subcutaneous small arteries has been identified. Adipocytes release adiponectin, which maintains endothelial NO production and thus reduces vascular tone. In obesity and MS, however, the anti-contractile capacity of PVAT is lost, because individual adipocyte size is greater and consequently there is local tissue hypoxia and inflammation. Such an environment provides the stimulus for adipocyte and macrophage release of inflammatory cytokines. Both hypoxia and inflammation are known to reduce adiponectin production from adipocytes and, in human and animal preparations, it has been demonstrated that these stimuli inhibit the vasorelaxant properties of PVAT through increased oxidative stress. The reduction in adiponectin generation or function is likely to be responsible for the observed down-regulation of NO synthase that contributes to endothelial dysfunction.[27] Finally, TNF-α is released by the macrophage and acts on the endothelium to impair endothelial NO production and induce apoptosis, thus leading to endothelial damage.[28]

It has been reported that bariatric surgery (BS) reverses the obesity-induced damage to PVAT anti-contractile function in the cutaneous circulation and restores the function of PVAT by reducing adipose inflammation and increasing local adiponectin and NO bioavailability.[29]

1.2.1. Lung function and inflammation

In obese subjects, a significant relationship between low-grade systemic inflammation and pulmonary inflammation in obese subjects has been reported.[23,26,30–32] Most of these studies included individuals with OSAS [30,31,33] an entity which is in fact believed to be the main pathogenic driver of the observed pulmonary inflammation. However, *Arismendi et al.*[23,32] have extended and complemented these previous reports by recently, showing

that there was a significant connection between systemic and pulmonary biomarkers, which is not different in obese patients with or without OSAS (and/or MS, or smoking). Alternatively, BS not only reduced systemic inflammation but had a similar anti-inflammatory effect in the lungs as well. The fact that pulmonary inflammation is significantly reduced after BS further supports the key role-played by obesity in the pathogenesis of both systemic and pulmonary inflammation.[23]

1.3. Lung function and obesity

Since 1956, when obesity was first associated with hypoventilation, arterial hypoxemia, polycythemia and right heart failure,[34] several studies have been focused in the effects of obesity in lung function. Nowadays, it is well known that obesity has a direct effect on respiratory well-being, since it increases oxygen consumption and carbon dioxide production, while at the same time it stiffens the respiratory system and increases the mechanical work needed for breathing.[35]

1.3.1. Lung volumes

The most consistently reported effect of obesity on lung function is a reduction in the functional residual capacity (FRC) and expiratory reserve volume (ERV)[35–37] (**Figure 1**).[38] This effect may reflect a shift in the balance of inflationary and deflationary pressures on the lung provoked by mechanical factors.

Adipose tissue around the rib cage and abdomen and in the visceral cavity induces both thoracic external compression and cephalic migration of the diaphragm, hence reducing chest volume. Accordingly, there is an exponential relationship between BMI increases (even with modest weight gain) and decreases in FRC and ERV.[36]

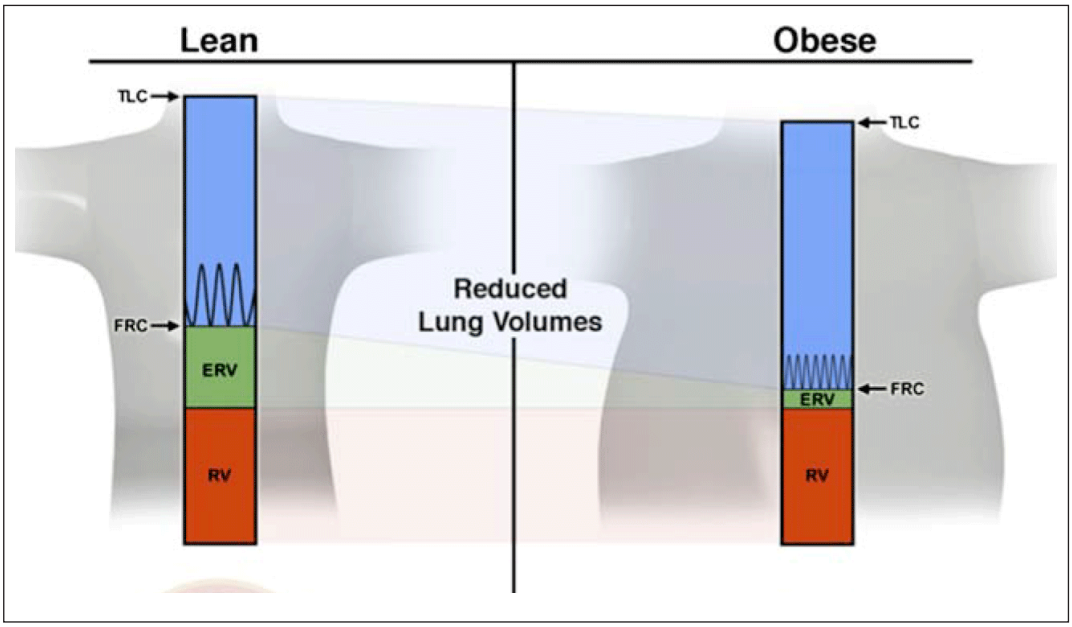


Figure 1. Lung volumes in normal-weight (lean) and obese individuals. Obesity leads to a marked reduction of expiratory reserve volume (ERV) and functional respiratory capacity (FRC). TLC, total lung capacity; RV, residual volume (taken, in part, from ref. 38)

In fact, FRC and ERV decrease approximately 3% and 5%, respectively, for each unit increase in BMI from 20 to 30 kg/m^2; and, above a BMI of 30 kg/m^2, both FRC and ERV decrease approximately 1% for each unit increase in BMI (**Figure 2**).[36] However, the effects of obesity on the extremes of lung volumes, at total lung capacity (TLC) and residual volume (RV), are modest. The TLC reduction is associated with BMI increases but the changes are small, and TLC is usually maintained within the normal range, even in severe obesity.[35] The RV is usually well preserved, and the RV/TLC ratio remains normal or slightly increased.[36]

Spirometric variables, such as forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC), tend to decrease with increasing BMI.[35] However, this effect is small and both FEV_1 and FVC are usually maintained within the normal range in 'healthy' obese adults.[35] The FEV_1/FVC ratio is usually well preserved or increased, even in morbid obesity, indicating that both FEV_1 and FVC are affected to the same extent.[39] This finding implies that the major effect of obesity is on lung volumes without any direct effect on airway obstruction.

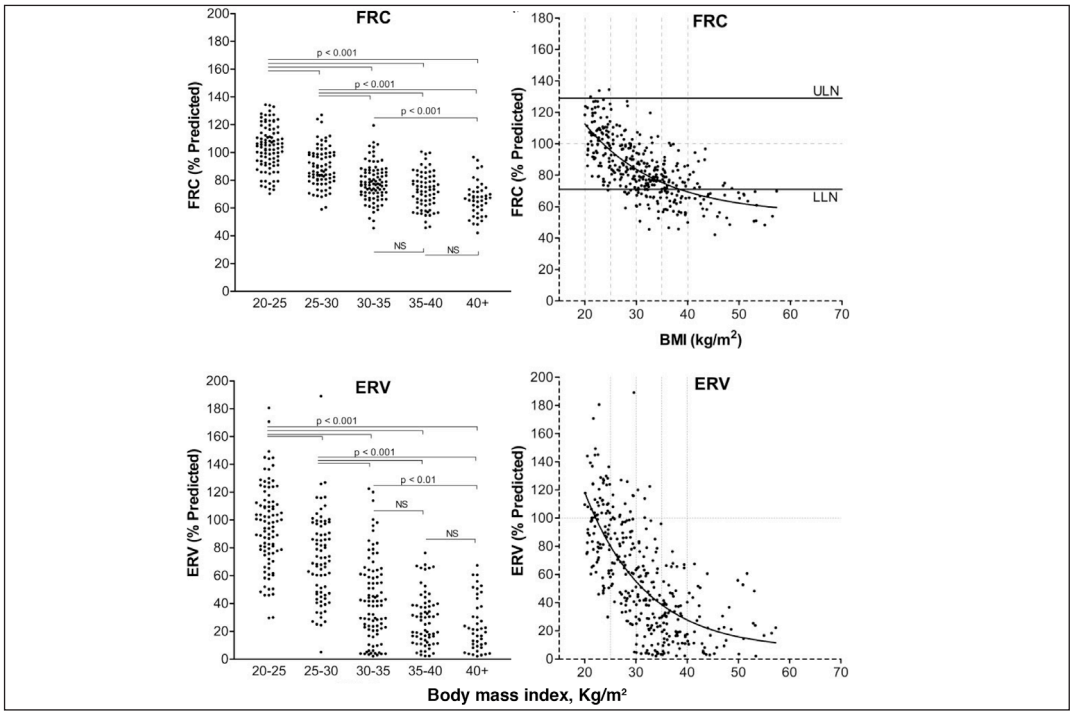


Figure 2. There is an exponential relationship between body mass index increase and the decrease of functional residual capacity (FRC) and expiratory reserve volume (ERV). ULN, upper limit of normal; LLN, lower limit of normal; NS, not significant (taken, in part, from ref. 36)

Moreover, it is important to note that the regional distribution of fat, specifically in central obesity, has an important effect on lung function. Therefore, the larger is WC and WHR, the more marked is the ERV reduction.[40,41] In addition, increases in central obesity also induce a decrease in FEV_1 and FVC, but it does not affect the relationship between the two parameters.[39] According to the latter information, it has been reported a positive, independently of BMI, relationship between lung function impairment and the MS, which is characterized by central obesity and higher levels of systemic inflammation. Likewise, several studies, have reported higher levels of serum biomarkers (leptin and CRP) in subjects with central obesity and lung function impairment.[42]

1.3.2. Pulmonary gas exchange

Severe obesity is associated with impaired pulmonary gas exchange, which may increases the risk of post-operative pulmonary complications and have a deleterious effect on obesity-related multi-morbidities.[40]

Mild increases in the alveolar-arterial oxygen difference ($AaPO_2 \geq 15$ mmHg) and mild arterial hypoxemia (arterial oxygen pressure [PaO_2] < 80 mmHg) are frequently associated with obesity in poorly defined populations.[41,43,44] In a review of 626 morbidly obese subjects at upright, mean PaO_2 (81 mmHg) and $AaPO_2$ (23 mmHg) were 17 mmHg lower and higher each than predicted normal values (PaO_2, 98 mmHg; and $AaPO_2$, 6 mmHg), respectively.[45] Moreover, according to lung volume behavior, PaO_2 and $AaPO_2$ are related to both overall BMI and central obesity (WC and WHR).[40,46] Even though WHR seemed to be better correlated with gas exchange than WC in morbidly obese subjects, the latter outcome has been demonstrated to be the best marker of central obesity. In fact, the increased levels of WC explain 36% of PaO_2 reduction.[40] Consequently, it has been reported that men who have higher incidence of central obesity than women have a poorer gas exchange status than women with the same BMI.[46] Therefore, in severe obese individuals at rest, there is a difference of −10 mmHg difference in PaO_2, of +8 mmHg difference in $AaPO_2$ and of −1% difference in $\%SaO_2$, but not in $PaCO_2$ according to sex, with women showing less gas exchange worsening than males.[45]

The abnormally high $AaPO_2$ and low PaO_2 at rest may be attributed to ventilation-perfusion (V_A/Q) abnormalities due to both basal lung compression and airway closure. On the one hand, the external compression induced by excessive thoracic and intra-abdominal fat, which raises intra-abdominal pressure causing upper migration of the diaphragm, reduces chest volume. This reduction is mainly centered in the lower parts of the lung, which tends to collapse and hence become *atelectatic* (absence of gas). On the other hand, this situation further reduces FRC and promotes airway closure in dependent lung regions.

Airway closure is a normal phenomenon that occurs during expiration, with re-opening of airways during the succeeding inspiration (**Figure 3**).[47] During normal breathing, it is more likely that airways will close during tidal breathing in older normal weight subjects (65–70 yrs) when upright, and even in 50 years-old subjects when supine.[48,49] Moreover, the reduction of FRC promotes airway closure in dependent lung regions and this reduction is more marked in *Trendelenburg* and head-down positions, in which there is a cranial displacement of the diaphragm.

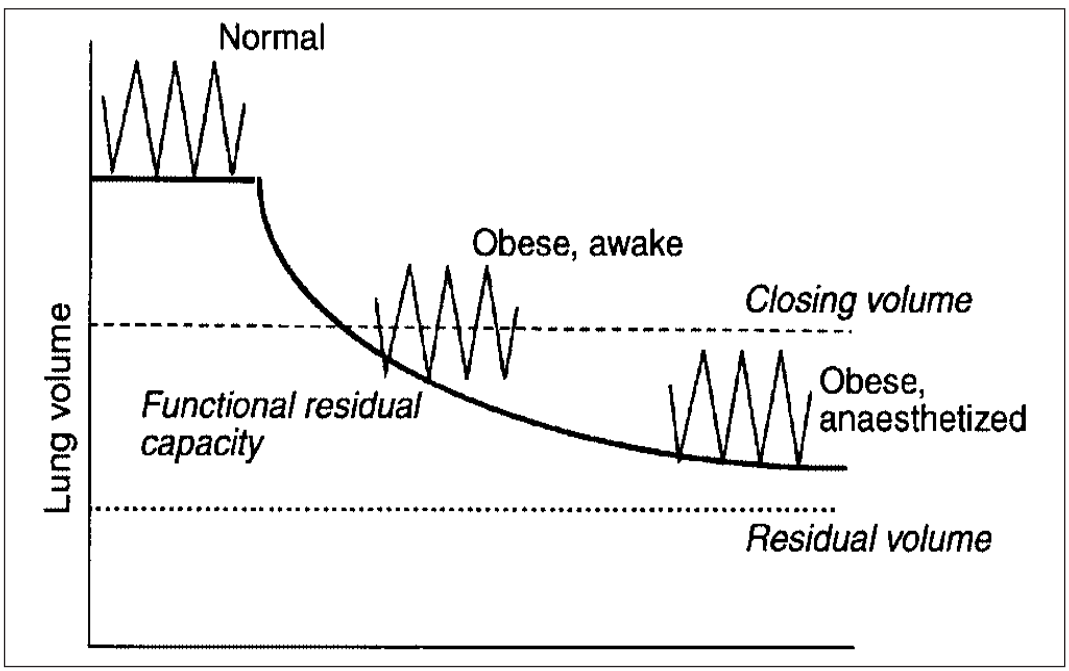


Figure 3. Contrary to normal-weight subjects but similar to anesthesia effect, severe obesity is associated with a reduction in functional residual capacity resulting in airway closure during normal tidal ventilation (taken, in part, from ref. 47)

As it has been previously alluded to, FRC is reduced in obese subjects and airway closure can occur within tidal breathing (**Figure 3**) inducing a decrease in dependent regions ventilation, while pulmonary blood flow continues to go preferentially through dependent regions, hence leading to airway collapse, alveolar *atelectasis* and V_A/Q mismatching.

In the 1960s, *Holley et al.*[50] reported the regional distribution of V_A/Q relationships by using radioactive xenon technique in 8 severe obese subjects (BMI, 39±[SD]4 kg/m^2) when upright. In all subjects the distribution of perfusion was predominantly diverted to the lower lung zones. Four subjects had a normal distribution of ventilation, increasing progressively from the apex to the basal lung areas. However, in the remaining 4 subjects who had a lower ERV (ERV < 0.4 L; 21% of predicted) distribution of ventilation was reversed, being the upper lung regions relatively better ventilated than the lower ones and the dependent zones relatively under-ventilated while inducing the development of airway collapse and *atelectasis*. Two years later, *Barrera et al.*[51] reported oxygen-*shunt* (Q_S/Q_T) measurements in 8 severe obese subjects and suggested that arterial hypoxemia was related to over-perfusion of under-ventilated areas and/or to perfusion of completely non-ventilated areas (*i.e.*, *shunt*). *Farebrother et al.*[43] hypothesized that, in severe obesity, when closing volume (CV) exceeds ERV (ERV-CV is negative), airway closure will occur within the range of tidal breathing so there are greater chances of under-ventilation in dependent lung regions, and hence developing hypoxemia. They observed in a subset of 8 severe obese individuals (4 men; BMI 42 kg/m^2; 7 current smokers), a reduction in ERV below CV and a positive relationship between ERV and PaO_2. Likewise, they demonstrated a close correlation between PaO_2 and the extent of airway closure within the range of tidal breathing, as a mechanism of basal lung hypoventilation, already previously acknowledged by *Holley et al.*[50]

1.3.3. Postural change effects on lung volumes and pulmonary gas exchange

Gravity plays an influential role in the relationship between alveolar ventilation and pulmonary blood flow. In the healthy normal individual, at upright, gravity generates a pleural pressure gradient that increases alveolar ventilation in pulmonary bases. Similar to this, gravity also creates a hydrostatic pressure gradient in pulmonary circulation between the apices and the lower lung regions, which receive a larger volume of blood flow.

Therefore, in healthy subjects postural-induced changes in pulmonary gas exchange from seated to supine postures would result from the interaction of three factors: *1*) the increased cardiac output in the supine position[52,53]; 2) the increased uniformity of perfusion distributions and of regional V_A/Q mismatching in the supine lung; and, *3*) the increased of "airway closure" volume when supine, tending to lower dependent zone V_A/Q imbalance.[44]

In normal-weight subjects, both FRC and CV were almost unchanged when supine so that there were no postural-induced effects on gas exchange.[53] In obese subjects, as we previously alluded to (see, "*Lung volumes"*), both TLC and FRC were both reduced when seated compared to normal-weight subjects. Moreover, it has been demonstrated that morbidly obese subjects suffer a marked FRC reduction induced by high intra-abdominal pressures. However, the latter study was performed in sedated and paralyzed morbidly obese subjects so that the methodology approach did not allow to extrapolate these findings to spontaneously breathing subjects.[37] More recently, *Watson et al.*[54] demonstrated that in obese subjects breathing spontaneously reductions in TLC and FRC were smaller (TLC, −80 ml; FRC, −70 ml, respectively) than in lean subjects (TLC, −190 ml; FRC, − 730 ml; respectively) when moved from upright to supine.[54,55] These findings suggest that increased extra-pulmonary mass load and the reduced compliance of the respiratory system are the presumptive causes of the restrictive lung disease in obese subjects when seated, but apparently they do not increase when supine.[54] Moreover, *Steier et al.*[55] have investigated postural-induced changes in lung volumes and gastric and esophageal pressures in 9 obese (BMI, 47±17 kg/m^2) and 9 normal weight subjects. They observed that FRC was reduced in the obese subjects while seated but was similar between the groups when supine. Thus, from seated to supine FRC decreased about 500 mL in normal-weight and remained unchanged in obese subjects. Moreover, at FRC, end-expiratory esophageal and gastric pressures were higher in the obese group when seated and supine. Both groups have similar increase in esophageal pressure and decrease in gastric

pressure when moved from seated to supine position. In summary, in obese subjects increased intra-abdominal and subsequent increased intra-thoracic pressure, in particular at end-expiration when diaphragm is relaxed, reduces FRC, ERV and pulmonary compliance. Although there is a negative effect of the supine posture on lung volumes in obese individuals, postural changes in intra-abdominal and intra-thoracic pressures become more important in obesity due to reduced ERV, thereby increasing their work of breathing, already increased due to the inspiratory threshold load caused by high intra-thoracic pressures.[55]

In terms of pulmonary gas exchange, there are very few studies in regards to the effect of postural changes in obese individuals. In normoxemic ($PaO_2 \geq 80$ mmHg) obese subjects, PaO_2 is lower when supine than at upright.[43,56] Two studies investigated 8 normoxemic ($PaO_2 \geq 80$ mmHg) obese subjects, each, and observed that PaO_2 and ERV were lower at supine than at upright, both before and after BS. Accordingly, ERV is significantly decreased and the negative value of ERV-CV is significantly greater when supine than at upright.[43] However, in 22 mild hypoxemic obese women, no postural effects on arterial blood gases were observed before and then at the third day after conventional surgery.[44]

The effects of weight loss on lung volumes and arterial blood gases will be addressed below (See "*Effects on lung function").*

Table 6. Pulmonary gas exchange studies using arterial blood gases in obese subjects

Author, year	N Subjects	Main Findings
Farebrother MJ. et al., 1974[43]	8	PaO_2 and ERV were lower at supine than at upright, both before and after weight loss.
Vaughan RW. et al., 1975[44]	22	In mild hypoxemic obese women, there were no postural effects on ABGs before and at the third day after conventional surgery.
Vaughan RW. et al., 1981[82]	64	Significant weight loss is associated with gas exchange improvement. There is a relationship between ERV increase and PaO_2 and $AaPO_2$ improvement.
Thomas PS. et al., 1989[81]	29	Significant weight loss is associated with marked increases in ERV accompanied by slightly but significantly increases in FEV_1 and FVC. Initial hypoxemia improves after weight loss to normal values.
Hakala K. et al., 2000[56]	13	In normoxemic ($PaO_2 \geq 80$ mmHg) obese subjects with mild-to-moderate obstructive sleep apnea syndrome, PaO_2 is lower when supine than in upright posture, before and after weight loss..
*Zavorsky GS. e*t al., 2007[46]	25	Variance in $AaPO_2$ (32%) and PaO_2 (36%) were explained by the WHR. Men had larger WHR ($p < 0.01$) and poorer gas exchange ($p = 0.06$) compared to women
Zavorsky GS.et al., 2008[41]	42	At rest, obese subjects have lower than predicted PaO_2 and higher than predicted $AaPO_2$ compared to normal-weight healthy controls. Morbidly obese men have worse ABGs values than women. Worse gas exchange in men is related to their higher WHR compared to women.
Zavorsky GS. et al., 2008[45]	768	Mean PaO_2 (81 mmHg) and $AaPO_2$ (23 mmHg) were 17 mmHg lower and higher than predicted values (98 mmHg and 6 mmHg, respectively). Significant weight loss is associated with gas exchange improvement. On average, for every 5–6 kg reduction in weight, PaO_2 is increased by 1 mmHg and $AaPO_2$ is reduced by 1 mmHg.
Gabrielsen A-M. et al., 2010[40]	149	There is a weak association between increased BMI (r, −0.28) and WC (r, −0.33) and decreased PaO_2 ($p<0.001$ each). The increased levels of WC explain 36% of PaO_2 reduction

N, Subjects, size of the subset; ERV, expiratory reserve volume; ABGs, arterial blood gases; AaPO2, alveolar to arterial PO_2 difference; FEV_1, forced expiratory volume in 1 s; FVC, functional residual capacity; WHR, waist-to-hip ratio; BMI, body mass index; WC, waist circumference.

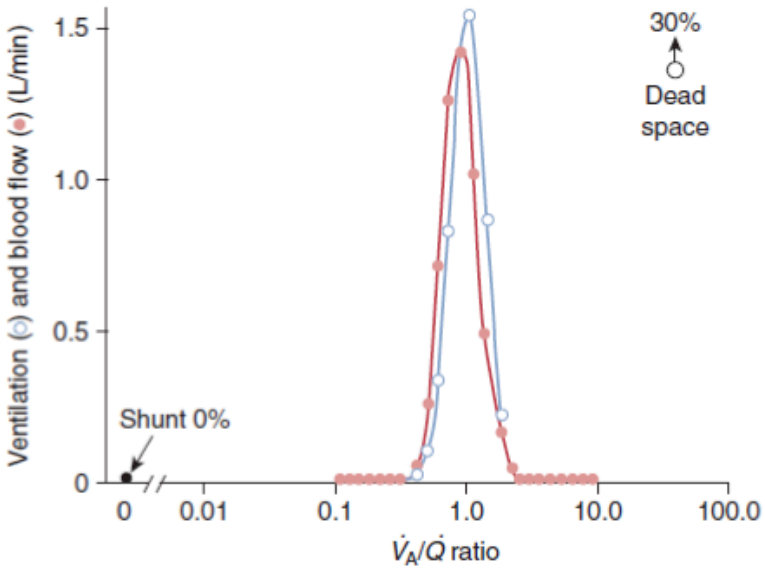
1.3.4. Multiple inert gas elimination technique (MIGET)

The multiple inert gas elimination technique (MIGET) was developed in the mid 1970s to overcome several limitations of the traditional approach of pulmonary gas exchange using physiologic (respiratory) blood gases (oxygen [O_2] and carbon dioxide [CO_2]). It is still nowadays considered the most robust tool to investigate pulmonary gas exchange in human beings. MIGET has three fundamental advantages: (*1*) it gives both quantitative and qualitative estimates of the pulmonary blood flow and alveolar ventilation and calculates the mismatch of V_A/Q relationships; (2) it partitions the $AaPO_2$ into determinants of intrapulmonary *shunt*, V_A/Q imbalance and diffusion limitation to O_2; and, (*3*) it facilitates the unraveling of the complex interplay between pulmonary (namely the three factors in [2]) and nonpulmonary (*i.e.*, inspired PO_2, overall ventilation, cardiac output (Q_T) and O_2 consumption) determinants governing pulmonary gas exchange. It is also important to note its major ability to perform measurements at any fractional inspired oxygen concentration (F_IO_2) without itself modulating the airway caliber or pulmonary vascular tone.[57]

The MIGET uses simultaneously venous infusion of six inert gases in trace concentrations (Sulfurhexafluoride [SF_6], ethane, cyclopropane, enflurane, diethyl ether, and acetone). The range of partition coefficient of the 6 gases allows to identify V_A/Q ratios from zero to 0.005 (SF_6) until 100 to infinitely great (acetone) in the 50 compartments, in which the whole lung is divided. For each inert gas the retention (R) is calculated as the ratio between arterial partial pressure and mixed venous partial pressure and the excretion (E) as the ratio between mixed expired partial pressure and mixed venous partial pressure. By using a multi-compartmental approach the retentions of the 6 inert gases allow the estimation of the pulmonary blood flow distribution, while the excretions of inert gases provide an estimation of the alveolar ventilation distribution.[57,58] The mathematical model of the technique has been also extensively reported.[59]

The most representative outcome of MIGET is a quantitative picture of distribution of

V_A/Q ratios (**Figure 4**).[57] As you can observe, each data point represents a particular amount of blood flow (close circles) or alveolar ventilation (open circles). The sum of the respective data point corresponds to overall pulmonary perfusion and total ventilation, respectively, the connecting lines being drawn for a better visual comprehension. These quantities (distributions) are plotted against a broad range (50) of V_A/Q ratios, from zero (intrapulmonary *shunt*) to above 100 (dead space), on a log scale.



F***igure 4. V_A/Q distributions. The frequency distribution (y axis) of both ventilation (open circles) and perfusion (close circles) in each compartment are plotted against V_A/Q ratio on a logarithmic scale (x axis). Both ventilation and perfusion distributions are centered on a V_A/Q ratio 1.0 narrow and symmetrical. There was no perfusion to low V_A/Q units (between 0.005 to 0.1) or ventilation to high V_A/Q units (>10.0). Note the absence of shunt (taken from ref. 62)***

The graphical representation gives a general overview of the distribution. It identifies domains with low, normal and high V_A/Q ratios and suggests patterns of distribution as unimodal, bimodal or even trimodal. Moreover, to quantify the degree of V_A/Q inequal-

ity the *first moment of distribution* is used, *i.e.* the mean abscissa value of each curve of ventilation (mean V) and perfusion (mean Q) (normal values, 1.0). Likewise, the *second moment of distribution* about its respective means (on a logarithmic scale) named Log SDV and Log SDQ for alveolar ventilation and pulmonary blood flow, respectively, is also commonly used. Normal values are 0.3-0.65 and 0.3-0.60 for Log SDV and Log SDQ, respectively.[60] We define the distributions as broad or well suited if they are located within normal ranges. Furthermore, we can use the fractions of total ventilation and blood flow within defined ranges of V_A/Q ratios. Thus, in normal young subjects, distributions are always located within the spectrum of $V_A/Q = 0.1$ through $V_A/Q = 10.0$. Abnormal low V_A/Q regions indicate an increase of pulmonary blood flow in units whose V_A/Q ratios are located between the range <0.1 and >0.005 (i.e., low V_A/Q units); and, abnormal high V_A/Q regions correspond to an increase of alveolar ventilation in units having V_A/Q ratios >10.0 and <100.0. Thus, an additional advantage of MIGET is the ability to identify the presence of lung units over the V_A/Q ratio 0.005 to 100.0. Thereby, areas with very low ratio (<0.005) (in practice considered zero V_A/Q) are defined as intrapulmonary *shunt*. *Shunt*, can be due to perfusion of unventilated or very poorly ventilated lung regions or directly to vascular communications (right-to-left *shunt*). In a corresponding manner, all ventilation in units of V_A/Q ratio >100.0 or even unperfused (V_A/Q infinitely high) is referred as dead space. Contrary to the venous admixture ratio (based exclusively on the oxygen-*shunt* measurement), MIGET's intrapulmonary *shunt* is insensitive to the presence of *post-pulmonary shunt* (corresponding to both bronchial and *Thebesian* circulations). In addition, inert-gas physiologic dead space is also slightly lower than *Bohr* dead space because it does not include the alveolar units that have an alveolar PCO_2 lower than arterial PCO_2.[58,61,62] Likewise, MIGET algorithm uses the derived V_A/Q distributions to estimate the amount of arterial hypoxemia that V_A/Q inequality should produce.

The inert gas methodology has been used in many categories of disease states, under different clinical conditions and interventions, to both dissect and sort out the relative

importance of all nonpulmonary factors and thus their interaction with key pulmonary governing of gas exchange. From a clinical viewpoint, three are the main mechanisms of altered arterial physiological gases during spontaneous breathing in any pulmonary disease: V_A/Q imbalance, increased intrapulmonary *shunt* and alveolar hypoventilation. The diffusion limitation to O_2 only plays a role in patients with pulmonary fibrosis and in very special situations, such as high altitude.[62] In general terms, V_A/Q inequalities play a main role in chronic lung diseases, namely chronic obstructive pulmonary disease (COPD) and bronchial asthma, which have in common expiratory airflow limitation and large pulmonary volumes. By contrast, increased intrapulmonary *shunt* is the vital component of arterial hypoxemia in conditions of both acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), clinical entities characterized by the development of small lung volumes.[62]

Chronic respiratory diseases

In COPD the principal pulmonary determinants of gas exchange abnormalities encompass different degrees of V_A/Q imbalance, characterized by the increase of the dispersion of blood flow (low V_A/Q ratio areas), alveolar ventilation (high V_A/Q ratios areas) or both, associated with different increased levels of physiologic dead space. These findings are consistent with the underlying principal structural derangement of COPD in the airways and alveolar spaces. It is important to notice that, on the one hand, increased intrapulmonary *shunt* is almost negligible during stable conditions as opposed to dead space, commonly increased.[63] Alternatively, although a reduced diffusing capacity (DL_{CO}) is a common finding at different stages of COPD, so far all inert gas studies have consistently excluded the presence of alveolar to end-capillary diffusion limitation to O_2 (predicted and measured PaO_2 are not significantly different).[63,64] During COPD exacerbations, with or without the need of noninvasive or invasive ventilatory support, the levels of intrapulmonary *shunt* rarely exceed 10% of Q_T. Moreover, the response to high inspired O_2 fractions either during stable or acute COPD increases PaO_2 usually above 450-500 mmHg,

and always deteriorates V_A/Q imbalance, characteristically assessed by a significant increase in the amount of alveolar units with low V_A/Q ratios and, consequently, in the dispersion of pulmonary blood flow (Log SDQ), without affecting intrapulmonary *shunt* nor the dispersion of alveolar ventilation (Log SDV). This further worsens V_A/Q relationships and clearly suggests (indicates) attenuation (release or reversion) of hypoxic pulmonary vasoconstriction (HPV), in spite of the fact that both pulmonary arterial pressure and vascular resistance can remain unaltered.[63]

In stable bronchial asthma, there is an abnormally elevated degree of V_A/Q imbalance, with increases dispersion of pulmonary blood flow (Log SDQ) about twice the normal average, including clinical conditions quite often with relatively well-preserved spirometry. Intrapulmonary *shunt* is always absent or negligible, and the areas with high V_A/Q ratios and *dead space* are within normal limits or reduced even in the most airways obstructed conditions. Nonetheless, during the most life-threatening asthma attacks, there is a predominance of regions with low V_A/Q ratios, because severe airways obstruction produces regions of low V_A/Q units that remain perfused but poorly ventilated. This must increase blood flow dispersion more than ventilation dispersion, other things being equal. Unexpectedly, intrapulmonary *shunt* is absent or very modest due to the efficiency of collateral ventilation and HPV;[63,65] and *dead space* is normal. Breathing 100% oxygen induces mild *shunt* (less than 10% of Q_T) and V_A/Q mismatch substantially deteriorates, as shown by a marked increase of the dispersion of pulmonary blood flow. Once again, this response to high concentrations of O_2 suggests that HPV is released or reversed.

Acute respiratory diseases

In acute respiratory failure (ALI or ARDS), the main cause of arterial hypoxemia is increased intrapulmonary shunt (*i.e.*, approximately 20% or more of Q_T). In some patients there are areas with low V_A/Q ratios, because blood flow is distributed to areas of the lung with reduced ventilation (usually less than 10% of Q_T). This V_A/Q profile reflects that

pulmonary perfusion is diverted to two lung areas: those with ventilation that is normal and proportional to blood flow and those that are unventilated (so-called, an *all or none phenomenon*). *Dead space* is increased in most patients. There is no limitation of diffusion to O_2, as reflected by the close agreement between predicted and measured PaO_2.[66] Interestingly, and at variance with chronic respiratory conditions, during 100% oxygen breathing PaO_2 increases modestly (≤250 mmHg) whereas intrapulmonary *shunt* increases considerably (about a mean of 35% of Q_T) suggesting the development of *reabsorption atelectasis* induced by alveolar denitrogenation.[66] The limited increase in PaO_2, while breathing 100% oxygen, indicates that increased intrapulmonary *shunt* is the fundamental determinant of arterial hypoxemia in ALI or ARDS while the dispersions of blood flow (Log SDQ) and alveolar ventilation (Log SDV) remains essentially unchanged. Thus, the increase in intrapulmonary *shunt* without release (reversion) of HPV (namely, absence of change in the Log SDQ) in severe acute respiratory failure is compatible with the concept that, at any level of inspired O_2 fraction, regions with low V_A/Q ratios cannot redistribute blood flow from areas with *shunt* or very low V_A/Q ratios, as their local vascular resistance remains unvaried.[67]

Table 7. Characteristics of pulmonary gas exchange in common chronic and acute respiratory diseases

Condition	Basal conditions	100% Oxygen effects
COPD	Absence of or mild intrapulmonary *shunt* (≤10% of Q_T	Marked increase in PaO_2 (≥ 450 mmHg)
	Mild-to-severe V_A/Q mismatch (several patterns)	Absence of intrapulmonary *shunt* increase
	Absence of diffusion limitation to O_2	Reversion of HPV
Asthma	Absence of intrapulmonary *shunt*	Marked increase in PaO_2 (≥ 450 mmHg)
	Severe V_A/Q mismatch (uniform pattern)	Mild to moderate increase in shunt
	Absence of diffusion limitation to O_2	Reversion of HPV
ALI/ARDS	Severe intrapulmonary *shunt* (≥20% of Q_T)	Moderate increase in PaO_2 (≤ 250 mmHg)
	Absent of mild V_A/Q mismatch	Mild to moderate increase in shunt
	Absence of diffusion limitation to O_2	Absence of HPV reversion

COPD, Chronic obstructive pulmonary disease, ALI, acute lung injury; Log SDQ, dispersion of pulmonary blood flow; Log SDV, dispersion of alveolar ventilation; Q_T, cardiac output; HPV, hypoxic pulmonary vasoconstriction (taken, in part, from refs. 62 and 63).

1.4. Bariatric Surgery

There are currently no truly effective pharmaceutical agents to treat obesity, especially in the morbidly obese individual.[68,69] Moreover, diet therapy, with and without the support of health-care organizations, is relatively ineffective in treating severe obesity in the long-term. In 1991, the *National Institutes of Health* established the indications of BS in morbid obesity (BMI ≥ 40 or BMI ≥ 35 kg/m^2 in the presence of significant multi-morbidities).[70]

Bariatric surgery is the best available therapy to achieve a sustained and significant weight loss in severe obese subjects in whom the efforts of a medical therapy have failed.[71] Bariatric surgery is considered successful if the postoperatively percentage of excess body weight loss (EWL) calculated as follows: [(initial BMI - final BMI)/(initial BMI - 25)]*100) is equal to or higher than 50%.[71,72]

Both laparoscopic Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) are currently the most effective and widely used surgical techniques,[70,73,74] both with similar outcomes in achieving a successful weight loss, resolution or amelioration of multi-morbidities, and peri-operative complications in short-term studies (one year follow-up after BS).[70,71] Likewise, both have shown better results than gastric banding or gastroplasty.[70]

In RYGB, the surgical technique consists of the creation of a small gastric pouch (15 mL) along the lesser curvature of the stomach, and the connection of a 100-cm Roux-en-Y limb as an entero-enterostomy to the jejunum 50 cm from the ligament of Treitz. The resultant gastric-pouch-jejunal anastomosis is about 1 to 1.5 cm in diameter[75] (**Figure 5A**).[76] It is a restrictive and malabsortive technique. In SG, the greater curvature including the complete fundus is resected from the distal antrum (5 cm proximal to the pylorus) to the angle of His. A laparoscopic stapler is used to divide the stomach parallel to and alongside a 46-50 French bougie placed against the lesser curvature of the stomach[77] (**Figure 5B**).[76]

Although SG is regarded as a restrictive procedure, it is increasingly recognized as a metabolic apporach[70,78] and, after one year, it leads to greater EWL than RYGB (69.7 ± [SD] 14.6 *versus* 60.5 ± 10.7%; p = 0.05).[72,79] However, after a median follow-up of 4.5 years, this effect disappears and higher weight regain is associated with SG, leading more commonly to a final EWL <50 %, as compared to RYGB.[80]

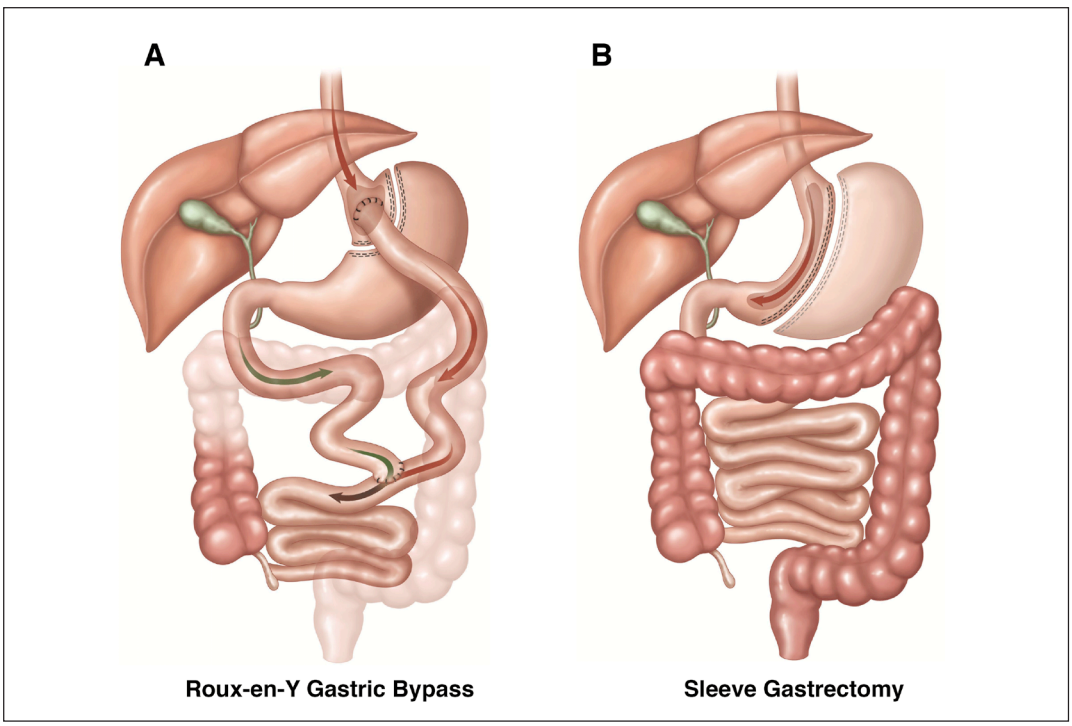


Figure 5. Most common surgical techniques of bariatric surgery (taken, in part, from ref. 76)

Bariatric surgery strengths are based on two major outcomes. The first one, is the effective weight loss achieved, which is able to induce (*1*) a marked amelioration or resolution of diabetes, hyperlipidemia, systemic arterial hypertension, OSA; and (*2*) the reduction of the relative risk of death by 89% (95% CI, 73% - 96%) after 5-years follow-up, with an absolute mortality reduction of 5.5% (p <001).[78] The second one is the low peri-operative morbidity and mortality. Mortality at 30 or less days is 0.28% (95% CI, 0.22 - 0.34) in subjects undergoing sleeve, 0.5% for gastric bypass procedures, and 1.1% in bilio-pancreatic diversion or duodenal switch. Mid-term (between 30-days to 2-years) overall mortality of

BS is also less tan 1% (0.35% [95% CI, 0.12 - 0.58). Analyses of subgroups yield clear verification that the very obese subjects (BMI ≥ 50kg/m^2), men and the elderly have a higher mortality risk.[73] Moreover, higher mortality and post-operative complications in hospitals with a low rate of BS procedures has also been reported (less than 50 BS procedures per year).[68]

1.4.1. Effects on lung function

Successful BS (EWL ≥ 50%) is associated with marked increases in FRC and ERV accompanied by slight but significantly increases in FEV_1 and FVC. Consequently, significant weight loss is associated with gas exchange improvement[81] such that on average for every 5–6 kg reduction in weight, PaO_2 was increased by 1 mmHg and $AaPO_2$ was reduced by 1 mmHg, respectively.[41] Moreover, a relationship between WC and $AaPO_2$ decreases and between ERV increases and PaO_2 and $AaPO_2$ improvements has been shown, indicating that ERV increases from surgical weight loss because of the reduction in WC.[43,81]

1.5. High resolution thoracic computed tomography

Thoracic imaging with computed tomography (CT) scanning is considered the gold standard for evaluation of pulmonary structure and lung volumes.[83] In 1995, the definition of *atelectasis* as non-aerated lung volume (NALV) with a threshold density in CT scan between – 100 to + 100 HU interval was established. This density analysis allows the quantification of *atelectasis* in regards to the total lung area. Using this density analysis, several studies have shown that impaired gas exchange in morbidly obese individuals during general anesthesia is related to higher development of *atelectasis* than the lower percentage observed in lean subjects. In addition, these areas of *atelectasis* resolve more slowly in the post-operative period in obese subjects.[84] Moreover, other studies were addressed to investigate different ventilation strategies during general anesthesia in obese subjects who undergo BS in order to minimize *atelectasis* development.[85–87] Thus, it was

shown that the application of positive end-expiratory pressure (PEEP) at the induction[85] as well as, the use of recruitment manoeuvres followed by PEEP during general anesthesia[86,87] are effective procedures to prevent, or decrease, *atelectasis* formation in morbidly obese patient.

Recently, a new approach of non-contrast thoracic CT imaging to quantify change in pulmonary vascular volume was described to evaluate and assess the concept of lung tissue volume (*Vtiss*). This technique describes the volume of the lung not occupied by air. If large airways and vessels are excluded from the overall measurement, *Vtiss t*herefore represents the volume occupied by parenchymal tissue and pulmonary small vessels and capillaries.[88] Lung tissue volume is estimated as previously described:

Vtiss = Lung volume x (1 + CT number /1,000).[89]

Changes in *Vtiss* reflect all fluid changes within the lung including blood volume and interstitial fluids. Accordingly, *Vtiss* accounts for increases in pulmonary capillary blood volume and in the segmented vascular volume. The rest of the increase in lung tissue volume is likely induced by an increase of fluid in the interstitial space. By using non-contrast CT scanning to unravel the individual contributions of pulmonary blood and parenchymal tissue,[90] it was possible to demonstrate an association between cross-sectional area of pulmonary vessels with lung function[91] or pulmonary hemodynamics in selected cohorts of patients.[92]

1.6. Summary:

- Obesity is recognized as a state of low-grade chronic inflammation induced by the increase of pro-inflammatory adipocytokine and mediators along with a decreased synthesis of anti-inflammatory adipocytokine (adiponectin). This reduced production of adiponectin inhibits the vasorelaxant properties of perivascular adipose tissue through increased oxidative stress, hence inducing widespread endothelial dysfunction.

- The principal effects on lung function of severe obesity not associated with chronic respiratory multi-morbidities encompass a marked decrease of expiratory reserve volume together with a mild reduction in functional residual capacity. These lung volume decrements are associated with mild pulmonary gas exchange abnormalities, namely decreased arterial PO_2 and increased alveolar-to-arterial PO_2.

- The principal mechanism of abnormal gas exchange has been linked indirectly to ventilation-perfusion imbalance related to the presence of under-ventilated and over-perfused areas in low lung regions induced by the diaphragm compression and the development of airway closure within the tidal volume. However, the latter ventilation-perfusion mismatching has been only demonstrated with the use of old thoracic scintigraphic images while breathing ambient air. The effects of 100% oxygen breathing were never explored.

- Lung tissue volume, namely the volume occupied by parenchymal tissue and pulmonary small vessels, assessed by thoracic computed tomography has been related to lung function or pulmonary hemodynamics in a few chronic respiratory conditions but not in severe obesity.

- The effects of postural changes on gas exchange in obese individuals remain insufficiently explored. Very few studies, with modest conventional methodology and

without adequate comparisons of control subjects, have been published.

- Bariatric surgery is the best available therapy to achieve a sustained and significant weight loss in severe obese subjects with improvements in lung volumes and pulmonary gas exchange, but assessed only with conventional arterial blood gases.

Several questions, however, remain to be answered:

- Which is the most characteristic pattern of pulmonary gas exchange abnormalities in the presence of severe obesity not associated with chronic respiratory multi-morbidities, i.e. the roles played by ventilation-perfusion imbalance, increased intrapulmonary *shunt* and/or alveolar-capillary diffusion limitation.

- Which are the effects of breathing 100% oxygen and of postural changes on pulmonary gas exchange abnormalities in severe obesity.

- Which are the effects on thoracic computed tomography and more specifically on lung tissue volume.

- Which are the overall long-term effects of bariatric surgery on pre-operative pulmonary gas exchange abnormalities (at basal conditions), as well as those induced by changes in oxygen breathing and in posture.

2. HYPOTHESES

HYPOTHESES

Severe obesity is associated with impaired pulmonary gas exchange, which may be due to pulmonary inflammation related to low-grade chronic systemic inflammation and also to lung compression induced by the excess of thoracic and intra-abdominal adipose tissue. Bariatric surgery would reverse all these aspects including systemic and pulmonary inflammation and normalize pulmonary gas exchange.

First Manuscript

Severe obesity is associated with pulmonary gas exchange disturbances while breathing ambient air, more specifically abnormal ventilation-perfusion ratio distributions and an abnormal pulmonary vasculature, likely with subsequent development of reabsorption *atelectasis* during oxygen breathing.

Bariatric surgery could not only reduce body mass index and systemic inflammation but also improve overall pulmonary gas exchange abnormalities.

Second Manuscript

Recumbency would aggravate these pulmonary gas exchange abnormalities due to increased intrapulmonary *shunt* and further ventilation-perfusion imbalance other factors being equal.

Bariatric surgery could reverse these postural-induced effects on pulmonary gas exchange abnormalities.

Third Manuscript

The increase of pulmonary perivascular adipose tissue and changes in extra-vascular lung water likely resulting from the low-grade chronic systemic and lung inflammation could be reflected by changes in lung tissue volume and in the cross-sectional area of pulmonary vessels measured by thoracic computed tomography.

3. OBJECTIVES

OBJECTIVES

The overall objective of the current thesis is centered on the most comprehensive investigation of the pulmonary and nonpulmonary determinants of gas exchange, using the multiple inert gas elimination technique, in severe obesity before and after bariatric surgery. Complementarily, clinical, biomarkers and imaging data were also assessed. This overall principal objective was sub-divided into three sub-studies, each with their respective specific objectives:

First Manuscript

- **First objective:** To investigate pulmonary gas exchange abnormalities using the multiple inert gas elimination technique while breathing ambient air and 100% oxygen to further explore the pulmonary vascular response.

- **Second objective:** To assess the long-term effects of bariatric surgery on the previous outcomes and their potential interaction.

Second Manuscript

- **First objective:** To investigate the determinants of pulmonary gas exchange abnormalities breathing ambient air in severe obese individuals when upright and supine.

- **Second objective:** To assess the effects of bariatric surgery on postural-induced pulmonary gas exchange abnormalities.

Third Manuscript

- **First objective:** To assess lung tissue volume and pulmonary vascular volumes using thoracic computed tomography in morbidly obese, before and after bariatric surgery.

- **Second objective:** To assess the effects of bariatric surgery on lung tissue volume.

4. RESULTS

RESULTS

4.1. Manuscript 1. *Ventilation/perfusion distribution abnormalities in morbidly obese subjects before and after bariatric surgery*

Authors**: Rivas, E**, Arismendi, E, Agustí, A, Sanchez, M, Delgado, S, Gistau, C, Wagner, PD, Rodriguez-Roisin, R

Chest 2015; 147: 1127-1134. doi: 10.1378/chest.14-1749.

Impact Factor: **7.483**

Cited by 3 articles

4.2. Manuscript 2. *Postural effects on pulmonary gas exchange abnormalities in severe obesity before and after bariatric surgery***.**

Authors: **Rivas, E,** Arismendi, E, Agustí, A, Gistau, C, Wagner, PD, Rodriguez-Roisin, R.

Minerva Anestesiologica 2015 Jun 9 [Epub ahead of print]. PMID: 26054299.

Impact Factor: **2.134**

4.3. Manuscript 3. *Lung tissue volume is elevated in obesity and reduced by bariatric surgery.*

Authors: Santos, A (*) and **Rivas, E** (*), Rodríguez-Roisin, R, Sánchez, M, Ruiz-Cabello, J, Arismendi, E, Venegas, JG.

(*): Arnoldo Santos and Eva Rivas contributed equally to this article.

Obesity Surgery 2016 March 21 [Epub ahead of print]. doi: 10.1007/s11695-016-2137-9.

Impact Factor: **3.747**

4.1. Manuscript 1. *Ventilation/perfusion distribution abnormalities in morbidly obese subjects before and after bariatric surgery*

Authors**: Rivas, E**, Arismendi, E, Agustí, A, Sanchez, M, Delgado, S, Gistau, C, Wagner, PD, Rodriguez-Roisin, R

Chest 2015; 147: 1127-1134. doi: 10.1378/chest.14-1749.

Impact Factor: **7.483**

Cited by 3 articles

[Original Research **Pulmonary Physiology**]

CHEST

Ventilation/Perfusion Distribution Abnormalities In Morbidly Obese Subjects Before and After Bariatric Surgery

Eva Rivas, MD; Ebymar Arismendi, MD; Alvar Agustí, MD; Marcelo Sanchez, MD; Salvadora Delgado, MD; Concepción Gistau, MsC; Peter D. Wagner, MD; and Roberto Rodriguez-Roisin, MD

BACKGROUND: Obesity is a global and growing public health problem. Bariatric surgery (BS) is indicated in patients with morbid obesity. To our knowledge, the effects of morbid obesity and BS on ventilation/perfusion ($\dot{V}_A/\dot{Q}$) ratio distributions using the multiple inert gas elimination technique have never before been explored.

METHODS: We compared respiratory and inert gas ($\dot{V}_A/\dot{Q}$ ratio distributions) pulmonary gas exchange, breathing both ambient air and 100% oxygen, in 19 morbidly obese women (BMI, 45 kg/m^2), both before and 1 year after BS, and in eight normal-weight, never smoker, age-matched, healthy women.

RESULTS: Before BS, morbidly obese individuals had reduced arterial Po_2 (76 $\pm$ 2 mm Hg) and an increased alveolar-arterial Po_2 difference (27 $\pm$ 2 mm Hg) caused by small amounts of shunt (4.3% $\pm$ 1.1% of cardiac output), along with abnormally broadly unimodal blood flow dispersion (0.83 $\pm$ 0.06). During 100% oxygen breathing, shunt increased twofold in parallel with a reduction of blood flow to low $\dot{V}_A/\dot{Q}$ units, suggesting the development of reabsorption atelectasis without reversion of hypoxic pulmonary vasoconstriction. After BS, body weight was reduced significantly (BMI, 31 kg/m^2), and pulmonary gas exchange abnormalities were decreased.

CONCLUSIONS: Morbid obesity is associated with mild to moderate shunt and $\dot{V}_A/\dot{Q}$ imbalance. These abnormalities are reduced after BS. CHEST 2015; 147(4):1127-1134

Manuscript received July 16, 2014; revision accepted September 15, 2014; originally published Online First October 9, 2014.

ABBREVIATIONS: A-aPo_2 = alveolar-arterial Po_2 difference; BS = bariatric surgery; D_{LCO} = diffusing capacity of the lung for carbon monoxide; ERV = expiratory reserve volume; Log SDQ = dispersion of blood flow distribution; Log SDV = dispersion of alveolar ventilation distribution; MIGET = multiple inert gas elimination technique; $\dot{Q}t$ = cardiac output; $\dot{V}_A/\dot{Q}$ = ventilation/perfusion

AFFILIATIONS: From Servei d'Anestesiologia (Dr Rivas); Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and Fundació Clínic per a la Recerca Biomédica (FCRB) (Drs Rivas, Arismendi, Agustí, Sanchez, Delgado, and Rodriguez-Roisin); Servei de Pneumologia (Institut Clínic del Tòrax [ICT]) (Drs Agustí and Rodriguez-Roisin and Ms Gistau); CIBER Enfermedades Respiratorias (CIBERES) (Drs Arismendi, Agustí, and Rodriguez-Roisin and Ms Gistau); Centre de Diagnòstic per la Imatge (CDI) (Dr Sanchez); Servei de Cirurgia Gastrointestinal (Dr Delgado), Hospital Clínic, Universitat de Barcelona, Barcelona, Spain; and the Department of Medicine (Dr Wagner), University of California, San Diego (UCSD), San Diego, CA.

Part of this study has been presented in abstract form at the American Thoracic Society Annual Meeting, May 21, 2014, San Diego CA.

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Obesity has become a global and rising public health challenge, affecting millions of adults and children. Current estimates indicate that > 12% of the world population is obese, as defined by a BMI > 30 kg/m^2, and that this figure is on the rise.[1,2] Bariatric surgery (BS) causes a significant and sustained reduction of BMI, with minor morbidity and mortality, in morbidly obese subjects.[3,4]

Many previous studies have investigated the effects of obesity on lung function. The excess adipose tissue in the abdomen and around the rib cage reduces functional residual capacity, as shown by a marked decrease in the expiratory reserve volume (ERV).[5] In addition, a widened alveolar-arterial Po_2 difference (A-aPo_2) is frequently reported in morbidly obese subjects.[6] However, to our knowledge, except for radioactive measurements of regional ventilation/perfusion ($\dot{V}A/\dot{Q}$) distributions in a few obese subjects,[7] the effects of obesity on $\dot{V}A/\dot{Q}$ relationships and the potential influence of BS have not before been reported.

We hypothesized that (1) morbid obesity is associated with abnormal $\dot{V}A/\dot{Q}$ ratio distributions and (2) BS reduces them. To test this hypothesis, we used the multiple inert gas elimination technique (MIGET) in morbidly obese individuals, before and after BS, breathing ambient air and 100% oxygen to further explore the pulmonary vascular response. The results of this study have been reported previously in abstract form.[8]

Materials and Methods

Participants, Study Design, and Ethics

Morbidly obese BS candidates (BMI ≥ 40 kg/m^2 or ≥ 35 kg/m^2, with obesity-related comorbidities) were recruited prospectively and studied 24 h prior to and 1 year after BS (median, 51 weeks). Exclusion criteria were the presence of moderate to severe sleep apnea[9] (by polysomnography) and other chronic respiratory (asthma, COPD, bronchiectasis), cardiovascular, and/or mental illnesses. Normal-weight, sex- and age-matched never smokers were enrolled and served as control subjects. Obese subjects and control subjects were studied while seated at rest, during ambient air and 100% oxygen breathing (30 min each), in random order, after they had refrained from any medication during the prior 24 h. One hundred percent oxygen breathing, inert gas, and hemodynamic measurements were not determined in control individuals. All participants signed informed consent. The study was approved by the ethics committee of the Hospital Clínic (Protocol 2008/4015).

Measurements

Lung Function: Forced spirometry (before and after bronchodilation), static lung volumes by body plethysmography, and single-breath diffusing capacity of the lung for carbon monoxide (DLCO) (Master Screen Body; Jaeger, CareFusion) were determined before and after BS according to international guidelines. Reference values were those of a Mediterranean population.[10-12]

Respiratory Gas Exchange: Arterial and mixed venous blood sample gases were analyzed in duplicate for pH, Po_2, and Pco_2 (Ciba Corning 800), and A-aPo_2 values were calculated using a standard formula.[13] Oxygen uptake and CO_2 production were calculated from mixed expired fractions of oxygen and CO_2 (Medical Graphics Corporation), respectively. Minute ventilation was measured using a Wright spirometer and corrected to body temperature and pressure saturated (Respirometer MK8; BOC Healthcare).

Hemodynamic Measurements: Heart rate and systemic and pulmonary arterial pressures were continuously monitored (HP 1001A-1006A monitor; Hewlett-Packard Company) as previously described.[13] Systemic and pulmonary vascular resistances were calculated according to standard formulae.

$\dot{V}A/\dot{Q}$ Distributions: The MIGET was used to estimate the distributions of $\dot{V}A/\dot{Q}$ ratios within the 24 h prior to surgery, as reported previously.[13,14] To calculate these, we used arterial, mixed venous, and mixed expired inert gas concentrations, and cardiac output ($\dot{Q}t$) determined by thermodilution, in obese patients with Pao_2 < 80 mm Hg (range, 55-79 mm Hg; n = 13), whereas they were estimated without mixed venous sampling, and $\dot{Q}t$ was determined by bioimpedance (PhysioFlow; Manatec Biomedical), as described previously[15] in those with normal Pao_2 (range, 82-97 mm Hg; n = 6).

Circulating Inflammatory Biomarkers: Serum samples were obtained by centrifugation of venous blood and were stored at −80°C until analysis. C-reactive protein was quantified using an immunoturbidimetry method (Advia Chemistry; Siemens AG) and leptin, adiponectin, soluble tumor necrosis factor-receptor 1, and IL-8 serum levels were measured using an enzyme-linked immunosorbent assay (Diagnostics Biochem Canada Inc, US BIOLOGIC, IBL International, and Anogen), respectively.

Statistical Analysis

Results are presented as mean ± SE, median, or percentage, as appropriate. To compare obese and control subjects, we used the unpaired Student *t* test (or the Mann-Whitney test for nonnormally distributed data) and the χ^2 test. Obese individuals before and after surgery were compared using the paired Student *t* test (or the Wilcoxon test for nonnormally distributed data) and the McNemar test. Pearson and Spearman tests were used, as appropriate, to explore bivariate correlations among variables of interest. A *P* value < .05 was considered statistically significant.

Results

Characterization of Participants

We studied 19 middle-aged, morbidly obese women (17 never smokers and two former smokers) and eight normal-weight, never smoker, healthy women. Table 1 presents their main demographic, serum biomarker, and lung function values at recruitment. As expected, both BMI and waist circumference were greater in obese than in control subjects. The prevalence of arterial hypertension (42% vs 0%), diabetes mellitus (26% vs 0%), and metabolic syndrome (37% vs 0%) ($P < .05$ each) was also higher in obese participants. The apnea/hypopnea index in obese individuals was 10 ± 2/h. All serum biomarker concentrations except adiponectin were significantly higher in obese than in control individuals (Table 1).

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TABLE 1] Demographic, Inflammatory Biomarker, and Lung Function Findings

Outcomes	Control Individuals (n = 8)	*P* Value[a]	Obese Individuals (n = 19)		*P* Value[b]
			Before BS	After BS	
Age, y	50 ± 3	.98	51 ± 2	52 ± 2	< .001
Weight, kg	62 ± 4	< .001	114 ± 3	78 ± 2	< .001
BMI, kg/m^2	25 ± 2	< .001	45 ± 1	31 ± 1	< .001
Waist circumference, cm	82 ± 5	< .001	125 ± 2	97 ± 3	< .001
Waist to hip ratio	0.83 ± 0.03	.08	0.90 ± 0.01	0.88 ± 0.02	.48
Leukocytes, 10^9/L	6.7 (5.1-7.4)	.18	7.7 (5.5-9.3)	6.4 (5.0-7.0)	.005
Neutrophils, %	64 (49-68)	.66	63 (57-70)	57 (51-61)	.002
C-reactive protein, mg/L	2.5 (0.8-4.5)	.001	10.5 (8.0-14.1)	1.4 (1.0-3.7)	< .001
Fibrinogen, mg/dL	255 (240-330)	< .001	400 (350-460)	345 (303-350)	.002
Leptin, ng/mL	19.2 (8.8-37.2)	< .001	59.3 (50.6-69.4)	21.9 (14.5-31.2)	< .001
Adiponectin, μg/mL	21.9 (15.6-24.3)	.05	11.8 (7.1-21.1)	21.0 (13.1-26.2)	.009
sTNF-R1, ng/mL	0.6 (0.5-0.8)	.005	1.2 (1.0-1.4)	0.8 (0.5-1.2)	.008
IL-8, pg/mL	2.0 (2.0-2.0)	.03	3.6 (2.0-22.1)	2.0 (2.0-22.5)	.83
Pao_2, mm Hg	87 ± 1	.001	76 ± 2	89 ± 2	< .001
$Paco_2$, mm Hg	37 ± 1	.29	39 ± 1	40 ± 1	.40
A-aPo_2, mm Hg	18 ± 1	.002	27 ± 2	15 ± 2	< .001
pH	7.42 ± 0.01	.74	7.41 ± 0.01	7.41 ± 0.01	.45
FVC, % predicted	98 ± 2	.10	91 ± 3	105 ± 3	< .001
FVC, L	3.25 ± 0.11	.26	3.00 ± 0.14	3.31 ± 0.11	< .001
FEV_1, % predicted	96 ± 3	.69	94 ± 4	105 ± 2	< .001
FEV_1, L	2.48 ± 0.05	.71	2.43 ± 0.12	2.62 ± 0.10	.001
FEV_1/FVC, %	77 ± 1	.09	81 ± 1	79 ± 1	.12
FRC, % predicted	101 ± 9	.003	77 ± 3	103 ± 4	< .001
FRC, L	2.91 ± 0.26	.002	2.18 ± 0.08	2.89 ± 0.12	< .001
ERV, % predicted	95 ± 16	.001	40 ± 6	106 ± 8	< .001
ERV, L	1.01 ± 0.17	< .001	0.41 ± 0.07	1.04 ± 0.08	< .001
TLC, % predicted	97 ± 3	.06	90 ± 2	101 ± 3	< .001
TLC, L	5.20 ± 0.24	.10	4.76 ± 0.14	5.27 ± 0.18	< .001
Dlco, % predicted	82 ± 2	.05	90 ± 2	95 ± 3	.07
Dlco, mL/min/mm Hg	18.8 ± 0.5	.10	20.6 ± 0.6	21.7 ± 0.9	.08

Data are presented as mean ± SE or median (interquartile range) unless indicated otherwise. A-aPo_2 = alveolar-arterial Po_2 difference; BS = bariatric surgery; Dlco = diffusing capacity of the lung for carbon monoxide; ERV = expiratory reserve volume; FRC = functional residual capacity; sTNF-R1 = soluble tumor necrosis factor-receptor 1; TLC = total lung capacity.

[a]*P* values for comparisons between control and obese individuals before surgery.

[b]*P* values for comparisons between pre- and postoperative conditions in obese individuals.

Findings Before BS

Ambient Air Breathing: All dynamic and static lung volume (except ERV) and Dlco values in obese participants were lower than in control subjects but were still within their normal reference value range (Table 1). Bronchodilator response was negative. Compared with control subjects, obese participants had enlarged A-aPo_2 and lower Pao_2, with normal $Paco_2$ and pH. In obese subjects, the mean shunt ($\dot{V}A/\dot{Q}$ ratios < 0.005) was mildly to moderately increased (range, 1% to 16% $\dot{Q}t$), and the dispersion of pulmonary blood flow distribution (Log SDQ) (upper normal limit ≤ 0.60)[16] was abnormally broad, albeit unimodal (range, 0.43-1.35) (Fig 1), with negligible amounts of low $\dot{V}A/\dot{Q}$ ratio areas (< 0.1, excluding shunt) (1.5% ± 0.5% $\dot{Q}t$) (Table 2). The dispersion of alveolar ventilation distribution (Log SDV) (upper normal limit ≤ 0.65) was also unimodal and abnormally broad (range, 0.45-0.98),[16] and alveolar units

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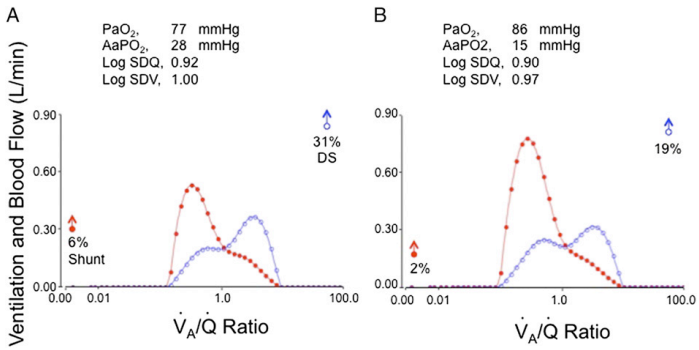

Figure 1 – *Ambient air. Distributions of alveolar ventilation (open blue circles) and pulmonary blood flow (solid red circles) plotted against $\dot{V}_A/\dot{Q}$ ratio from a representative, mildly hypoxemic, obese participant (age, 56 y; BMI, 42 kg/m²) during ambient air breathing. A, Before bariatric surgery (BS). B, After BS. Before surgery, the pattern of $\dot{V}_A/\dot{Q}$ ratio distributions was abnormally broadly unimodal, with mild to moderate shunt. Note that after surgery (BMI, 29 kg/m²), shunt decreases without noticeable changes in Log SDQ and Log SDV. $AaPO_2$ = alveolar-arterial Po_2 difference; DS = dead space; Log SDQ = dispersion of blood flow distribution; Log SDV = dispersion of alveolar ventilation distribution; $\dot{V}_A/\dot{Q}$ = ventilation/perfusion.*

with $\dot{V}A/\dot{Q}$ ratios > 100, expressed as a percentage of alveolar volume (dead space) were reduced. A global index of $\dot{V}A/\dot{Q}$ inequality that combines Log SDQ and Log SDV (DISP R-E*),[17] was moderately to severely increased (9.2 ± 0.7; range, 4.0-15.4) (upper normal limit ≤ 3.0). There was no oxygen diffusion limitation, as reflected by the close agreement between predicted Pao_2 by the MIGET and measured Pao_2. The median residual sum of squares, the descriptor of the quality of MIGET results, was 2.3, below the expected value of 5.4, indicating a high quality of inert gas data.[14]

Ventilatory, systemic and pulmonary hemodynamics, and metabolic variables were within normal limits in all obese participants (Table 2). We observed that the waist to hip ratio correlated with Pao_2 (ρ, −0.72), A-aPo_2 (ρ, 0.67), and shunt (ρ, 0.57) ($P < .05$ each) (Fig 2); likewise, waist circumference was correlated with shunt (ρ, 0.57; $P < .02$). As expected, shunt was correlated with both Pao_2 (ρ, −0.70) (Fig 2) and A-aPo_2 (ρ, 0.70) ($P < .002$ each). Similarly, a lower ERV was associated with more arterial hypoxemia (ρ, 0.52; $P < .05$).

100% Oxygen Breathing: On the on hand, 100% oxygen breathing increased arterial and mixed venous Po_2 and A-aPo_2 (that already reached full equilibration at 15 min), without changes in $Paco_2$, pH, or ventilatory parameters, and systemic arterial pressure and systemic vascular resistance. In parallel, 100% oxygen breathing reduced $\dot{Q}t$, cardiac index, pulmonary artery pressure, and pulmonary vascular resistance (Table 2). Moreover, shunt increased twofold as compared with ambient air, an increase that was inversely related to the reduction of Log SDQ (Fig 3) and the increase in Pao_2 ($r = -0.63$, $P < .01$).

Findings After BS

Two standard BS procedures, sleeve gastrectomy (n = 13) and Roux-en-Y gastric bypass (n = 6) were used as clinically indicated. There were no differences in any of the outcomes using the two procedures. All obese individuals were discharged from hospital after BS without complications. BMI decreased from 45 ± 1 kg/m² to 31 ± 1 kg/m² ($P < .001$), representing a percentage of excess weight loss, a common surrogate marker of BS success, of 70% ± 4%.[4] Arterial hypertension (except in two subjects), diabetes mellitus, and metabolic syndrome were resolved in all obese individuals. All inflammatory serum biomarkers except adiponectin decreased significantly, although fibrinogen, leptin, and soluble tumor necrosis factor-receptor 1 levels remained slightly elevated (like BMI) after surgery (Table 1).

Ambient Air Breathing: As compared with preoperative conditions, forced spirometry, static lung volumes, $Dlco$, Pao_2, and A-aPo_2improved significantly, without changes in $Paco_2$ and pH (Tables 1, 2). Similarly, most of the abnormal $\dot{V}A/\dot{Q}$ descriptors improved, as shown by the significant decreases in intrapulmonary shunt without changes in Log SDQ (Fig 1, Table 2). By contrast, Log SDV increased (deteriorated), and dead space remained unchanged. The increased Log SDV could be related to the significant fall in pulmonary artery pressure. This suggests less apical perfusion in the lung, hence facilitating the development of high $\dot{V}A/\dot{Q}$ regions after surgery, which would increase the Log SDV, other things being

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TABLE 2] Pulmonary Gas Exchange, Ventilatory, Hemodynamic, and Metabolic Findings Breathing Ambient Air and Oxygen-Induced Changes in Obese Individuals Before and After BS

	Before BS (n = 19)		After BS (n = 19)			
Outcomes	21% O_2[a]	100% O_2[b]	21% O_2[a]	100% O_2[b]	*P* Value[c]	*P* Value[d]
Pao_2, mm Hg	76 ± 2[e]	519 ± 22[e]	89 ± 2[f]	583 ± 20[f]	< .001	.007
$Paco_2$, mm Hg	39 ± 1	39 ± 1	40 ± 1	39 ± 2	.40	.32
A-aPo_2, mm Hg	27 ± 2[e]	155 ± 21[e]	15 ± 2[f]	96 ± 19[f]	< .001	.007
pH	7.41 ± 0.01	7.42 ± 0.01	7.41 ± 0.01[f]	7.43 ± 0.01[f]	.45	.12
$P\bar{v}o_2$, mm Hg	38 ± 1[e]	48 ± 1[e]	39 ± 1[f]	48 ± 1[f]	.18	.15
Shunt,[g] % $\dot{Q}t$	4.3 ± 1.1[e]	9.8 ± 1.7[e]	1.9 ± 0.4[f]	3.7 ± 1.0[f]	.005	.001
Log SDQ	0.83 ± 0.06	0.91 ± 0.08	0.71 ± 0.06	0.78 ± 0.07	.09	.92
Log SDV	0.69 ± 0.04	0.76 ± 0.07	0.87 ± 0.07[f]	0.71 ± 0.06[f]	.03	.02
Dead space,[h] % $\dot{V}A$	23.8 ± 3.5[e]	30.2 ± 1.9[e]	24.8 ± 1.6[f]	34.6 ± 3.3[f]	.85	.47
$\dot{V}E$, L/min	6.7 ± 0.5	7.5 ± 0.6	5.9 ± 0.5[f]	6.8 ± 0.6[f]	.04	.70
$\dot{Q}t$, L/min	6.6 ± 0.3[e]	6.0 ± 0.3[e]	5.2 ± 0.2[f]	4.5 ± 0.2[f]	< .001	.20
Cardiac index, L/min/m^2	2.6 ± 0.1[e]	2.4 ± 0.1[e]	2.1 ± 0.1[f]	1.8 ± 0.1[f]	< .001	.17
Psa, mm Hg	100 ± 2[e]	105 ± 3[e]	91 ± 3[f]	99 ± 2[f]	.001	.12
PAP, mm Hg	19 ± 1[e]	16 ± 1[e]	13 ± 1	13 ± 1	< .001	.15
PCWP, mm Hg	8 ± 1	8 ± 1	6 ± 1	7 ± 1	.04	.82
SVR, dyn × s/cm^5	1,197 ± 78[e]	1,373 ± 76[e]	1,295 ± 84[f]	1,733 ± 89[f]	.09	.003
PVR, dyn × s/cm^5	132 ± 16[e]	106 ± 15[e]	99 ± 13	105 ± 12	.01	.08
$\dot{V}o_2$, mL/min	251 ± 14	NA	191 ± 11	NA	< .001	NA

Data are presented as mean ± SE. Log SDQ = dispersion of blood flow distribution; Log SDV = dispersion of alveolar ventilation distribution; NA = not available; O_2 = oxygen; PAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; Psa = mean systemic arterial pressure; $P\bar{v}o_2$ = mixed venous Po_2; PVR = pulmonary vascular resistance; $\dot{Q}t$ = cardiac output; SVR = systemic vascular resistance; $\dot{V}A$ = alveolar volume $\dot{V}E$ = minute ventilation; $\dot{V}o_2$ = oxygen consumption. See Table 1 for expansion of other abbreviations.

[a]Breathing ambient air.

[b]Breathing 100% oxygen.

[c]*P* values for comparisons during ambient air breathing between pre- and postoperative conditions.

[d]*P* values for comparisons between pre- and postoperative differences from 21% to 100% oxygen breathing.

[e]$P < .05$ for comparisons before surgery, between ambient air and 100% O_2 breathing.

[f]$P < .05$ for comparisons after surgery, between ambient air and 100% O_2 breathing.

[g]Unventilated units ($\dot{V}A/\dot{Q}$ ratios < 0.005), expressed as % of $\dot{Q}t$.

[h]Alveolar units with $\dot{V}A/\dot{Q}$ ratios > 100, expressed as % of $\dot{V}A$.

equal. The DISP R-E* descriptor remained moderately abnormal (8.7 ± 0.9; range, 3.3-17.2). Except for systemic vascular resistance, ventilatory, metabolic, and hemodynamic outcomes decreased after BS (Table 2).

The reduction in BMI after BS was significantly related to higher functional residual capacity (ρ, −0.64) and lower Log SDQ (ρ, 0.54) (improvement) values ($P < .05$ each). The increase in Pao_2 after BS was related to Log SDQ reduction (ρ, −0.52; $P < .01$). The increase in ERV after BS was associated with lower leptin levels (ρ, −0.59; $P < .01$).

100% Oxygen Breathing: As compared with ambient air, arterial and mixed venous Po_2, arterial pH, and A-aPo_2 increased, without accompanying changes in $Paco_2$. It is of note that the increase in Pao_2 observed during 100% oxygen breathing was significantly higher than that observed before surgery ($P < .01$), in keeping with a lower increase in shunt ($P < .01$) (Table 2). While breathing 100% oxygen, systemic hemodynamic changes were close to those observed during ambient air breathing, except for a greater increase in systemic vascular resistance (Table 2), without changes in pulmonary hemodynamics. The negative preoperative correlation observed between oxygen-induced changes in shunt and Log SDQ was lost after surgery ($r = -0.45$, $P = .06$) (Fig 3).

Discussion

The principal results of this study confirm our working hypothesis by showing that morbidly obese subjects exhibit mild to moderate shunt, together with an abnormal

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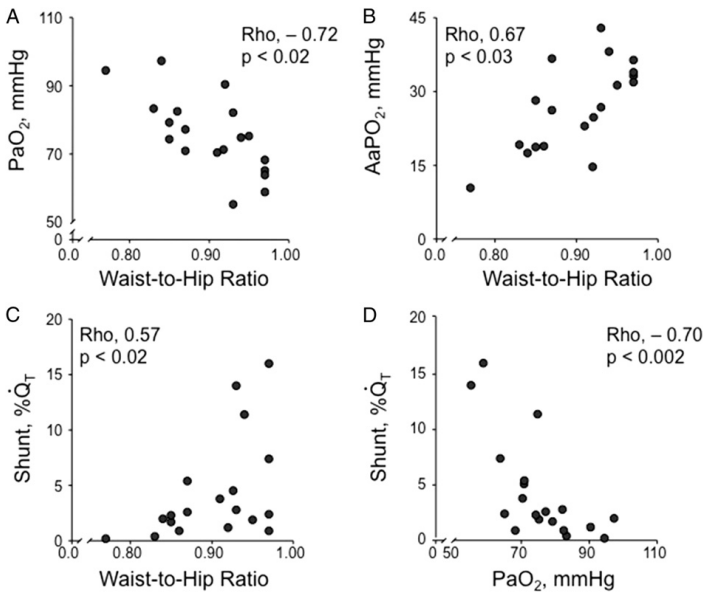

Figure 2 – *Preoperative correlations between gas exchange descriptors and central obesity and between gas exchange indexes. The greater the waist to hip ratio, the worse the gas exchange abnormalities. A, Lower arterial Po_2. B, Greater $AaPO_2$. C, Intrapulmonary shunt. D, Likewise, the lower the arterial Po_2, the greater the amount of shunt. $\dot{Q}_T$ = cardiac output. See Figure 1 legend for expansion of other abbreviation.*

pulmonary blood flow dispersion, that are reduced after BS.

Previous Studies

Although several previous papers have investigated the effects of obesity and BS on lung function, to our knowledge this is the first study that uses the MIGET to investigate $\dot{V}_A/\dot{Q}$ ratio distributions under these clinical circumstances. In keeping with some,[5,6] but not all, of them,[18,19] we observed that static lung volumes were relatively well preserved in the subjects studied herein (Table 1). Discrepancies are likely explained by our stringent recruitment criteria, which excluded the coexistence of moderate to severe comorbidities, such as sleep apnea. Previous studies also reported the presence of widened A-aPO_2 and mild arterial hypoxemia in obese subjects,[6,20] in keeping with our observations (Table 1), as well as significant associations between waist circumference and waist to hip ratio with lung volumes and gas exchange indexes,[21] also in agreement with our current findings. Although no previous study has used the MIGET to investigate the distribution of $\dot{V}_A/\dot{Q}$ ratios in these patients, a study in the 1960s reported oxygen-shunt measurements in a few obese individuals and indicated that arterial hypoxemia was related to perfusion of unventilated areas (ie, shunt).[22]

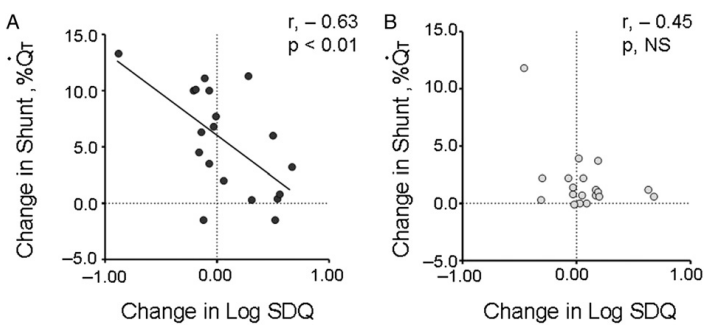

Figure 3 – *Pre- and postoperative correlations between oxygen-induced changes in shunt, expressed as a percentage of $\dot{Q}_T$, (y-axis) and in Log SDQ (x-axis) (dimensionless). A, Before surgery. B, After surgery. Preoperative oxygen-induced increases in shunt (dark gray circles) were inversely associated with reductions in Log SDQ, a correlation (light gray circles) that was lost postoperatively. See Figure 1 and 2 legends for expansion of other abbreviations.*

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Another study using radioactive tracer, also in a few obese subjects, demonstrated that perfusion was maximal in the lower zones, whereas ventilation was significantly reduced, a regional $\dot{V}A/\dot{Q}$ imbalance closely associated with the parallel reduction in ERV.[7] The amounts of shunt observed in our study are in agreement with these findings.

Interpretation of Findings

Three main novel gas exchange findings of our study deserve specific discussion: (1) the pattern of pulmonary gas exchange abnormalities observed before BS, (2) the differential effects of 100% oxygen breathing before and after BS, and (3) the observation that all $\dot{V}A/\dot{Q}$ abnormalities except the Log SDV values were significantly ameliorated after BS.

First, despite minor impairment of lung volumes, very severe obesity was associated with mild to moderate shunt and increased dispersion of both Log SDQ and Log SDV. This pattern of gas exchange disturbances is similar to that shown in patients with pleural effusion that physically compresses the underlying normal lung parenchyma.[23] Accordingly, we propose that the excessive adipose tissue that accumulates in morbid obesity causes an excessive and unopposed intraabdominal pressure that compresses the dependent regions of the lungs and results in mild amounts of shunt and low $\dot{V}A/\dot{Q}$ ratios (increased Log SDQ). Alternatively, systemic inflammation can alter pulmonary vascular tone,[24] hence also influencing $\dot{V}A/\dot{Q}$ ratio distributions. Unfortunately, we cannot unravel which of these mechanisms is more relevant, because BS reduced both body weight and systemic inflammation (Table 1).

Second, during oxygen breathing, the increase in shunt was paralleled by a fall in Log SDQ, hence indicating pulmonary blood flow redistribution. This dynamic response mimics that shown in patients with acute lung injury and is likely to be related to the development of reabsorption atelectasis without reversion of hypoxic pulmonary vasoconstriction.[25,26] Our contention is that this may reflect weaker and more rigid pulmonary vessels because of excessive adipose tissue-induced endothelial dysfunction,[24] because both leptin and adiponectin modulate vascular tone by increasing nitric oxide bioavailability in healthy but not obese individuals.[27] Our findings are, therefore, consistent with abnormal pulmonary vascular contractility in morbidly obese subjects.[24]

Finally, in parallel with weight loss and reduction of systemic inflammation after BS, abnormal $\dot{V}A/\dot{Q}$ distributions were also significantly reduced but not abolished, akin to the remnant of mild obesity and systemic inflammation. Although mechanical factors can undoubtedly play a role, the fact that the correlation between oxygen-induced changes in shunt and Log SDQ after BS (Fig 3) was lost suggests improvement in pulmonary blood flow redistribution. This would be in keeping with experimental evidence indicating that perivascular adipose tissue-induced vasodilatation of small arteries can be restored after BS.[24] Hence, our observations could support a causal role of obesity on pulmonary gas exchange and vascular tone abnormalities.

Strengths and Limitations

Our study has strengths and limitations. Among the former is the fact that we used the MIGET for the first time to assess pulmonary gas exchange disturbances in this clinical scenario. The MIGET is the most robust tool to investigate the pulmonary and nonpulmonary determinants of gas exchange in humans. Among the latter is the fact that we studied only women because of the sex differences described in pulmonary gas exchange in morbidly obese individuals[28,29] and the higher prevalence of this disease in females. Hence, our results may not be extrapolated directly to men.

Conclusions

This study shows that even in the absence of major lung volume alterations, morbidly obese individuals have abnormal $\dot{V}A/\dot{Q}$ distributions that are reduced after BS.

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Author contributions: R. R.-R. is the guarantor of the manuscript and takes responsibility for the integrity of the data and the accuracy of the data analysis. E. R., E. A., M. S., S. D., C. G., and R. R.-R. contributed to the conduction of the experimental work and the acquisition and analysis of the data; E. R., A. A., P. D. W., and R. R.-R. contributed to the conception and design of the study and interpretation of the data; E. R., E. A., M. S., S. D., C. G., and R. R.-R. contributed to the planning and coordination of the study; and E. R., A. A., P. D. W., and R. R.-R. contributed to the writing of the article and/or had substantial involvement in its critical revision before submission.

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ORIGINAL ARTICLE

Postural effects on pulmonary gas exchange abnormalities in severe obesity before and after bariatric surgery

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ABSTRACT

BACKGROUND: We hypothesized that in morbid obesity, pulmonary gas exchange abnormalities will worsen when supine and that bariatric surgery (BS) will mitigate this effect.
METHODS: Gas exchange was investigated in 19 morbidly obese and 8 non-obese, age-matched control females, spontaneously breathing ambient air, both upright and supine, before and one year after BS.
RESULTS: In control non-obese individuals, no postural changes in arterial blood gases (ABGs) were observed. While obese subjects had more altered PaO_2, SaO_2 and $AaPO_2$ values than controls ($P<0.05$ each) when upright, the values unexpectedly remained unchanged when supine. This was also the case in the subset of 6 normoxemic obese but the remaining 13 hypoxemic individuals actually improved ABGs when supine: PaO_2 (by +2.7±1.3 mmHg, $P=0.06$), SaO_2 (by +1.5±0.6 %), pH (by +0.01±0.01) and $AaPO_2$ (by -3.4±1.4 mmHg); and cardiac output increased (by +0.4±0.2 $L \cdot min^{-1}$) ($P<0.05$ each). After BS, PaO_2 (from 75.5±2.4 to 89.4±2.4 mmHg) and $AaPO_2$ (from 27.0±2.0 to 15.4±2.1 mmHg) ($P<0.05$ each) and pulmonary gas exchange were improved compared to before BS when upright, but ABGs worsened when supine (PaO_2, by –4.6±1.7 mmHg; $AaPO_2$, by +4.2±1.6 mmHg) ($P<0.05$ each).
CONCLUSIONS: Before BS, ABGs are not altered in normoxemic obese subjects moving from upright to supine, even improving in those with hypoxemia when supine. After successful BS, pulmonary gas exchange improved when upright in all subjects but ABGs deteriorated when supine. However, the important clinical observation is the lack of gas exchange deterioration when supine, which may have implications for critical care and anesthesia settings.

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Key words: Gases - Obesity - Pulmonary gas exchange - Bariatric surgery.

Current clinical guidelines recommend that critically ill obese patients remain upright during perioperative and intensive care conditions to avoid and/or minimize supine-induced gas exchange worsening due to excessive intra-abdominal pressure on dependent lung regions.[1, 2] We have recently shown that prior to bariatric surgery (BS), compared to healthy

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subjects, morbidly obese individuals increased intrapulmonary shunt and ventilation-perfusion (V_A/Q) imbalance inducing low arterial PO_2 (PaO_2) and increased alveolar-arterial PO_2 difference ($AaPO_2$) when upright, whereas after BS overall gas exchange disturbances were substantially improved in relation to their own pre-operative data.[3] Here, we hypothesized that prior to BS recumbency would aggravate these pulmonary gas exchange abnormalities due to increased intrapulmonary shunt and further ventilation-perfusion (V_A/Q) imbalance other factors being equal and that BS would improve them.

We tested this hypothesis in the same non-obese and obese individuals reported previously,[3] but separately, given the complexity of the study design. This prompted us to focus exclusively on the postural effects of pulmonary gas exchange before and after BS.

Materials and methods

Study population, study design and ethics

The principal characteristics and inclusion criteria of 8 control and 19 obese participants, all females, without major multi-morbidities, such as severe-to-moderate sleep apnea syndrome, have been previously reported elsewhere.[3] Control subjects were normal weight, age-matched females who required thoracic computerized tomography (CT) scans for screening and follow- up of *in situ* cutaneous melanoma; otherwise, these subjects were completely healthy. We herein report the effects of posture on gas exchange. All measurements in both control and obese subjects were always performed when upright with the legs down and when supine using a TotalCare® bed (Hillrom, Pluvigner, France) for 30 min each, in random order. All subjects refrained from any medication during the prior 24 h, before and after BS (median, 51 weeks). All participants signed informed consent. The study was approved by the Ethics Committee of the Hospital Clínic (Protocol 2008/4015).

Measurements

Physiologic blood gases

Arterial and mixed venous blood samples gases were analyzed for pH, PO_2 and PCO_2 (CIBA Corning 800. Medfield, MA, USA), and the alveolar-arterial PO_2 gradient ($AaPO_2$) was calculated using the measured exchange respiratory ratio as reported previously.[3] Oxygen uptake (VO_2) was calculated using standard formulae.[3]

Ventilatory and hemodynamic measurements

Minute ventilation was measured using a Wright spirometer (Respirometer MK8, BOC-Healthcare. Essex, UK). The electrocardiogram, heart rate, mean arterial pressures and oxygen saturation through a pulseoximeter were continuously monitored (HP M 1001A-1006A &B, 1012A, 1020A, 1046A, 1166A; Hewlett-Packard, Waltham, MA, USA) to ensure safety conditions. An 18-gauge plastic cannula was inserted into the radial artery for monitoring systemic arterial pressure and for arterial blood gas sampling. In obese subjects with PaO_2<80 mmHg (range, 55-79 mmHg; n, 13), a 7-French triple-lumen thermodilution balloon-tipped pulmonary artery catheter (Edwards; Baxter Healthcare Corporation, Irvine, CA, USA) was inserted under echography guidance to exclude the presence of pulmonary arterial hypertension. Pulmonary artery, capillary wedge and right atrial pressures were monitored and pulmonary (PVR) and systemic vascular resistances (SVR) were calculated using standard formulae. Accordingly, in hypoxemic obese individuals, V_A/Q distributions were calculated using arterial, mixed venous and mixed expired inert gases concentrations and cardiac output (Q_T) was determined by thermodilution.[3] In obese individuals without arterial hypoxemia (PaO_2 ≥80 mmHg; range, 82-97 mmHg; n, 6), V_A/Q distributions were estimated without mixed venous sampling and cardiac output was measured by bioimpedance (PhysioFlow®, Manatec Biomedi-

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cal. Paris, France).[4] Paired inert gas studies were completed in all obese participants.

V_A/Q RATIO DISTRIBUTIONS

Measurements of V_A/Q distributions were estimated by the multiple inert gas elimination technique (MIGET) in the customary manner.[5, 6] Arterial and mixed venous blood samples and mixed expired inert gases were collected through a metallic heated box by duplicate. The dispersion of pulmonary distribution blood flow and that of alveolar ventilation distribution on a logarithmic scale (Log SDQ and Log SDV; upper normal limits, 0.60 and 0.65, respectively) [7] were calculated. Shunt and dead space were defined, respectively, as the fraction of blood flow perfusing units with V_A/Q ratios <0.005 and the fraction of alveolar ventilation distribution with V_A/Q ratios >100. All measurements were determined under steady-state conditions, defined by stability (±5%) of both ventilatory and hemodynamic variables, and by the close agreement (±5%) between duplicate measurements of mixed expired and arterial oxygen and carbon dioxide.

Statistical analysis

Results are presented as mean±SEM. We used the unpaired Student *t*-test to compare control and obese subjects before BS. A generalized linear mixed model was applied to all obese participants such that all their data were analyzed considering three interventions (BS, inspired oxygen fraction, and posture). In this model each individual was considered as a random factor to assess the effects induced by each intervention and their potential interactions. Given the presence of significant consistent interactions among them, we then focus the analysis on the postural effects while breathing ambient air, before and after BS, using paired Student *t*-test. Corrections were made for multiple comparisons of interventions in obese individuals. A P<0.05 value was considered significant at all instances.

Results

We enrolled 19 morbidly obese women (age, 51±[SE]2 years; Body Mass Index [BMI], 45±1 kg/m^2), all but one never-smokers and 8 non-obese (BMI, 25±2 kg/m^2), sex- and age-matched (50±3 years) never-smokers.

Findings before BS

Arterial blood gases (ABGs), pH, oxygen saturation (SaO_2), inert gases and hemodynamic values in the upright and supine postures are set out in Tables I, II, respectively. Note that clinical and lung function tests, including ABGs, pulmonary and systemic hemodynamics and inert gas exchange measurements for all obese individuals when upright have been previously reported [3] but herein reproduced for convenience in both tables for comparison with those measured when supine. ABGs in non-obese participants were within normal limits (PaO_2, 86.5±1.4 mmHg; $PaCO_2$, 37.1±1.2 mmHg; $AaPO_2$, 18.4±1.4 mmHg; pH, 7.42±0.01; SaO_2, 99±1%) when upright without significant changes (PaO_2, -0.2±2.7 mmHg; $PaCO_2$, +1.4±0.8 mmHg; $AaPO_2$, -1.0±2.6 mmHg; pH, +0.01±0.01; SaO_2, +0.5±0.5 %) when supine. Similarly, in all obese individuals considered together, PaO_2 (+0.6±1.6 mmHg), $AaPO_2$ (–1.1±1.6 mmHg) and SaO_2 (+0.6±0.5 %) did not deteriorate when supine. Except for PCWP and SVR, systemic and pulmonary hemodynamic values and V_A/Q descriptors were not different from upright to supine (Table II). However, there were different postural ABGs effects in obese participants according to the presence or absence of arterial hypoxemia at enrollment (Figure 1). While no postural changes were observed in the 6 normoxemic obese subjects, the 13 hypoxemic individuals (Table I, Figures 1, 2) improved ABGs from seated to supine. Arterial pH (by +0.01±0.01), and SaO_2 (by +1.5±0.6 %) increased and $AaPO_2$ decreased (by -3.4±1.4 mmHg) (P<0.05 each) when supine, without significant changes in PaO_2 (by +2.7±1.3 mmHg; P=0.06) and $PaCO_2$. Except for a small increase in Q_T (from 6.6±0.4 to 7.0±0.4, L·min^{-1}) and in PCWP (by

RESULTS

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TABLE I.—*Gas exchange findings at upright and supine in obese individuals before and after bariatric surgery.*

	Before surgery			After surgery		
	All obese individuals (N.=19)					
	Upright	Supine	P*	Upright	Supine	P‡
PaO_2, mm Hg	75.5 ± 2.4†	76.1 ± 2.6	NS	89.4 ± 2.4†	84.8 ± 3.1	0.02
$PaCO_2$, mm Hg	39.0 ± 1.0	39.7 ± 1.0	NS	39.8 ± 1.2	39.7 ± 1.3	NS
$AaPO_2$, mm Hg	27.0 ± 2.0†	25.9 ± 2.0	NS	15.4 ± 2.1†	19.6 ± 2.1	0.02
pH	7.41 ± 0.01	7.41 ± 0.01	NS	7.41 ± 0.01	7.42 ± 0.01	0.01
SaO_2, %	96 ± 1†	96 ± 1	NS	99 ± 1†	99 ± 1	NS
	Normoxemic obese individuals (N.=6)					
	Upright	Supine	P*	Upright	Supine	P‡
PaO_2, mm Hg	88.4 ± 2.7†	84.3 ± 5.7	NS	100.1 ± 3.8†	97.2 ± 5.8	NS
$PaCO_2$, mm Hg	35.8 ± 1.7	35.9 ± 1.6	NS	36.3 ± 2.0	34.6 ± 2.3	NS
$AaPO_2$, mm Hg	17.9 ± 2.2†	22.1 ± 4.3	NS	9.6 ± 2.7†	13.9 ± 2.9	NS
pH	7.45 ± 0.01	7.43 ± 0.01	NS	7.43 ± 0.01	7.44 ± 0.01	NS
SaO_2, %	99 ± 1	98 ± 1	NS	100 ± 0	100 ± 0	NS
	Hypoxemic obese individuals (N.=13)					
	Upright	Supine	P*	Upright	Supine	P‡
PaO_2, mm Hg	69.5 ± 1.9†	72.3 ± 2.3	0.06	84.4 ± 1.9†	79.1 ± 2.4	0.01
$PaCO_2$, mm Hg	40.5 ± 1.1	41.5 ± 0.9	NS	41.4 ± 1.2	42.1 ± 1.1	NS
$AaPO_2$, mm Hg	31.2 ± 1.9†	27.8 ± 2.0	0.03	18.1 ± 2.5†	22.2 ± 2.4	0.07
pH	7.39 ± 0.01	7.41 ± 0.01	0.04	7.40 ± 0.01	7.41 ± 0.01	0.01
SaO_2, %	94 ± 1†	96 ± 1	0.03	99 ± 1†	98 ± 1	NS

Values are expressed as mean±SEM. $AaPO_2$: alveolar to arterial PO_2 difference; * denotes P-values for comparisons between upright and supine before surgery; † denotes P<0.05 for comparisons at upright between pre- and postoperative conditions; ‡ denotes P-values for comparisons between upright and supine after surgery.

TABLE II.—*Pulmonary gas exchange, ventilatory, hemodynamic and metabolic findings at upright and supine in all obese (N.=19) individuals before and after bariatric surgery.*

	Obese individuals					
	Before		P*	After		P‡
	Upright	Supine		Upright	Supine	
PvO_2, mm Hg	38 ± 1	39 ± 1	NS	39 ± 1	39 ± 1	NS
Shunt, $\%Q_T$	4.3 ± 1.1†	3.8 ± 0.6	NS	1.9 ± 0.4†	2.0 ± 0.3	NS
Log SDQ	0.83 ± 0.06	0.82 ± 0.05	NS	0.71 ± 0.06	0.82 ± 0.05	NS
Log SDV	0.69 ± 0.04†	0.73 ± 0.05	NS	0.87 ± 0.07†	0.72 ± 0.05	0.03
Dead space, $\%V_A$	23.8 ± 3.5	24.1 ± 2.6	NS	24.8 ± 1.6	23.9 ± 1.1	NS
V_E, $L \cdot min^{-1}$	6.7 ± 0.5†	6.5 ± 0.5	NS	5.9 ± 0.5†	5.4 ± 0.4	NS
Q_T, $L \cdot min^{-1}$	6.6 ± 0.3†	6.9 ± 0.4	NS	5.2 ± 0.2†	5.5 ± 0.3	NS
CI, $L \cdot min^{-1} \cdot m^2$	2.6 ± 0.1†	2.7 ± 0.1	NS	2.1 ± 0.1†	2.2 ± 0.1	NS
Psa, mm Hg	100 ± 2†	98 ± 3	NS	91 ± 3†	87 ± 3	NS
PAP, mm Hg	19 ± 1†	22 ± 2	NS	13 ± 1†	17 ± 1	<0.001
PCWP, mm Hg	8 ± 1†	11 ± 1	0.04	6 ± 1†	10 ± 1	<0.001
SVR, $dyn \cdot s \cdot cm^{-5}$	1197 ± 78	1079 ± 71	0.01	1295 ± 84	1236 ± 100	NS
PVR, $dyn \cdot s \cdot cm^{-5}$	132 ± 16†	122 ± 14	NS	99 ± 13†	108 ± 14	NS
VO_2, $mL \cdot min^{-1}$	251 ± 14†	254 ± 17	NS	191 ± 11†	182 ± 10	NS

Values are expressed as mean±SEM; PvO_2: mixed venous PO_2; shunt: unventilated units (V_A/Q) ratios <0.005, expressed as % of Q_T); Log SDQ: dispersion of blood flow distribution and Log SDV, dispersion of ventilation distribution (both dimensionless); dead space: alveolar units with V_A/Q ratios >100, expressed as % of alveolar volume (V_A); V_E: minute ventilation; Q_T: cardiac output; CI: Cardiac Index; Psa: mean systemic arterial pressure; PAP: mean pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; SVR: systemic vascular resistance; PVR: pulmonary vascular resistance; VO_2: oxygen consumption; * denotes P-values for comparisons between upright and supine before surgery; † denotes P<0.05 for comparisons at upright between pre- and postoperative conditions; ‡ denotes P-values for comparisons between upright and supine after surgery.

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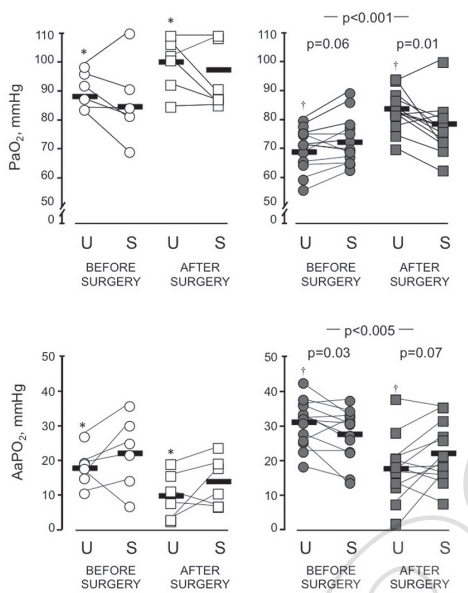

Figure 1.—Individual arterial PO_2 (PaO_2) and alveolar-arterial PO_2 difference ($AaPO_2$) values at upright (U) and supine (S) postures in obese individuals with normal (open symbols) and low PaO_2 (closed symbols), before (circles) and after (squares) bariatric surgery. Solid bars represent mean values. The 3 hypoxemic individuals who did not improve PaO_2 before BS did not show any distinct features from the remainder 10 hypoxemic subjects who did increase PaO_2 when supine.

+3±1 mmHg) associated with a decrease in SVR (by -118±40 dyn·s·cm^{-5}) (P<0.05 each), no other effects were observed on pulmonary (V_A/Q ratio distributions) (Figure 2) and/or other non-pulmonary (minute ventilation and/or VO_2) factors governing pulmonary gas exchange and on systemic and pulmonary hemodynamics. Moreover, in hypoxemic obese individuals, postural-induced changes in Q_T and mixed venous PO_2 were linearly correlated (r, 0.62; P<0.03). The median residual sum of squares, the descriptor of the quality of MIGET results, was 2.0, below the expected value of 5.4, indicating high quality of inert gas data.[5]

Findings one year after BS

BS was successful in all obese subjects, as shown by a significant BMI reduction (31 kg/m^2) (P<0.001)[8]. Overall, obese individuals increased PaO_2 and decreased $AaPO_2$ (P<0.05 each) post-BS in the upright posture[3] (Table I, Figure 1). Likewise, all abnormal V_A/Q descriptors but the alveolar ventilation dispersion (Log SDV) improved and all hemodynamic outcomes (systemic arterial pressure, Q_T, PAP, PCWP, and PVR) and VO_2 decreased (P<0.05 each) after BS as previously reported (Table II).[3] However, ABGs worsened in the supine position: PaO_2 decreased (by -4.6±1.7 mmHg), and $AaPO_2$ (by +4.2±1.6 mmHg) and pH (by +0.01±0.01) increased (P<0.05 each) without changes in $PaCO_2$ and SaO_2. While no postural effects were observed in normoxemic obese subjects, hypoxemic obese individuals decreased PaO_2 (by -5.3±1.7 mmHg; P=0.01) and increased pH (by +0.02±0.01; P=0.01) when supine without changes in $AaPO_2$ (by +4.2±2.1 mmHg; P=0.07), $PaCO_2$ and SaO_2. Moreover, except for a decrease in Log SDV (improvement), intrinsically related to increases in both PAP and PCWP (by +4±1 mmHg each) (P<0.05 each), no other changes in V_A/Q descriptors (Figure 2) and systemic and pulmonary hemodynamics were observed.

Discussion

This study shows that, in a selected population of morbidly obese female candidates for BS without major multi-morbidities, pulmonary gas exchange abnormalities including ABGs remain essentially unchanged when moved from upright to supine postures as was also observed in control subjects. Unexpectedly, hypoxemic obese individuals actually improved arterial oxygenation in the supine position. After BS, all obese participants considered together considerably improved pulmonary gas exchange when upright (compared to pre-BS), but then ABGs deteriorated alone when supine. All pre- and post-operative ABG findings were small and associated with few hemodynamic and V_A/Q changes.

Previous studies

Our findings in normoxemic obese individuals are at variance with those shown by Fare-

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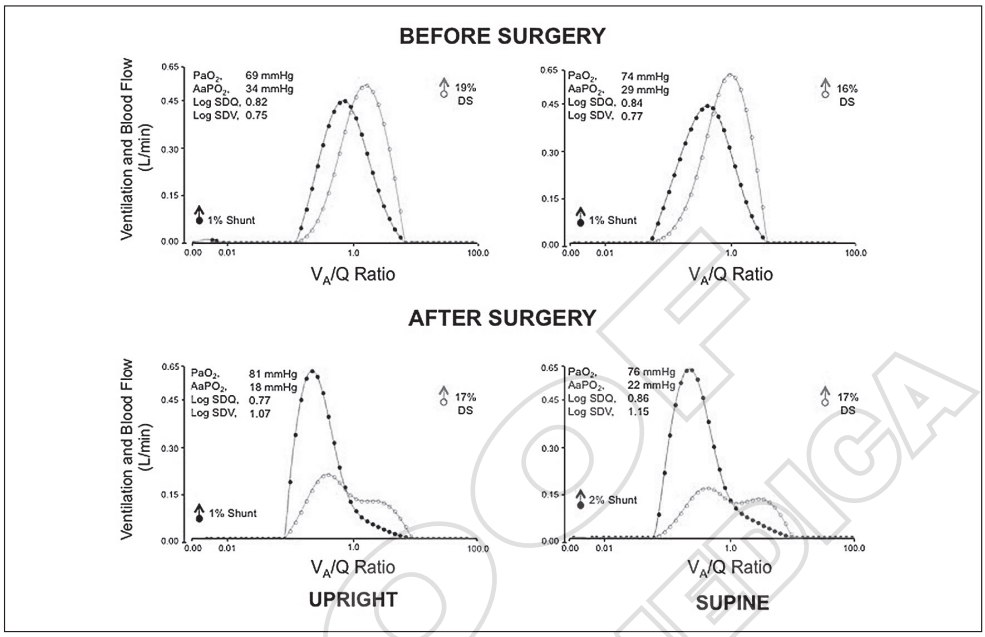

Figure 2.—Distributions of alveolar ventilation (V_A) (open circles) and pulmonary blood flow (Q) (solid circles) plotted *versus* V_A/Q ratio from a representative hypoxemic obese female (age, 55 yrs) when upright (left panels) and when supine (right panels), before (BMI, 38 kg/m²) and after BS (BMI, 30 kg/m²). Note that, at each time point, both V_A/Q distributions were always broadly unimodal. Note also that before BS (top), PaO_2 increased and $AaPO_2$ decreased (*i.e.*, arterial oxygenation improved) without changes in intrapulmonary shunt and V_A/Q imbalance when supine. All inert dead space values were reduced. After BS (bottom), PaO_2 increased and $AaPO_2$ decreased (*i.e.*, arterial oxygenation improved) while all but Log SDV improved when upright; by contrast, PaO_2 decreased and $AaPO_2$ increased (*i.e.*, arterial oxygenation worsened) while no other changes in V_A/Q descriptors were observed when supine (for further explanation, see text).
$AaPO_2$: alveolar-arterial PO_2 difference; DS: dead space; Log SDQ: dispersion of blood flow distribution; Log SDV: dispersion of alveolar ventilation distribution; V_A/Q: ventilation-perfusion.

brother *et al.*[9] who investigated normoxemic obese smokers of both sexes, before and after starvation, and showed PaO_2 decreases when supine before and after weight loss. Vaughan *et al.*[10] observed no effects on ABGs in mild hypoxemic obese young nonsmoking females, before and at the third day after conventional surgery. Because these two studies differed considerably in design and inclusion criteria, direct comparisons with our results are difficult. Discrepancies can be also likely explained by our inclusion criteria, which excluded the coexistence of moderate to severe multi-morbidities.

Interpretation of findings

Contrary to our original hypothesis, *i.e.* pulmonary gas exchange disturbances in obese participants would worsen in parallel to the expected deterioration of shunt and pulmonary vascular disturbances from upright to supine postures before BS, pulmonary gas exchange abnormalities in our obese subset considered as a single group remained unchanged as well as in obese subjects with normal PaO_2 from upright to supine. By contrast, in hypoxemic obese individuals, arterial oxygenation improved when supine so that it worsened when upright, a phenomenon characteristically seen, although of slightly more magnitude, in patients with hepatopulmonary syndrome (HPS).[11, 12] Nevertheless, the underlying mechanisms differ. In HPS, upright-induced arterial deoxygenation or orthodeoxia is caused by further V_A/Q imbalance without changes in non-pulmonary (*i.e.,* minute ventilation, car-

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diac output and/or oxygen uptake) determinants of gas exchange,[13] likely produced by an abnormal pulmonary vasculature with more heterogeneous gravitational blood flow redistribution to dependent lung areas.[11, 12] By contrast, in hypoxemic morbidly obese subjects, orthodeoxia is induced by decreased cardiac output without ensuing gravitational negative changes in shunt and V_A/Q mismatching, likely aggravated by the coexistence of a pulmonary vasculature absent of hypoxic pulmonary vasoconstriction[3]. Should increased cardiac output in the hypoxemic morbidly obese when supine be accompanied by higher increases in intrapulmonary shunt, it is most likely that the net effect would have offset the observed improvement in arterial oxygenation.

Alternatively, the absence of ABG changes in all obese subjects when supine can be related to the combination of small postural-induced lung volume changes [14, 15] along with the absence of noticeable Q_T effects. In a recent study in morbidly obese individuals, lung volumes were more restricted than in healthy individuals along with important reductions in expiratory reserve volume and in end-tidal functional residual capacity (FRC) when upright, but similar when supine.[14] In another study investigating supine changes in obese and lean subjects, total lung capacity (TLC) and its subdivisions, it was shown that despite the increased extra-pulmonary mass load in obese subjects, further falls in TLC and FRC were negligible when supine.[15] Likely, this was not the case in the subset of hypoxemic obese subjects in whom the increase in Q_T played a positive influential effect on PaO_2 through mixed venous PO_2. It remains unknown though what can be the duration of this supine-induced PaO_2 improvement in the clinical setting of the real-world of morbidly obese individuals. It may be plausible that after a few hours at supine, the increase in Q_T is limited so that the presence of upright-induced arterial deoxygenation is not patent any more.

In parallel to the substantial post-operative weight loss,[3] all gas exchange indices but Log SDV in all obese individuals improved when upright. However, after BS, PaO_2 decreased in all obese and in pre-operatively hypoxemic subjects when supine. Although these supine-induced deleterious effects on arterial oxygenation remain ultimately unsettled, we have to consider that the Log SDV improvement was the result of considerable pulmonary vascular changes within the context of a post-operative remnant of mild obesity.[3, 16] Whether the current findings can improve or worsen potential ABG changes during sleep, anesthesia, surgery and critical care medicine settings remains unknown so that further investigations need to be conducted.

Strengths and limitations

There were strengths and shortcomings in our study. First, this is the first study that so far has addressed the effects of postural changes on pulmonary gas exchange before and after BS through a comprehensive approach, including measurements of systemic and pulmonary hemodynamics and inert gas studies. Second, the same participants were studied before and one year after BS, thus providing a robust comparative insight into the difficult interplay of pulmonary and non-pulmonary factors governing pulmonary gas exchange.[13] However, there were some limitations. One was the small number of patients due to the stringent complexity of our study design. Another shortcoming was the exclusive inclusion of females given the fact that males have worse gas exchange than females due to the different regional distribution of adipose tissue. Accordingly, our current data cannot be necessarily extrapolated to very severe obese males or to those who associated severe multi-morbidities.

Conclusions

Before BS, in normoxemic obese subjects arterial oxygenation is not altered but in hypoxemic individuals is paradoxically improved when supine. After successful BS, pulmonary gas exchange considerably improved while arterial oxygenation increased in all obese and in pre-operatively hypoxemic individuals when upright but decreased when supine. Postural-induced ABG changes are small and of limited

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clinical relevance. However, the finding that a short period of time (up to 30 min) when supine does not induce arterial deoxygenation prior to surgery can be of interest to anesthesiologists for their daily clinical practice. Obese individuals are usually placed on supine to induce anesthesia, intubation and instrumentation before any surgical intervention.

Key messages

— Prior to bariatric surgery, arterial oxygenation in a cohort of obese females without serious multi-morbidities is not altered but in hypoxemic individuals improved when supine.

— Preoperative supine-induced arterial oxygenation improvement is associated to an increase in cardiac output without ensuing changes in shunt and ventilation- perfusion mismatching.

— After successful bariatric surgery, arterial oxygenation significantly increased in all obese and in pre-operative hypoxemic individuals when upright but worsen when supine.

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Authors' contributions.—Eva Rivas, Ebymar Arismendi, Concepción Gistau and Roberto Rodriguez-Roisin conducted the experimental work and were involved in the acquisition and analysis of the data. Roberto Rodriguez-Roisin, Peter D. Wagner, Alvar Agustí and Eva Rivas contributed to the conception, design, and data interpretation of the study. Eva Rivas, Ebymar Arismendi, Concepción Gistau and Roberto Rodriguez-Roisin were involved in the planning and coordination of the study. Eva Rivas, Alvar Agustí, Peter D. Wagner and Roberto Rodriguez-Roisin contributed to the writing of the article and/or had substantial involvement in its critical revision before submission.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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4.3. Manuscript 3. *Lung tissue volume is elevated in obesity and reduced by bariatric surgery.*

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(*): Arnoldo Santos and Eva Rivas contributed equally to this article.

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ORIGINAL CONTRIBUTIONS

Lung Tissue Volume is Elevated in Obesity and Reduced by Bariatric Surgery

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Abstract

Background Bariatric surgery (BS) in severely obese subjects causes a significant reduction of body weight with lung function improvement. We have shown that abnormalities in pulmonary gas exchange in morbidly obese subjects are substantially improved with BS. These abnormalities were thought to be related to reduced lung volumes as well as to abnormal endothelial function induced by low-grade chronic inflammation linked to perivascular adipose tissue (PVAT). In this study, we used computed tomography (CT) to assess whether BS also caused measurable structural changes in the lung tissue volume (*Vtiss*) and cross-sectional vessel analysis, hypothesizing that these measures could be related to the previously reported lung functional changes.

Methods This is a post hoc analysis of a previous reported prospective study. Pulmonary vessels and lung volumes, including *Vtiss*, were quantified in thoracic CT scans. We compared findings in 12 obese women before and after BS and in 8 healthy lean women.

Results Vtiss was significantly elevated in obese subjects before BS compared to control subjects and systematically reduced after BS (by 8 %); other CT lung volumes or vascular areas were not affected in a consistent manner. No relationship was observed between BS-induced individual changes in *Vtiss* and pulmonary vessel area.

Conclusions *Vtiss* is elevated in morbidly obese subjects, compared to lean individuals of similar body height, and is systematically reduced by BS. These effects do not appear related to vascular changes but may be caused by

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elevated extravascular lung water, due to low-grade inflammation, and/or hypertrophic PVAT in severe obesity.

Keywords Bariatric surgery · Lung tissue volume · Obesity · Pulmonary hemodynamics · Thoracic computed tomography

Abbreviations

$AaPO_2$	alveolar-arterial PO_2 difference
BMI	body mass index
BS	bariatric surgery
CT	computed tomography
EELV	end-expiratory lung volume
ERV	expiratory reserve volume
HALV	hypo-aerated lung volume
HU	Hounsfield unit
NALV	non-aerated lung volume
PAP	pulmonary artery pressure
PVAT	perivascular adipose tissue
PVR	pulmonary vascular resistance
Q_T	cardiac output
RV	residual volume
V_A/Q	ventilation-perfusion
Vtiss	lung tissue volume

Introduction

Bariatric surgery (BS) in severely obese people causes a significant reduction of body weight [1] and improvement in lung function [2]. We previously showed that ventilation-perfusion (V_A/Q) imbalance substantially improved in morbidly obese subjects 1 year after BS [3]. Pulmonary gas exchange abnormalities were thought to be related to abnormal lung volumes as well as to endothelial dysfunction induced by low-grade chronic inflammation possibly linked to perivascular adipose tissue (PVAT) [4]. In the present study, we used CT to assess whether BS caused structural changes in the lung consistent with inflammation and PVAT. We focused in lung tissue volume (*Vtiss*) and cross-sectional vessel analysis, hypothesizing that these measurements could be related to the lung functional changes previously reported.

CT has been used for evaluation of *Vtiss* that describes the volume of the lung not occupied by air [5]. If large airways and vessels are excluded, *Vtiss* includes the parenchymal tissue, small pulmonary vessels, and capillaries [6]. In non-contrast CT, the individual contributions of pulmonary blood and parenchymal tissue cannot be differentiated. However, CT has been able to characterize changes in *Vtiss* in a number of lung conditions [7, 8] and relationships between cross-sectional area of pulmonary vessels with lung function [9] or pulmonary hemodynamics in cohorts of patients [10].

We hypothesized that if PVAT of the pulmonary vasculature was elevated in morbid obesity [3], it could be detected as increases in *Vtiss* and in the cross-sectional area of pulmonary vessels measured by CT. Similarly, potential increases in extravascular lung water resulting from the observed low-grade chronic inflammation in severe obesity could be reflected by changes in *Vtiss*.

Thus, the goals of this study were to compare *Vtiss* and the pulmonary vascular volumes in morbidly obese subjects undergoing BS against matched non-obese healthy controls and to test whether these measurements were different 1 year after BS. Our study evaluates imaging data obtained from individuals in a previous study that exclusively focused on the effects of BS on pulmonary gas exchange [3].

Materials and Methods

Participants, Study Design, and Ethical Issues

This is a post hoc analysis of data obtained in a previously reported prospective study [3]. The principal characteristics and inclusion criteria of 8 normal-weight and age-matched control and 12 obese (body mass index [BMI] $\geq$ 40 kg/m^2 or $\geq$ 35 kg/m^2 with associated morbidities), all females, never-smokers were previously reported and herein included for convenience (Table 1) [3]. Two standard bariatric surgery (BS) procedures, sleeve gastrectomy [11] (n = 10) and Roux-en-Y gastric bypass [12] (n = 2) were used as clinically indicated. Cardiac output was measured using either thermodilution or bioimpedance, and pulmonary artery pressure was determined with a Swan-Ganz catheter in hypoxemic (PaO_2 < 80 mmHg) obese participants alone, before and after BS (52 ± 1 weeks; median, 53 weeks; range, 40–67 weeks). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The study was approved by the ethics committee of the hospital clinic. There were no incidents or significant events during the study [13, 14].

Measurements

Thoracic CT. Scans without contrast were acquired at the end of a full expiration maneuver to residual volume (RV) in order to evaluate the presence of gas trapping. During scanning, patients were in supine position breathing air. The same scanner was always used in the study (Sensation 64, Siemens,

Table 1 Demographic, lung function, systemic and pulmonary hemodynamic findings, and serum biomarkers in control and obese participants, before and after bariatric surgery

	Control individuals (*n*, 8)		Obese individuals (*n*, 12)		
		*p**	Before	*p***	After
Age, years	50 ± 3	NS	48 ± 3		49 ± 3
Weight, kg	62 ± 4	<0.001	118 ± 4	<0.001	80 ± 3
Height, m	1.59 ± 0.02		1.58 ± 0.02		
BMI, kg/m^2	25 ± 2	<0.001	47 ± 1	<0.001	32 ± 1
Waist circumference, cm	82 ± 5	<0.001	125 ± 2	<0.001	98 ± 4
FVC, % predicted	98 ± 2	NS	92 ± 5	<0.01	103 ± 3
FEV_1, % predicted	96 ± 3	NS	91 ± 5	<0.005	103 ± 4
FEV_1/FVC, %	77 ± 1	NS	79 ± 2	NS	79 ± 2
FRC, % predicted	101 ± 9	<0.05	80 ± 4	<0.001	107 ± 4
RV, % predicted	103 ± 8	NS	92 ± 7	NS	108 ± 8
ERV, % predicted	95 ± 16	<0.05	52 ± 8	<0.001	113 ± 10
PaO_2, mm Hg	87 ± 1	NS	80 ± 3	<0.001	94 ± 3
$AaPO_2$, mmHg	18 ± 1	NS	25 ± 3	<0.001	15 ± 3
Q_T, L · min^{-1}	NA		7.0 ± 0.5	<0.005	5.3 ± 0.3
PAP, mmHg	NA		19 ± 1	<0.01	13 ± 1
PVR, dyn · s · cm^{-5}	NA		115 ± 9	NS	85 ± 15
Leucocytes, 10^9/L	6.7 (5.1–7.4)	NS	8.2 (5.9–10.9)	<0.05	6.5 (5.9–6.9)
Neutrophils, %	64 (49–68)	NS	67 (63–73)	<0.01	59 (54–65)
C-reactive protein, mg/L	2.5 (0.8–4.5)	<0.001	12.7 (8.5–14.9)	<0.005	1.9 (1.3–3.8)
Fibrinogen, mg/dL	255 (240–330)	<0.001	410 (358–453)	<0.05	345 (293–350)
Leptin, ng/mL	19.2 (8.8–37.2)	<0.005	61.8 (51.5–64.9)	<0.005	21.9 (10.8–33.1)
Adiponectin, μg/mL	21.9 (15.6–24.3)	NS	19.2 (9.3–20.9)	0.05	22.8 (13.9–26.6)
sTNF-R1, ng/mL	0.6 (0.5–0.8)	<0.05	1.2 (0.9–1.3)	NS	0.9 (0.3–1.2)
IL-8, pg/mL	2.0 (2.0–2.0)	NS	2.0 (2.0–10.3)	NS	2.0 (2.0–2.4)

Data are expressed as mean ± SEM or median (interquartile range)

*Denotes *p* values for comparisons between control and obese individuals before surgery; ** denotes *p* values for comparisons between pre- and post-operative conditions in obese individuals

BMI, body mass index, *FRC* functional residual capacity, *RV* residual volume, *ERV* expiratory reserve volume, $AaPO_2$ alveolar to arterial PO_2 difference, Q_T cardiac output, *PAP* mean pulmonary artery pressure and *PVR* pulmonary vascular resistance determined in hypoxemic (PaO_2 < 80 mmHg: *n*, 6) obese individuals alone, *sTNF-R1* soluble tumor necrosis factor-receptor 1, *NA* not available, *NS* not significant

Germany). Collimation was 64 × 0.6 mm, scanning voltage 120 Kv, and 200 effective mAs. Images were reconstructed with a section thickness of 1.0 mm and 0.7 mm increment using a smooth reconstruction kernel at a 512 × 512 matrix. The CT scanner was calibrated twice a day and its stability was validated every 6 months or after major maintenance work.

Lung volumes and density were quantified using a Leonardo workstation (Siemens. Erlangen, Germany) that generated a region of interest with a window of −1000 to +100 HU that defined the lungs and also excluded large airways and major vessels. The end-expiratory lung volume (EELV), non-aerated lung (NALV), hypo-aerated lung (HALV), and gas trapping were estimated by densitometry using thresholds previously reported [15, 16]. Lung tissue volume (*Vtiss*) was estimated as [17]:

$$\text{Vtiss} = \text{Lung volume} \times (1 + \text{CT number} / 1000).$$

In addition to potential calibration drifts in the scanner, a change in chest wall soft tissue resulting from BS could potentially affect the CT numbers within the lung due to beam hardening and scatter [18, 19]. These potential effects were corrected for using the average values of CT numbers obtained from the trachea and in the aorta. These values were obtained before and after BS from 5 consecutive cross-sectional

slices at the same level of the thorax using anatomic markers in the spine. The corrected CT number (CTcorr) was defined as

$$\mathrm{CTcorr} = [\mathrm{CTn} \times (\mathrm{CTa} - \mathrm{CTt})/1000 + (\mathrm{CTa} + 0.05 \times \mathrm{CTt})]/1.05,$$

where CTn or CT number is the average lung region of interest (ROI) and CTa and CTt are the average CT numbers measured in the aorta and in the trachea, respectively.

Pulmonary Vascular Analysis

Each scan was analyzed using Image J 1.48v (a public domain Java image processing program available at http://imagej.nih.gov/ij). According to the methodology previously described (9), in CT scans acquired at total lung capacity, vessels were identified as round filled elements present after thresholding the slice with a window of (−720 HU) [9]. Since the CT scans of this study were acquired at much lower lung volumes, the use of that window excluded large regions with high density from the analysis of the dependent lungs. For that reason, we used in our analysis a window of −300 HU that included these parts of the lung. Only vessels between 3 and 30 mm^2 were selected for the analysis. The cumulative vessel area was defined as the cumulative sum of vessel number multiplied by its respective cross-sectional area. Vessels were grouped in 9 bins (3-mm^2 width) for describing their distribution. Vessels with area <5 mm^2 were characterized since that size was shown to be affected in COPD [9] and pulmonary hypertension [10]. We took the change in area in vessel <5 mm^2 as a surrogate of small vessel blood volume to explore if changes in *Vtiss* by BS were associated with changes in blood volume.

Serum Inflammatory Markers

As inflammation can be related to PVAT and extravascular lung water, values of serum inflammatory markers previously reported [3, 20] are replicated herein for convenience. C-reactive protein was quantified using an immunoturbidimetry method (Advia Chemistry; Siemens AG) and leptin, adiponectin, soluble tumor necrosis factor-receptor 1 (sTNF-R1), and IL-8 levels were measured using an enzyme-linked immunosorbent assay (Diagnostics Biochem Canada Inc., US BIOLOGIC, IBL International, and Anogen).

Statistical Analysis

Data were expressed as mean ± standard error. A paired *t* test or Wilcoxon's test (for non-normally distributed data) was used to compare measurements before and after BS. Non-paired *t* test or Mann–Whitney *U* test (for non-normally distributed data) was used to compare measurements on obese before BS versus control subjects. Pearson test (or Spearman's for not-normally distributed data) was used to evaluate associations between changes between before and after BS in Vtiss and in vessel area of vessels <5 mm^2 or hemodynamic variables. To compare obese and control individuals, *Vtiss* was standardized by body height. This was based on the observed relationship between *Vtiss* and body height in control and post-operative obese participants, a finding consistent with previous reports (17). Correlations between changes in *Vtiss* and area of vessels <5 mm^2 were determined with and without body height standardization. Significance was considered with a p value <0.05.

Results

Bariatric surgery resulted in a reduction in BMI from 47 ± 1 to 32 ± 1 kg/m^2 ($p < 0.001$) representing a successful excess weight loss of 69 ± 5 %. No differences were observed between the two surgical approaches. Individuals were discharged from hospitalization after BS without complications. C-reactive protein, fibrinogen, and leptin were elevated in obese BS comparing with control individuals and decrease after BS. Leucocytes and neutrophils decreased after BS. Serum levels of sTNF-R1 were elevated in obese subjects before BS but remained unchanged after BS; adiponectin increased after BS (Table 1).

The pre-operative CT of obese subjects revealed that both HALV and *Vtiss* (both with or without height standardization) were significantly higher than those of control subjects, while gas/tissue ratio was lower (Table 2). The cumulative distributions of vessel size, total vessel area, and that of vessels with area <5 mm^2 were not significantly different between obese before BS and control participants (Fig. 1).

Following BS, overall lung function and pulmonary and systemic hemodynamics improved (Table 1) and *Vtiss* significantly decreased (by 8 %) in obese subjects, with a trend towards the values shown in control participants (Table 2 and Fig. 2). By contrast, BS did not affect other CT lung volumes or pulmonary vascular areas (Table 2). No correlations were observed between changes after BS in pulmonary vascular areas and systemic and pulmonary hemodynamic against those in *Vtiss*.

Discussion

This is the first study assessing structural changes of the lung in morbidly obese females who underwent BS and in a control group of lean never-smokers. Our novel finding was that pre-operative *Vtiss*, standardized or not by body height, was higher than in control subjects. After BS, there was a systematic reduction of *Vtiss* among the participants.

Table 2 Thoracic CT and vessel quantification findings

	Control individuals (*n*, 8)		Obese individuals (*n*, 12)		
		*p**	Before	*p***	After
EELV (ml)	2728 ± 287	NS	2339 ± 109	NS	2291 ± 152
Gas trapped (%)	11.7 ± 4.2	NS	3.9 ± 1.2	NS	4.6 ± 0.9
HALV (%)	6.9 ± 1.2	<0.05	12.7 ± 1.5	NS	9.8 ± 1.0
NALV (%)	1.7 ± 0.2	NS	2.1 ± 0.2	NS	2.2 ± 0.1
Gas volume (ml)	1994 ± 257	NS	1484 ± 97	NS	1518 ± 123
Vtiss (ml)	728 ± 32	<0.01	859 ± 27	<0.001	778 ± 25
Gas/tissue ratio	2.65 ± 0.24	<0.05	1.75 ± 0.12	NS	1.94 ± 0.10
Total area (mm^2)	13,664 ± 1231	NS	12,353 ± 575	NS	12,163 ± 436
<5 mm^2	2613 ± 199	NS	2465 ± 109	NS	2418 ± 115

Data are expressed as mean ± SEM

*Denotes *p* values for comparisons between control and obese individuals before surgery; **denotes *p* values for comparisons between pre- and post-operative conditions in all obese individuals

EELV end-expiratory lung volume, *Gas trapped* areas with lung density ranging between −860 to −950 HU, *HALV* hypo-aerated lung volume (ranging between −500 and −100 HU), *NALV* non-aerated lung volume (ranging between −100 to +100 HU), *Vtiss* lung tissue volume, *Gas/tissue ratio* defined as the ratio between gas volume and Vtiss, *Total area* the cumulative area of vessels between 3 and 30 mm^2, *<5 mm^2* the cumulative area of vessels between 3 and 5 mm^2, *NS* not significant

In contrast, BS was not associated with changes in the total area of CT-visible vessels and the post-operative changes in *Vtiss* were not associated with changes in systemic and pulmonary hemodynamics or small vessel areas (3 mm^2 < area < 5 mm^2). As observed in a former study of healthy subjects [17], individual *Vtiss* values in the current study were within the normal limits and significantly correlated with the corresponding subjects' body height. Based on this empirical relationship, we standardized the value of *Vtiss* to the average body height of our subsets of participants before conducting further comparisons between groups. Lung tissue volume has been previously evaluated with CT in several diseases [6, 21]. Given that the pulmonary vascular blood volume remained unchanged, variations in *Vtiss* should track the changes in volume of the lung parenchymal tissue.

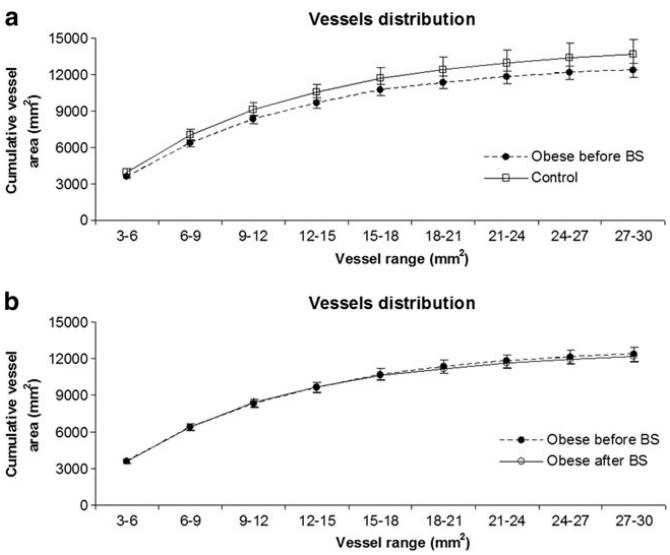

Fig. 1 Mean ± SE values for cumulative areas (normalized by subject's height) per bin and vessels grouped in bins of 3 mm^2 each (range, 3–30 mm^2) in all obese (*closed circles*) and control (*open squares*) individuals (**a**) before bariatric surgery (BS). **b** corresponds to all obese participants before (*closed circles*) and after (*open circles*) BS

Fig. 2 Individual changes in lung tissue volume (standardized by subject's height) (*Vtiss*) in all obese compared to control individuals, before and after bariatric surgery (BS)

Previous studies showed that BS improved lung function and pulmonary gas exchange in the morbidly obese subjects [3, 22, 23] possibly due to a increase in transpulmonary pressure [24, 25]. Based on the possibility that BS-induced changes in *Vtiss* could be due to variations in vascular blood volume, we tested whether changes in *Vtiss* were correlated with variations in the area of small vessels, as a surrogate of possible changes in blood volume. Such analysis showed no significant reductions in the post-operative area of the pulmonary vessels <5 mm^2. Similarly, there were no correlations between changes in *Vtiss* and any pulmonary hemodynamic changes following BS. Therefore, we need to conclude that factors other than changes in the blood volume of small vessels were mostly responsible for the observed changes in *Vtiss* by BS.

It is conceivable that systemic (and pulmonary) inflammation could have increased extravascular lung water and thus been in part responsible for the elevated *Vtiss* in obese subjects before BS compared with controls and for its subsequent reduction after BS. Accordingly, our findings are consistent with increased serum inflammatory biomarkers before BS and their post-operative reduction (Table 1). In addition, changes in *Vtiss* could have also been related to a potential reduction in PVAT after BS [4], as suggested by the improvement in V_A/Q distributions shown previously [3]. From our data, however, it is not possible to unravel which of these mechanisms was more influential.

The fact that changes in *Vtiss* and in vascular area were not correlated may suggest that most of the variations in *Vtiss* were likely not induced by changes in small vessel size. However, if pulmonary vascular changes were in part causing changes in *Vtiss*, then other confounding factors could have modulated vessel size. For example, the accumulation of PAVT could have made the vessels apparently thicker preoperatively but inter-subject variability in pulmonary vasomotor tone and trans-mural vascular pressure could have obscured potential changes in vascular area by BS. It has been demonstrated that obesity enhances the anti-contractile properties induced by PVAT [4, 26], an effect that is restored after BS [4]. Such abnormalities could affect the pulmonary vascular wall tone and vessel size. Taken together, these competing effects may have hidden a direct relation between vessel area and *Vtiss*.

Lastly, and contrary to our expectations, there was no significant reduction in post-operative hypo-aerated lung areas. This may be a relevant issue as part of the explanation underlying post-BS lung function improvement may be related to more complex mechanisms, such as V_A/Q distribution improvement, as previously demonstrated by our group [3].

Strengths and Limitations

The strength of our study was its longitudinal design in a cohort of morbidly obese subjects to provide comparative insights of the interplay between lung volumes and pulmonary vascular factors. There were however some shortcomings. One was the small number of participants due to the stringent requirements of our study design and the density of the data collected, although it is remarkable that significant associations of *Vtiss* with individual body height and significant differences in *Vtiss* could be detected in spite of the small number of subjects. Another limitation was the exclusive study of obese females, expected to have better gas exchange than males due to different distributions of adipose tissue [27]. Moreover, we cannot overlook that pre-operative areas of atelectasis and/or hypo-aerated lung volume could have been present, hence limiting the reliability of the threshold-based segmentation algorithms and reducing the actual lung area included. This potential weakness could have also been important for the CT vessel quantification. To minimize these confounding effects, we carefully evaluated CT scans to ensure that areas of atelectasis, if present, were included. Even so, if there was a systematic exclusion of atelectatic areas during segmentation, that effect would have probably induced an underestimation in pre-operative *Vtiss* since atelectasis would have been more likely present before BS, other factors being equal. The quantification algorithm identified vessels as round elements within the CT slices. Thus, this approach only measured the area of

vessels running perpendicular to the CT gantry. However, because the CT slices were axial in all scans, the measurement is expected to track changes in vessel numbers and size distributions. Although the latter limitations tone down the conclusions on potential vascular effects on *Vtiss*, they do not invalidate the primary endpoint of our study. Namely, the systematic decrease *Vtiss* in obese individuals after BS observed irrespective of the corrections for changes in CT calibration or changes in chest wall scattered effects.

Conclusions

In this subset of severely obese women, lung tissue volume was elevated compared to lean individuals and systematically decreased 1 year after BS. These differences could not be explained by changes in lung blood volume and may be related to elevated extravascular lung water due to low-grade systemic and pulmonary inflammation and/or to hypertrophic perivascular adipose tissue in severe obesity. This contention would be consistent with the hypothesized association between inflammation-induced endothelial dysfunction and the V_A/Q mismatching in obesity previously demonstrated by our group [3].

Authors' Contributions RR-R and JGV are the guarantors of the manuscript and take responsibility for the integrity of the data and the accuracy of the data analysis, respectively. ER, EA, MS, and RR-R were involved in the planning and coordination of the study. ER and MS conducted the data collection and experimental work. RR-R, JGV, AS, JR-C, and ER contributed to the design and implementation of the imaging analysis algorithms and interpretation of the results of study. AS, ER, RR-R, JR-C, and JGV contributed to the writing of the article.

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Compliance with Ethical Standards

Conflict of Interest Statements The authors declare that they have no conflict of interest.

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5. DISCUSSION

DISCUSSION

The three articles of the current thesis are centered on a comprehensive assessment of pulmonary gas exchange disturbances in severe obesity including their relationships with routine lung function, thoracic imaging, and systemic inflammation, before and after bariatric surgery.

Manuscript 1. Ventilation/perfusion distribution abnormalities in morbidly obese subjects before and after bariatric surgery.

The principal results of this study confirms our working hypothesis by showing that: *(1)* very severe obesity is associated with mild-to-moderate pulmonary gas exchange disturbances and an abnormal pulmonary vascular tone with the development of reabsorption *atelectasis* during oxygen breathing; and, *(2)* bariatric surgery not only reduces body mass index and systemic inflammation but also improves most ventilation-perfusion indices, hence supporting a cause-effect relationship between body weight, systemic inflammation and pulmonary gas exchange abnormalities.

Previous studies

To our knowledge, our study is the first to use a combined functional and biochemical research design to investigate overall lung function in morbidly obese individuals before and after BS. However, previous papers have explored some of these aspects isolately being in general our findings consistent with them. For instance, as in previous studies,[25] we found evidence of low-grade systemic inflammation. Similarly, surgery was well tolerated and had a profound weight-reduction effect.[72] In keeping with some[35,36] but not all previous studies[39,42] we observed that static lung volumes were relatively well preserved in our population, discrepancies likely being explained by our stringent recruitment criteria that excluded the coexistence of moderate-to-severe multi-morbidities, such as

OSAS. Also in keeping with our observations, previous studies reported the presence of widened $AaPO_2$ and mild arterial hypoxemia in obese participants,[35,45] as well as significant associations between WC and WHR with lung volumes and gas exchange indices.[40] Although no previous study has used the MIGET to assess the distribution of V_A/Q ratios in these subjects, an investigation in the 1960s using oxygen-*shunt* measurements in a few obese individuals indicated that arterial hypoxemia was related to perfusion of unventilated areas (*i.e.*, *shunt*).[51] Likewise, the use of a radioactive tracer in the lungs in obese subjects demonstrated that perfusion was maximal in the lower zones whereas ventilation was significantly reduced, a regional V_A/Q imbalance closely associated to the parallel reduction in ERV.[50] The amounts of *shunt* observed in our study are in agreement with these findings.

Interpretation of findings

Three main novel findings of our study deserve specific discussion: *(1)* the unique pattern of pulmonary gas exchange abnormalities observed before surgery; *(2)* the differential effects of 100% oxygen breathing before and after surgery; and, *(3)* the observation that all gas exchange abnormalities but the dispersion of alveolar ventilation distribution (Log SDV) were improved after surgery.

Pulmonary gas exchange abnormalities before bariatric surgery. Despite minor impairment of lung volumes, we observed that morbidly obese individuals had pulmonary gas exchange abnormalities characterized by widened $AaPO_2$ and mild arterial hypoxemia, due to mild-to-moderate *shunt* and increased dispersion of both pulmonary blood flow (Log SDQ) and alveolar ventilation (Log SDV) distributions. It is of note that both PaO_2 and $AaPO_2$ values in obese individuals were worse than in previous studies (76 *versus* 90 mmHg),[82] due to their older age (51±[SD]8 *versus* 38±10 yrs, respectively)[82] and to the enrolment of a large proportion of hypoxemic morbid obese.

The presence of mild-to-moderate intrapulmonary *shunt* during ambient air is similar to that shown in patients with extensive pleural effusion (volume range, 0.5-1 L) that physically compresses the underlying normal lung parenchyma. **Table 8** shows the gas exchange outcomes of 9 patients with pleural effusion before thoracentesis. Although PaO_2 was normal, $AaPO_2$ was slightly increased and they had broadened unimodal V_A/Q distributions, namely increased Log SDQ and mild intrapulmonary *shunt*, as cardinal mechanisms underlying their arterial hypoxemia.[93] Accordingly, we propose that the excessive abdominal adipose tissue that characterizes very severe obesity results in an excessive and unopposed intra-abdominal pressure that compresses the lower regions of the lungs and causes mild amounts of *shunt* and low V_A/Q ratios (increased Log SDQ). In addition, in the condition of pleural effusions there are marginal areas with low V_A/Q units, likely related to the different degree of lung collapse caused by the accumulation of fluid in the pleural cavity, also akin to our abnormal gas exchange findings.

Table 8. Pulmonary gas exchange findings in 9 patients with pleural effusion

Outcomes	Baseline
PaO_2, mm Hg	82 ± 10
$AaPO_2$, mm Hg	29 ± 10
***Shunt*, % Q_T**	6.9 ± 6.7
Low V_A/Q, %Q_T	1.4 ± 2.2
Log SDQ	0.72 ± 0.29

Definition of abbreviations: *$AaPO_2$, alveolar to arterial oxygen pressure (normal values, <15-20 mmHg; Shunt, non-ventilated units (V_A/Q· <0.005, expressed as % of Q_T; normal values, <1%); Low V_A/Q, poorly ventilated units (V_A/Q <0.1 excluding shunt, expressed as % of Q_T; normal values, %); Log SDQ, dispersion of pulmonary blood flow; normal values <0,60) (taken, in part, from ref. 93).*

A complementary explanation may be related to the presence of systemic inflammation, known to alter normal vascular contractile activity, and likely pulmonary vascular tone,[29] hence potentially influencing V_A/Q ratio distributions, as discussed below (see *"Effects of*

100% oxygen breathing"). Nonetheless, from our data we cannot differentiate which one of these two potential mechanisms is more operative or relevant since BS reduced both body weight and systemic inflammation.

To note that, in terms of nonpulmonary determinants of pulmonary gas exchange, *i.e.* hemodynamic and ventilatory parameters, were within normal limits in our subset of obese subjects before BS .

Effects of 100% oxygen breathing. During oxygen breathing, the bigger the *shunt* the greater the fall in the dispersion of pulmonary blood flow (Log SDQ), hence indicating abnormal pulmonary blood flow redistribution. This increment of *shunt* is likely to be related to the development of lung areas with reabsorption *atelectasis*, resulting from alveolar denitrogenation,[66,67] without reversion of hypoxic pulmonary vasoconstriction. Under conditions of high F_IO_2, it has been demonstrated that low inspired V_A/Q ratios alveolar units, named *"critical"*, become unstable and may ultimately collapse, thereby resulting in the development of *atelectasis*.[67] In addition, the simultaneous increase in mixed venous PO_2 also reduces the pulmonary vascular tone.[63] Our findings may therefore reflect more rigid pulmonary vessels due to increased systemic (and likely pulmonary) perivascular adipose tissue-induced endothelial dysfunction.

This gas exchange pattern characterized, on the one hand, by mild-to-moderate *shunt* during ambient air and, on the other, by oxygen-induced increased intrapulmonary *shunt* without reversion of HPV mimics that observed in patients with ALI,[66] a phenomenon related to underlying pulmonary vascular changes. **Figure 6** shows the effects of a 1 h period of 100% oxygen breathing on several pulmonary gas exchange indices in two subsets of patients (*i.e.*, ALI and COPD). Patients affected by ALI significantly increased intrapulmonary *shunt*, whereas Log SDQ remained unaltered; by contrast, those with COPD increased the Log SDQ with a trend to increase the areas of low V_A/Q ratios, without any change at all in intrapulmonary *shunt*. In ALI, hyperoxia-induced increased intrapul-

monary *shunt* is not associated with the release of HPV release as reflected by a lack of change (instead of an increase) of the dispersion of blood flow (Log SDQ).[66]

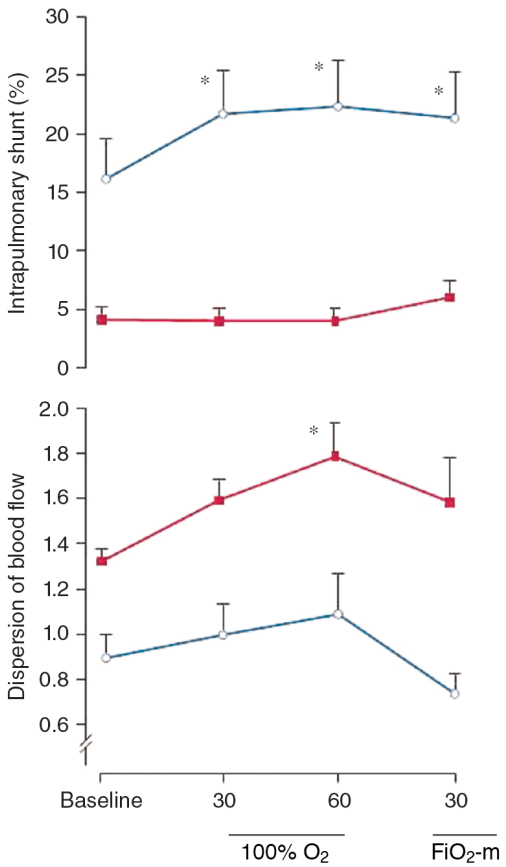

Figure 6. Time course (x axis, in minutes) of hyperoxia-induced effects on shunt (% of Q_T) and the dispersion of pulmonary blood flow distribution (Log SDQ) in patients with ALI (blue color) and in those with COPD (red color). Data points are mean±SEM values and asterisks denote significant differences ($p < 0.05$) between each time point and baseline value within each subset of patients. FIO_2-m = maintenance O_2 fraction (taken, in part, from refs. 63)

In healthy subjects, leptine and adiponectin cytokines secreted by adipocytes modulates systemic vascular tone by increasing NO bioavailability, but this is lost in obesity.[27] Thus, in obese subjects there is an accumulation of adipose tissue, so that the local inflammation induces a paradoxical inhibition of the beneficial perivascular adipose tissue vasodilation, more specifically evidenced in the cutaneous circulation.[29] Our findings can be, therefore, consistent with abnormal vascular contractility in morbidly obese subjects.[29]

Likewise, during 100% oxygen breathing there are hemodynamic changes. Indeed, both systemic vascular resistances and systemic arterial pressure increase, primarily driven by the oxygen-induced reduction in cardiac output (and cardiac index as well) following decreased oxygen consumption. This is an expected hemodynamic response, also observed in other respiratory conditions, such as COPD.[94]

Findings one year after bariatric surgery. In parallel with significant weight loss and reduction of systemic inflammation after BS, all pulmonary gas exchange (including V_A/Q distributions) abnormalities but the dispersion of alveolar ventilation (Log SDV) were also considerably improved but not abolished, akin to the remnants of mild obesity and systemic inflammation (serum inflammatory biomarkers decreased but still abnormal). Although mechanical factors con undoubtedly play a role, the fact that the correlation between oxygen-induced changes in *shunt* and Log SDQ after BS was lost suggests improvement in pulmonary blood flow redistribution. This would be in keeping with experimental evidence indicating that perivascular adipose tissue-induced vasodilatation of small arteries (likely including pulmonary vasculature) can be restored after BS.[29] Hence, our observations could support a causal role of obesity on pulmonary gas exchange and vascular tone abnormalities.

Except for systemic vascular resistance, ventilatory, metabolic and hemodynamic outcomes decreased after BS. Likewise, the higher arterial PO_2 increase while breathing 100% O_2 after BS is explained by the smaller *shunt* values (from 9.8±1.7, before surgery, to 3.7±1.0 $\%Q_T$) after surgery instead of the effect of hypoxic pulmonary vasoconstriction release because pulmonary vascular resistance remained unchanged. Finally, the decrease of pulmonary artery pressure after BS (19±1 to 13±1 mmHg, while breathing ambient air), suggests less apical perfusion in the lung and thus the development of high V_A/Q regions after surgery, which would increase the dispersion of alveolar ventilation distribution (Log SDV), other things being equal.

Strengths and limitations

Our study has strengths and limitations. Among the former, the fact that we used MIGET for the first time to assess very comprehensively pulmonary gas exchange disturbances in this clinical scenario. Multiple inert gas elimination technique is the most robust tool to investigate the pulmonary and nonpulmonary determinants of gas exchange in humans. Among the latter, the fact that we studied only females due to the sex differences related to pulmonary gas exchange in morbidly obese individuals[41,95] and the higher prevalence of this disease in females. Hence, our results may not be directly extrapolated to males.

Second Manuscript. Postural effects on pulmonary gas exchange abnormalities in severe obesity before and after bariatric surgery.

This second study shows that, in this overall subset of morbidly obese without major multi-morbidities candidates to BS, pulmonary gas exchange including arterial blood gases remain essentially unchanged when moved from upright to supine postures as was also observed in control subjects. Unexpectedly, hypoxemic obese individuals actually improved gas exchange when supine. By contrast, after BS, all obese participants considered together considerably improved pulmonary gas exchange when upright (compared to pre-BS), but then deteriorated when supine. All pre- and post-operative arterial blood gases changes were small and were not associated with hemodynamic and V_A/Q ratio significant changes. Yet, the positive effect of the supine posture in the hypoxemic morbidly obese should be considered when assessing obesity in the clinical settings of respiratory, sleep, anesthesia and critical care medicine.

Previous studies

Our findings in normoxemic obese individuals are at variance with those shown by *Farebrother et al.*[43] who investigated normoxemic obese smokers of both sexes, before and after starvation, and showed PaO_2 decreases when supine before and after weight loss. Alternatively, *Vaughan et al.*[44] observed no effects on arterial blood gases in mild hypoxemic obese young nonsmoking females, before and at the third day after conventional surgery. Because these two studies differed considerably in design and inclusion criteria, direct comparisons with our results are difficult. Discrepancies can be also likely explained by our recruitment criteria which excluded the coexistence of severe multi-morbidities.

Interpretation of findings

Contrary to our original hypothesis, *i.e.* pulmonary gas exchange disturbances in obese

participants before BS would worsen in parallel to the expected deterioration of *shunt* and of pulmonary vascular disturbances from upright to supine,[55] pulmonary gas exchange abnormalities in our obese subset considered as a single group remained unchanged as well as in obese subjects with normal PaO_2 from upright to supine. By contrast, in hypoxemic obese individuals, arterial oxygenation improved when supine, a feature typically observed, although of slightly more magnitude, in patients with hepatopulmonary syndrome.[96,97] Nevertheless, the underlying mechanisms differ. In hepatopulmonary syndrome, *orthodeoxia* (upright-induced arterial deoxygenation) is caused by further V_A/Q imbalance and increased intrapulmonary *shunt* without changes in non-pulmonary (*i.e.,* minute ventilation and cardiac output) determinants of gas exchange (**Figure 7**),[96,97] likely produced by an abnormal pulmonary vasculature with more heterogeneous gravitational blood flow redistribution to dependent lung areas.[96,97] Actually, *orthodeoxia* in patients with chronic hepatic diseases reflects an imperfect pulmonary vascular tone, more rigid and fixed, due to an abnormal pulmonary vasculature less liable to proportionately accommodate gravitational blood flow changes to ventilation in dependent alveolar units. This is consistent with the finding that the pulmonary circulation of cirrhotic patients behaves paradoxically, combining a lower, or even absent, hypoxic vascular response but also some degree of hypoxic vasoconstriction reversion during 100% oxygen.[63]

By contrast, in hypoxemic morbidly obese subjects, *orthodeoxia* is due to upright-induced decreased cardiac output without accompanying changes in pulmonary factors (increased *shunt* and V_A/Q mismatching) that modulate gas exchange. *Orthodeoxia* in morbidly obese population likely points to a more altered pulmonary vasculature due to excessive perivascular adipose tissue-induced endothelial dysfunction.[98] However, we can assume that in clinical conditions of more increases in cardiac output in the hypoxemic morbidly obese when supine should be accompanied by higher increases in intrapulmonary *shunt*, it is most likely that the net effect would have offset the observed improvement in arterial oxygenation.

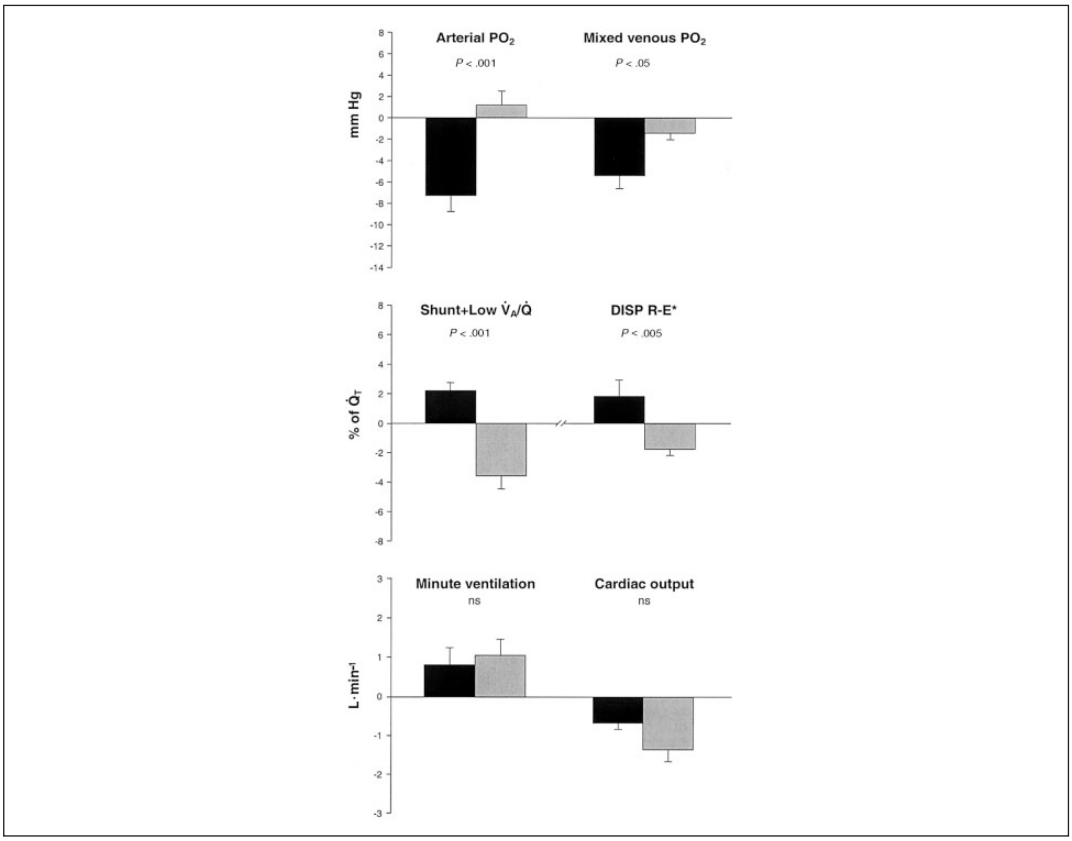

***Figure 7. Postural-induced changes on pulmonary gas exchange descriptors, minute ventilation and cardiac output in patients with hepatopulmonary sindrome with (solid bars) and without (gray bars) orthodeoxia. Orthodeoxia (upright-induced arterial deoxygenation) is caused by significantly further V_A/Q imbalance and increased* intrapulmonary *shunt* without changes in nonpulmonary gas exchange determinants (minute ventilation and cardiac output) (taken, in part, from ref. 96)**

Accordingly we suggest adding severe obesity to the list of etiologies of orthodeoxia, with or without underlying dyspnea (so-called, platypnea), as it has been very recently published **(Table 9)**.[99]

Alternatively, the absence of arterial blood gases changes in all obese subjects when supine can be related to the combination of small postural-induced lung volume changes[54,55] along with the absence of slightly more noticeable cardiac output effects.

Table 9. Etiology of orthodeoxia with or without dyspnea (platypnea): a pathophysiological basis

1.	**Intracardiac *shunts***
	Atrial septal defect
	Patent foramen ovale
2.	**Intrapulmonary *shunts***
	Hepatopulmonary syndrome
	Lower lobe arterial venous malformation
3.	**Ventilation-perfusion mismatch**
	Interstitial lung disease Antisynthetase syndrome; myositis-associated interstitial lung disease Idiopathic pulmonary fibrosis Nonspecific interstitial lung disease Interstitial fibrosis Cryptogenic fibrosing alveolitis
	Infections Cytomegalovirus pneumonia Pneumocystis jiroveci pneumonia
	Pulmonary embolism

Taken, in part, from ref. 99.

In a recent study in morbidly obese individuals, lung volumes were more restricted than in healthy individuals along with important reductions in expiratory reserve volume (ERV) and in end-tidal functional residual capacity (FRC) when upright, but similar when supine[55]. In another study investigating supine changes in obese and lean subjects, total lung capacity (TLC) and its subdivisions, it was shown that despite the increased extra-pulmonary mass load in obese subjects, further falls in TLC and FRC were negligible when supine.[54] This was not the case in our subset of hypoxemic obese subjects in whom the increase in cardiac output played a positive influential effect on PaO_2 through mixed venous PO_2. Of note that these minor postural changes were at variance with the conspicuous effects on gas exchange observed during 100% oxygen breathing when upright in our previous study.[98] It remains unknown though what can be the duration of this supine-induced PaO_2 improvement in the clinical setting of the real-world of morbidly obese individuals. It may be plausible that after a few hours at supine, the increase in cardiac

output is limited due to a re-accommodation of systemic and pulmonary blood flow to prolonged postural change, so that the presence of *orthodeoxia* is not patent any more.

In parallel to the substantial post-operative weight loss,[98] gas exchange indices but the alveolar ventilation distribution (Log SDV) in all obese individuals improved when upright. Interestingly, after BS, PaO_2 was reduced when supine in all obese and in pre-operatively hypoxemic subjects. It is most likely that this observation may be again related to the remnants of both mild obesity and systemic inflammation.[23,98] Likewise, our findings indicate that, in addition to the improvement in abnormal lung mechanics,[55] provoked by considerable weight loss, V_A/Q matching is restored. However, although the post-operative supine-induced deleterious effects on arterial oxygenation remain ultimately obscure, we have to consider that the Log SDV improvement alone was the result of considerable pulmonary vascular changes within the context of a post-operative remnant of mild obesity as alluded to in the first study.[23,98] Whether the current findings can improve or worsen potential arterial blood gas changes during sleep, anesthesia, surgery and critical care medicine settings remains unknown so that further investigations need to be conducted.

Strengths and limitations

There were strengths and shortcomings in our study. First, this is the first study that so far has addressed the effects of postural changes on pulmonary gas exchange before and after BS through a comprehensive design approach, including measurements of systemic and pulmonary hemodynamic and inert gas studies. Second, the same participants were studied before and one year after BS, thus providing a robust comparative insight into the difficult interplay of pulmonary and nonpulmonary factors governing pulmonary gas exchange.[63] However, there were some limitations. One was the small number of patients due to the stringent complexity of our study design. Another shortcoming was the exclusive inclusion of females given the fact that males have worse gas exchange than females

due to the different regional distribution of adipose tissue. Accordingly, our current data cannot be necessarily extrapolated to very severe obese males or to those with severe multi-morbidities.

Third Manuscript: Lung tissue volume is elevated in obesity and reduced by bariatric surgery.

This is the first study assessing structural changes of the lung in morbidly obese females who underwent BS and in a control group of lean never-smokers. Our novel finding was that pre-operative lung tissue volume (*Vtiss*), whether or not standardized by body height, in obese participants was higher than in control subjects. However, after BS there was a systematic reduction of *Vtiss* among the obese participants. In contrast, BS was not associated with changes in the total area of computed tomography (CT) visible vessels and the post-operative changes in *Vtiss* were not associated with changes in systemic and pulmonary hemodynamics or small vessel areas (3 mm^2 < area < 5 mm^2). As observed in a former study of healthy subjects,[100] individual *Vtiss* values in the current study were within the normal limits and significantly correlated with the corresponding subjects' body height. Based on this empirical relationship we standardized the value of *Vtiss* to the average body height of our subsets of participants before conducting further comparisons between groups. Lung tissue volume has been previously evaluated with CT in several diseases.[88,101] Given that the pulmonary vascular blood volume remained unchanged, variations in *Vtiss* should track the changes in volume of the lung parenchymal tissue.

Previous studies had shown that BS improved lung function and pulmonary gas exchange in the morbidly obese[98,102,103] possibly due to a increase in trans-pulmonary pressure.[54,55] Based on the possibility that BS-induced changes on *Vtiss* could be due to variations in vascular blood volume, we tested whether changes in *Vtiss* were correlated with variations in the area of small vessels, as a surrogate of possible changes in blood volume. Such analysis showed no significant reductions in the post-operative area of the pulmonary vessels < 5 mm^2. Similarly, there were no correlations between changes in *Vtiss* and any pulmonary hemodynamic changes following BS. Therefore, we need to conclude that factors other than changes in the blood volume of small vessels were mostly responsible

for the observed changes in *Vtiss* by BS.

It is conceivable that systemic (and pulmonary as well) inflammation could have increased extra-vascular lung water and thus been in part responsible for the elevated *Vtiss* in obese subjects before BS compared with controls and for its subsequent reduction after BS. Accordingly, our findings are consistent with the presence of increased serum inflammatory biomarkers before BS and their post-operative reduction (*see above, First Manuscript*). In addition, changes in *Vtiss* could have also been related to a potential reduction in perivascular adipose tissue after BS,[29] as suggested by the improvement in V_A/Q distributions shown in our first study.[98] From our data, however, it is not possible to unravel which of these mechanisms was more influential.

The fact that changes in *Vtiss* and in vascular area were not correlated may suggest that most of the variations in *Vtiss* were likely not induced by changes in small vessels size. However, if pulmonary vascular changes were in part causing changes in *Vtiss*, then other confounding factors could have modulated vessel size. For example, the accumulation of perivascular adipose tissue could have made the vessels apparently thicker pre-operatively but inter-subject variability in pulmonary vasomotor tone and trans-mural vascular pressure could have obscured potential changes in vascular area by BS. It has been demonstrated that obesity enhances the anti-contractile properties induced by perivascular adipose tissue,[27,29] an effect that is restored after BS.[29] Such abnormalities could affect the pulmonary vascular wall tone and vessel size. Taken together, these competing effects may have hidden a direct relation between vessel area and *Vtiss*.

Lastly, and contrary to our expectations, there was no significant reduction in post-operative hypo-aerated lung areas. This may be a relevant issue as part of the explanation underlying post-BS lung function improvement may be related to more complex mechanisms, such as V_A/Q distributions improvement, as previously demonstrated by our

group.[98] An alternatively explanation is that the power resolution of CT lung imaging, a state-of-the art topographical approach, is different from that offered by MIGET, a very robust functional approach. Accordingly, if we admit that MIGET is more sensitive than CT lung imaging to detect the amount of *shunt*, it is expected to see more noticeable changes in inert gas *shunt* and fewer in computed tomography lung hypo-aerated and non-aerated lung areas.

Strengths and limitations

The strength of our study was its longitudinal design in a cohort of morbidly obese subjects to provide comparative insights of the interplay between lung volumes and pulmonary vascular factors. There were however some shortcomings. One was the small number of participants due to the stringent requirements of our study design and the density of the data collected, although it is remarkable that significant associations of *Vtiss* with individual body height and significant differences in *Vtiss* could be detected in spite of the small number of subjects studied. Another limitation was the exclusive study of obese females, expected to have better pulmonary gas exchange than males due to different distributions of adipose tissue, a criticism already raised in the two previous studies.[41] Moreover, we cannot overlook that pre-operative areas of *atelectasis* and/or hypo-aerated lung volume could have been present, hence limiting the reliability of the threshold based-segmentation algorithms and reducing the actual lung area included. This potential weakness could have also been important for the CT vessel quantification. To minimize these confounding effects we carefully evaluated scans to ensure that areas of *atelectasis*, if present, were included. Even so, if there was a systematic exclusion of *atelectatic* areas during segmentation, an effect that would have probably underestimated pre-operative *Vtiss* since *atelectasis* would have been more likely present before BS, other factors being equal. The quantification algorithm identified vessels as round elements within the CT slices. Thus, this approach only measured the area of vessels running perpendicular to the CT gantry. However, because the CT slices were axial in all scans,

the measurement is expected to track changes in vessel numbers and size distributions. Although the latter limitations tone down the conclusions on potential vascular effects on *Vtiss*, they do not invalidate the primary end-point of our study. Namely the systematic decreased *Vtiss* in obese individuals after BS observed irrespective of the corrections for changes in CT calibration or changes in chest wall scattered effects.

6. CONCLUSIONS

CONCLUSIONS

First Manuscript

This study shows that even in the absence of major lung volume abnormalities, morbidly obese individuals have ventilation-perfusion unbalance. These ventilation-perfusion abnormalities are characterized by mild-to-moderate *shunt* and increased dispersions of both pulmonary blood flow and alveolar ventilation distributions. Moreover, during oxygen breathing, the bigger the *shunt* the greater the fall in the dispersion of pulmonary blood flow likely related to the development of lung areas with *reabsorption atelectasis*, without reversion of hypoxic pulmonary vasoconstriction. It is of note that after bariatric surgery all pre-operative pulmonary gas exchange disturbances alluded to were significantly reduced (***Rivas E et al. Ventilation/perfusion distribution abnormalities in morbidly obese subjects before and after bariatric surgery. Chest 2015; 147: 1127-1134. doi: 10.1378/chest.14-1749***).

Second Manuscript

Before bariatric surgery, in normoxemic obese subjects arterial oxygenation is not altered but in hypoxemic individuals is paradoxically improved when supine. After successful bariatric surgery, pulmonary gas exchange considerably improved while arterial oxygenation increased in all obese and in pre-operatively hypoxemic individuals when upright but decreased when supine. Postural-induced arterial blood gases changes are small and of limited clinical relevance. However, the finding that the supine posture supine does not induce arterial deoxygenation during a short period of time (less than one our) prior to surgery can be of interest to intensive care physicians and anesthesiologists for their daily clinical practice. Obese individuals are usually placed on supine to induce anesthesia, intubation and instrumentation before any surgical intervention (***Rivas E et al. Postural effects on pulmonary gas exchange abnormalities in severe obesity before and after bariatric surgery. Minerva Anestesiologica 2015 Jun 9 [Epub ahead of print]. PMID: 26054299***).

Third Manuscript

In this subset of severe obese women, lung tissue volume was elevated compared to lean individuals and systematically decreased one year after bariatric surgery. These differences could not be explained by changes in lung blood volume and may be related to elevated extra-vascular lung water due to low-grade systemic and pulmonary inflammation and/or to hypertrophic perivascular adipose tissue in severe obesity. This contention would be consistent with the hypothesized association between inflammation-induced endothelial dysfunction and ventilation-perfusion mismatching in obesity previously demonstrated in the first study of this thesis[98] (***Santos A and Rivas E et al. Lung tissue volume is elevated in obesity and reduced by bariatric surgery.*** **Obesity Surgery 2016 Mar 21 [Epub ahead of print]. doi: 10.1007/s11695-016-2137-9).**

7. SUMMARY

Manuscript 1. ***Ventilation/perfusion distribution abnormalities in morbidly obese subjects before and after bariatric surgery.***

Background: Obesity is a global and growing public health problem. Bariatric surgery (BS) is indicated in patients with morbid obesity. To our knowledge, the effects of morbid obesity and BS on ventilation/perfusion (V_A/Q) ratio distributions using the multiple inert gas elimination technique have never before been explored.

Methods: We compared respiratory and inert gas (V_A/Q ratio distributions) pulmonary gas exchange, breathing both ambient air and 100% oxygen, in 19 morbidly obese women (BMI, 45 ± 1 kg/m^2), both before and 1 year after BS, and in eight normal-weight, never smoker, age-matched, healthy women.

Results: Before BS, morbidly obese individuals had reduced arterial PO_2 (76 ± 2 mm Hg) and an increased alveolar-arterial PO_2 difference (27 ± 2 mm Hg) caused by small amounts of *shunt* (4.3 ± 1.1% of cardiac output), along with abnormally broadly unimodal blood flow dispersion (0.83 ± 0.06). During 100% oxygen breathing, *shunt* increased twofold in parallel with a reduction of blood flow to low V_A/Q units, suggesting the development of reabsorption *atelectasis* without reversion of hypoxic pulmonary vasoconstriction. After BS, body weight was reduced significantly (BMI, 31 ± 1 kg/m^2), and pulmonary gas exchange abnormalities were decreased.

Conclusions: Morbid obesity is associated with mild to moderate *shunt* and V_A/Q imbalance. These abnormalities are reduced after BS.

Manuscript 2. ***Postural effects on pulmonary gas exchange abnormalities in severe obesity before and after bariatric surgery*****.**

Background: We hypothesized that in morbid obesity, pulmonary gas exchange abnormalities will worsen when supine and that bariatric surgery (BS) will mitigate this effect.

Methods: Gas exchange was investigated in 19 morbidly obese and 8 non-obese, age-matched control females, spontaneously breathing ambient air, both upright and supine, before and one year after BS.

Results: In control non-obese individuals, no postural changes in arterial blood gases (ABGs) were observed. While obese subjects had more altered PaO_2, SaO_2 and $AaPO_2$ values than controls ($p<0.05$ each) when upright, the values unexpectedly remained unchanged when supine. This was also the case in the subset of 6 normoxemic obese but the remaining 13 hypoxemic individuals actually improved ABGs when supine: $AaPO_2$ (by –3.4 ± 1.4 mmHg), SaO_2 (by +1.5 ± 0.6 %), pH (by +0.01 ± 0.01); and cardiac output increased (by +0.4 ± 0.2 $L \cdot min^{-1}$) ($p<0.05$ each). After BS, PaO_2 (from 75.5 ± 2.4 to 89.4 ± 2.4 mmHg) and $AaPO_2$ (from 27.0 ± 2.0 to 15.4 ± 2.1 mmHg) ($p<0.05$ each) and pulmonary gas exchange were improved compared to before BS when upright, but ABGs worsened when supine (PaO_2, by –4.6 ± 1.7 mmHg; $AaPO_2$, by +4.2 ± 1.6 mmHg) ($p<0.05$ each).

Conclusions: Before BS, ABGs are not altered in normoxemic obese subjects moving from upright to supine, even improving in those with hypoxemia when supine. After successful BS, pulmonary gas exchange improved when upright in all subjects but ABGs deteriorated when supine. However, the important clinical observation is the lack of gas exchange deterioration when supine, which may have implications for critical care and anesthesia settings.

Manuscript 3. ***Lung tissue volume is elevated in obesity and reduced by bariatric surgery.***

Background: Bariatric surgery (BS) in severely obese subjects causes a significant reduction of body weight and improvement in lung function. We have shown previously that abnormalities in pulmonary gas exchange in morbidly obese are substantially improved with BS. These abnormalities were thought to be related to reduced lung volumes as well as to abnormal endothelial function induced by low-grade chronic inflammation linked to perivascular adipose tissue (PVAT). In this study we used computed tomography (CT) to assess whether BS also caused measurable structural changes in the lung. We focused in lung tissue volume (*Vtiss*) and cross-sectional vessel analysis hypothesizing that these measures could be related to the previously reported lung functional changes.

Methods: Pulmonary vessels and lung volumes, including *Vtiss,* were quantified in thoracic CT scans. We compared findings in 12 obese women before and after BS and in 8 healthy lean women.

Results: *Vtiss* was significantly elevated in obese subjects before BS compared to control subjects and systematically reduced after BS (by 8%); other CT lung volumes or vascular areas were not affected in a consistent manner. No relationship was observed between BS-induced individual changes in *Vtiss* and pulmonary vessel area.

Conclusions: *Vtiss* is elevated in morbidly obese, compared to lean individuals of similar body height, and is systematically reduced by BS. These effects do not appear related to vascular changes but may be caused by elevated extra-vascular lung water, due to low-grade inflammation, and/or hypertrophic PVAT in severe obesity.

8. SUMMARY IN SPANISH

Resumen. Primer artículo (*First Manuscript*). *Anomalías de las distribuciones ventilación-perfusión en la obesidad grave, antes y después de cirugía bariátrica.*

En la actualidad, la obesidad es un grave problema de salud pública a escala mundial. La cirugía bariátrica (CB) es un tratamiento efectivo de la obesidad grave. No existen trabajos previos sobre los efectos de la obesidad grave y la pérdida ponderal inducida por CB sobre el intercambio gaseoso pulmonar, específicamente en la distribución de las relaciones ventilación-perfusión (V_A/Q) con el empleo de la técnica de gases inertes múltiples (TEGIM).

Métodos: Se investigó el intercambio gaseoso pulmonar mediante la TEGIM en 19 mujeres obesas graves (índice de masa corporal [IMC] 45 ± 1kg/m^2) antes y después CB y, también en 8 mujeres con peso normal, no fumadoras, de la misma edad.

Resultados: Antes de la CB, las mujeres obesas tenían una PaO_2 reducida (76 ± 2 mm Hg) y un gradiente alveolo-arterial de oxígeno aumentado (27 ± 2 mm Hg) secundarios a un aumento discreto del *shunt* intrapulmonar (4 ± 1% del gasto cardiaco) y de la dispersión de la distribución de la perfusión pulmonar (Log SDQ, 0,83 ± 0,06 [valores normales ≤0,60]) (aire ambiente). Tras respirar oxígeno al 100%, el *shunt* se duplicó sin cambios acompañantes en la dispersión de la perfusión pulmonar (Log SDQ), todo ello sugestivo del desarrollo de *atelectasias* de reabsorción sin reversión de la vasoconstricción pulmonar hipóxica. Después de la CB, tanto el peso corporal (IMC, 31 ± 1 kg/m^2) como las alteraciones del intercambio gaseoso pulmonar se redujeron significativamente.

Conclusiones: La obesidad mórbida se asocia con la presencia de *shunt* intrapulmonar discreto asociado a un desequilibro leve-moderado de las relaciones V_A/Q. Estas anomalías se reducen tras CB.

Resumen. Segundo artículo (*Second Manuscript*). *Efecto de los cambios posturales sobre las anomalías del intercambio gaseoso pulmonar en la obesidad grave, antes y después de cirugía bariátrica*

Introducción: En este trabajo se planteó la hipótesis de que las alteraciones del intercambio gaseoso pulmonar empeorarían en posición supina en la obesidad grave , hallazgo que debería mejorar tras cirugía bariátrica (CB).

Métodos: Se investigó el intercambio gaseoso pulmonar (aire ambiente) en los dos grupos de mujeres, obesas graves y peso normal, en posición sentada y supina, antes y después de la CB.

Resultados: No se observaron cambios gasométricos posturales en el grupo control. Mientras que todas las mujeres obesas tenían valores de PaO_2, SaO_2 y $AaPO_2$ más alterados que las controles ($p<0,05$, respectivamente) en posición sentada, no se observaron cambios en supino. El mismo comportamiento gasométrico se observó en las mujeres obesas normoxémicas ($PaO_2 \geq 80$ mmHg). Por contra, la oxigenación arterial mejoró ($AaPO_2$, –3,4 ± 1,4 mmHg; SaO_2, +1,5 ± 0,6 %; pH, +0,01 ± 0,01) y el gasto cardiaco aumentó (+0,4 ± 0,2 $L \cdot min^{-1}$) ($p<0,05$, respectivamente) en supino en las 13 obesas hipoxémicas ($PaO_2<80$ mmHg). Tras CB , la PaO_2 (de 75,5 ± 2,4 a 89,4 ± 2,4 mmHg) y el $AaPO_2$ (de 27,0 ± 2,0 a 15,4 ± 2,1 mmHg) ($p<0,05$, respectivamente) y en general el conjunto del intercambio gaseoso mejoraron en la población obesa en posición sentada, si bien la gasometría arterial (PaO_2, –4,6 ± 1,7 mmHg; $AaPO_2$, +4,2 ± 1,6 mmHg; $p<0,05$, respectivamente) empeoró en supino.

Conclusiones: Antes de CB, la gasometría arterial no varió con los cambios posturales en las mujeres obesas normoxémicas e incluso mejoró en las hipoxémicas en supino. Tras una CB exitosa en todos los casos, el intercambio gaseoso pulmonar mejoró en posición sentada si bien la gasometría arterial empeoró en supino. El interés clínico de estos hallazgos radica en la ausencia de un deterioro del intercambio gaseoso en posición supina, lo que puede conllevar connotaciones prácticas en medicina intensiva y anestesiología.

Resumen. Tercer Artículo (Third Manuscript). Aumento del volumen de tejido pulmonar en la obesidad grave y su reducción tras cirugía bariátrica.

Introducción: En este tercer estudio, se empleó la tomografía computarizada (TC) torácica para valorar si la cirugía bariátrica (CB) es capaz de provocar cambios estructurales a nivel pulmonar, para centrarnos en el análisis del volumen de tejido pulmonar (*Vtiss*) y el área de corte de los pequeños vasos pulmonares. Nuestra hipótesis fue que estos parámetros podrían correlacionarse con los cambios funcionales pulmonares descritos previamente.

Métodos: Se cuantificaron mediante TC los volúmenes pulmonares, incluido el volumen de tejido pulmonar (*Vtiss*) y el área de los vasos pulmonares, en un subgrupo de 12 mujeres obesas mórbidas, antes y después de CB, así como en las 8 mujeres del grupo control ya referidas en los dos artículos previos.

Resultados: Los valores de *Vtiss* estaban aumentados en las mujeres obesas antes de CB, en comparación con el grupo control, y se redujeron (en un 8%) sistemáticamente un año después. No se observaron cambios en el resto de volúmenes pulmonares o en áreas vasculares. No se observó ninguna asociación entre los cambios inducidos por la CB en las áreas vasculares pulmonares ni en el *Vtiss*.

Conclusiones: El volumen de tejido pulmonar (*Vtiss*) está aumentado en las personas obesas graves antes de CB, en comparación con el de mujeres de peso normal y se reduce de forma sistemática tras CB. Estos efectos sobre el *Vtiss* no se relacionan con cambios vasculares pulmonares, pero podrían estar influidos con el aumento de agua pulmonar extravascular inducido por la inflamación sistémica y/o la hipertrofia del tejido adiposo perivascular subyacentes.

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10. ANNEXED PUBLICATIONS

This research project has generated two more publications, which were the basis of the Doctoral Thesis of Ebymar Arismendi. It was qualified as Sobresaliente *cum laude*.

10.1 Manuscript 4. *The Systemic Inflammome of Severe Obesity before and after Bariatric Surgery.*

Authors: Arismendi E, **Rivas E**, Agusti A, Vidal J, Ríos J, Barreiro E, Rodriguez-Roisin R.

Plos One. 2014; 9: e107859. doi: 10.1371/journal.pone.0107859. eCollection 2014.

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10.2 Manuscript 5. *Airway Hyperresponsiveness to Mannitol in Obesity Before and After Bariatric Surgery.*

Authors: Arismendi E, **Rivas E**, Vidal J, Barreiro E, Torralba Y, Rodriguez-Roisin R.

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PLOS ONE

The Systemic Inflammome of Severe Obesity before and after Bariatric Surgery

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Abstract

Introduction: Obesity is associated with low-grade systemic inflammation. The ''inflammome'' is a network layout of the inflammatory pattern. The systemic inflammome of obesity has not been described as yet. We hypothesized that it can be significantly worsened by smoking and other comorbidities frequently associated with obesity, and ameliorated by bariatric surgery (BS). Besides, whether or not these changes are mirrored in the lungs is unknown, but obesity is often associated with pulmonary inflammation and bronchial hyperresponsiveness.

Objectives: We sought to: *(1)* describe the systemic inflammome of morbid obesity; *(2)* investigate the effects of sex, smoking, sleep apnea syndrome, metabolic syndrome and BS upon this systemic inflammome; and, *(3)* determine their interplay with pulmonary inflammation.

Methods: We studied 129 morbidly obese patients (96 females; age 46±12 years; body mass index [BMI], 46±6 kg/m^2) before and one year after BS, and 20 healthy, never-smokers, (43±7 years), with normal BMI and spirometry.

Results: Before BS, compared with controls, all obese subjects displayed a strong and coordinated (inflammome) systemic inflammatory response (adiponectin, C-reactive protein, interleukin (IL)-8, IL-10, leptin, soluble tumor necrosis factor-receptor 1(sTNF-R1), and 8-isoprostane). This inflammome was not modified by sex, smoking, or coexistence of obstructive sleep apnea and/or metabolic syndrome. By contrast, it was significantly ameliorated, albeit not completely abolished, after BS. Finally, obese subjects had evidence of pulmonary inflammation (exhaled condensate) that also decreased after BS.

Conclusions: The systemic inflammome of morbid obesity is independent of sex, smoking status and/or comorbidities, it is significantly reduced by BS and mirrored in the lungs.

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Introduction

Obesity is a major and raising global health problem. Among others, it increases significantly the risk of cardiovascular disease and premature death [1]. A key mechanism explaining this association appears to be the release by adipocytes of the so-called adipokines, such as leptin and adiponectin [2], a family of mediators that influences body weight homeostasis, insulin resistance and inflammation, and eventually causes endothelial dysfunction and atherosclerosis [3]. In addition, external risk factors such as smoking often contribute to enhance the adverse effects of obesity on cardiovascular health [3].

Bariatric surgery (BS) results in significant and sustained weight loss in morbidly obese subjects with minor morbidity or mortality [4–6]. Previous studies indicate that systemic inflammation in obese subjects appears to be reduced after BS [4;7]. Notwithstanding, the inflammatory response is complex and includes the contribution of many different cells and mediators [8]. Network analysis allows a more comprehensive approach to complex biological systems [9] with the potential of unraveling novel interplays among apparently disconnected mediators and clinical manifestations [10;11]. This research strategy has already proved to be useful to dissect the biological and environmental determinants of obesity [12] and smoking [13], as well as to

characterize the systemic inflammatory pattern (so-called, inflammome) associated with smoking and chronic obstructive pulmonary disease (COPD) [8]. In this study we describe for the first time the *inflammome* of morbid obese individuals and test the hypothesis that it could be significantly worsened by smoking or other comorbidities frequently associated to severe obesity, such as the obstructive sleep apnea syndrome (OSAS) and the metabolic syndrome (MS) and be ameliorated after BS. Besides, given that obesity is often associated with pulmonary inflammation and bronchial hyperresponsiveness [14–16], we also sought to investigate potential relationships between systemic and pulmonary inflammation in the morbid obese, so that we also quantified a number of inflammatory markers in the exhaled gas condensate in morbid obese, both before and after BS.

Methods

For more information see Methods S1.

Study Design, Participants and Ethics

This was a prospective, observational study in which we enrolled: *(1)* 129 obese individuals (96 females/33 males; age 46±12 years) with a body mass index (BMI) ≥40 kg/m^2 (or ≥ 35 kg/m^2 in those with comorbidities) without major cardiovascular and chronic obstructive airway diseases, candidates to BS; and, *(2)* 20 healthy, normal weighted, sex- (16 females/4 males) and age-matched (43±7 years) non-smokers with normal spirometry, who served as controls. Obese participants were studied before (mean, 8±4 weeks; median, 5 weeks) and one year (15±4 months; median, 13 months) after BS. The project was approved by the Ethics Committee for Clinical Research (*Comitè Ètic d'Investigació Clínica*) of Hospital Clínic of Barcelona (2008/4015) and all participants signed their written informed consent.

Measurements

The following measurements were obtained in all obese subjects before and after BS. Forced spirometry, plethysmographic lung volumes, arterial blood gases and the 6-minute walking test (6MWT) were determined according to international recommendations [17–19]. Reference values were those of Roca *et al*. [20–22]. An apnea/hypopnea index (AHI) ≥15 events/h was considered indicative of OSAS [23].

Serum was obtained after overnight fasting by peripheral venopuncture followed by centrifugation and stored at −80°C until analysis. The serum concentration of adiponectin, C-reactive protein (CRP), interleukin (IL)-8, IL-10, leptin, soluble tumor necrosis factor-receptor 1(sTNF-R1), and 8-isoprostane were determined, as previously reported [24]. The serum concentration of C-reactive protein (CRP) were determined using an immunoturbidimetry method (Advia Chemistry, Siemens Tarrytown, NY, USA) and those of leptin (Diagnostic Biochem Canada Inc. Ontario, Canada), serum adiponectin, soluble tumor necrosis factor-receptor 1(sTNF-R1), interleukin (IL)-8, IL-10 and 8-isoprostane by ELISA (US Biological Salem, MA, USA; IBL international Hamburg, Germany; ANOGEN Ontario, Canada and Cayman Chemical Company, Ann Arbor, MI, US, respectively). All biomarkers were quantified in duplicate and their mean values were used for analysis. In some individuals serum biomarker concentrations were below the lower limit of quantification (LLQ). To avoid a downward bias of biomarkers, a nominal level of half of the LLQ value was used in the analysis in individuals with values below the LLQ [25]. Exhaled breath condensate samples were obtained using an EcoScreen condenser (Jaeger, Würzburg, Germany) following international recommendations [26;27] and the concentrations of IL-8, IL-10 and 8-isoprostane were measured by ELISA (Cayman Chemical Company, Ann Arbor, MI, US).

Forced spirometry and serum and exhaled biomarker concentrations were determined in control participants only once.

Statistical Analysis

Results are described as mean ± standard deviation (SD), median [interquartile range] [IQR] or absolute and relative frequencies (%), as appropriate. Quantitative variables, were tested for normality using a Kolgomorov-Smirnov test and parametric (paired and unpaired t-test) and non-parametric (Wilcoxon and Mann-Whitney tests) were used accordingly to compare quantitative variables between patients and controls (at baseline) and between patients before and after BS. Fisher's exact test and McNemar test were used for qualitative variables.

As described previously [8], we used the 95th (and 5th) percentile value determined in controls as the upper (and lower) normal levels, so biomarker concentrations beyond these threshold were considered abnormal in obese subjects. Cross tabulations between healthy and obese subjects, before and after BS and also in different subsets of obese individuals according to sex, smoking status and coexistence of OSAS and MS, were determined to analyze biomarker alterations and their interactions. All statistical tests were two-sided and a p value <0.05 was considered significant. Due to the observational characteristics of this study p values presented were nominal and not adjusted for multiplicity. Data analysis was carried out with SPSS 20.0 (IBM Corporation).

Results

Characterization of Participants

Table 1 presents the main demographic and clinical characteristics of participants. BMI, waist and waist-to-hip ratio were, as compared to control, higher in obese subjects, but age and proportion of females were similar in the two populations. Only 21 obese subjects were current smokers (≥10 pack-years). Most obese subjects were non- (<10 pack-years) or former (>1 year after cessation) smokers, and their level of dyspnea was mild-to-moderate. Comorbidities were common in obese individuals, especially OSAS (67%) and MS (78%). As shown in Table 2 obese, as compared to control participants, had reduced forced spirometric and pulseoximetry values, although within normal limits, along with diminished expiratory reserve volume (ERV) and functional residual capacity (FRC) values. Mean PaO_2 (range, 57-119 mmHg) was within normal limits and mean alveolar-arterial PO_2 difference ($AaPO_2$) was abnormally enlarged (range, 0–51 mmHg). The former two values were more abnormal in males than in females (Tables S1 and S2).

Systemic Inflammation

The mean concentration of most serum inflammatory biomarkers was significantly higher in obese than in control subjects, except for adiponectin, which was lower (Table 2). Figure 1 presents the frequency distribution of the number of abnormal serum biomarker values in obese individuals (>95th percentile of controls (or <5th percentile in the case of adiponectin) [8]. Not a single obese subject had a normal battery of biomarkers and the majority exhibited at least 5 or more abnormal biomarkers. We observed that BMI was significantly associated to the serum concentrations of CRP (Rho, 0.31; $p<0.001$) and leptin (Rho, 0.41; $p<0.001$) values, whereas descriptors of central adiposity, *i.e.* waist circumference, were associated to sTNF-R1 (Rho, 0.25; $p<0.01$) and adiponectin (Rho, -0.18; $p<0.05$) levels.

Table 1. Main demographic and clinical characteristics of control and obese participants (mean ± SD or n (%)).

	CONTROL SUBJECTS	OBESE SUBJECTS			
		P Value *	BEFORE BS	*P* Value †	AFTER BS
Demographics					
Age, years	43±7	0.7	46±12	0.68	47±12
Female,%	83	0.24	74	---	74
Body mass index, kg/m^2	22±3	<.001	46±6	<.001	30±5
Waist circumference, cm	80±8	<.001	130±14	<.001	99±13
Waist-to-hip ratio	0.84±0.09	0.001	0.93±0.09	0.016	0.89±0.09
Clinical features					
Non-Smokers, n (%)	20	<.001	75 (58)	<.001	75 (58)
Current smokers, n (%)	0	<.001	21 (16)	.08	17 (13)
Tobacco, pack-years	0	<.001	34±32	.36	35±32
Ex-smokers, n (%)	0	<.001	33 (26)	.43	37 (29)
Tobacco, pack-years	0	<.001	35±24	.97	35±24
Dyspnea level (mMRC)	0	<.001	1.2±0.8	<.001	0.1±0.3
Obstructive Sleep Apnea, n (%)	—	NA	87 (67)	<.001	13 (10)
Apnea Hypopnea Index, events/h	—	NA	60±34	<.001	17±15
Metabolic Syndrome, n (%)	0	<.001	100 (78)	<.001	20 (16)
Diabetes Mellitus type 2, n (%)	0	<.001	52 (40)	<.001	12 (9)
Hypertension, n (%)	0	<.001	77 (60)	<.001	37 (29)

Demographic and clinical characteristics of healthy and obese individuals, before and after bariatric surgery. NA: not applicable; * p-values for comparisons between controls individuals and obese subjects before bariatric surgery whereas † indicate p-values for comparisons between obese subjects before and after bariatric surgery.
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The systemic inflammome is a network representation (Figure 2) of the prevalence and relationships between the different inflammatory markers determined in serum [8;28]. In obese subjects, all nodes were significantly larger than in control participants (indicating a higher prevalence of abnormal values) and there were many significant interactions among the different inflammatory biomarkers determined (Figure 2). By contrast, in control subjects, nodes were by definition small, many of them were not linked to the others and, in any case, links were few and thin, indicating the virtual absence of systemic inflammation.

We explored the effects of a number of factors that can potentially influence the systemic inflammome of morbid obesity, including sex, smoking status, and coexistence of OSAS or MS [24;29–31]. By and large, differences between males and females (Figure S1), current or former and non-smokers (Figure S2), and/or participants with or without OSAS and/or MS (Figures S3 and S4, respectively) were modest or absent, indicating that morbid obesity by itself was the main driving force of the systemic inflammome in these patients. Nevertheless, some (small) changes deserve comment.

Pulmonary Inflammation

A large proportion of obese subjects had abnormal levels (>95th percentile of controls) of exhaled IL-8 (56%) and IL-10 (15%) so that their mean values were significantly higher in obese subjects (Table 2). On the other hand, albeit exhaled 8-isoprostane was within the normal range in all obese individuals, mean values were significantly lower (Table 2). We did not observe significant differences in any of these exhaled inflammatory markers according to sex, smoking status and/or presence of OSAS and/or MS. The AHI and the concentration of exhaled breath biomarkers were not significantly related. By contrast, we observed a positive correlation between the concentration of exhaled IL-8 and serum sTNF-R1 (Rho, 0.24; $p<0.01$) and between exhaled IL-8 and serum 8-isoprostane (Rho, 0.27; $p<0.01$) as well.

Findings One Year after Bariatric Surgery

Sleeve gastrectomy was performed in 68 (53%) and Roux-en-Y gastric bypass in 61 (47%) obese subjects. Ninety-one percent of obese subjects (n = 118) had an excess weight loss>50% (75±18%), a marker of BS success [4].

Tables 1 and 2 show that most clinical and functional outcomes improved and most inflammatory markers were reduced after BS. All lung function tests improved significantly after BS but it should be noted that they were already within normal limits before surgery. It is of note that the 6MWT substantially increased and this was a novel post-operative finding in morbid obese.

The effects of BS upon systemic inflammation are illustrated in Figures 1 and 2. There was a dramatic downward shift of the frequency distribution of obese subjects with abnormal biomarkers with a marked shrinking of the systemic inflammome after BS, both in terms of node size and link width. Of note, however, that some patients still remained inflamed after BS (Figures 1 and 2). Finally, we observed that the exhaled concentrations of IL-8 and IL-10 (but not those of 8-isoprostane) were also significantly reduced after BS (Table 2).

Discussion

This study describes, for the first time to our knowledge, the systemic inflammome associated with morbid obesity and shows that it is: *(1)* barely modified by sex, smoking status and/or

Table 2. Lung function and inflammatory markers in control and obese participants, before and after bariatric surgery (mean ± SD or median [interquartile range]).

	CONTROL SUBJECTS		OBESE PATIENS		
		P Value *	BEFORE BS	*P* Value †	AFTER BS
Lung function					
FVC,% pred	103±13	0.003	91±13	<.001	103±13
FEV_1,% pred	102±13	0.02	94±15	<.001	104±14
FEV_1/FVC,%	71±4	0.008	82±5	<.001	79±9
FRC,% pred	ND	---	73±13	<.001	113±25
ERV,% pred	ND	---	34±23	<.001	106±36
TLC,% pred	ND	---	92±10	<.001	106±13
RV/TLC,%	ND	---	35±7	0.14	36±8
SGaw, s-1·cmH2O-1	ND	---	0.11±0.04	0.002	0.13±0.10
PaO_2, mmHg	ND	---	82±12	<.001	93±11
$PaCO_2$, mmHg	ND	---	37±4	<.001	39±5
$AaPO_2$, mmHg	ND	---	23±10	<.001	9±11
SaO_2,%	98±1	0.046	97±3	0.36	97±7
6MWT, m	ND	---	471±75	<.001	546±76
Serum markers					
Leucocytes, 10^9/l	6,215 [5490–7682]	<.001	8,010 [6,825–9,395]	<.001	6,700 [5,600–7,855]
C-Reactive Protein, mg/l	0.40 [0.16–0.70]	<.001	7.80 [4.30–14.50]	<.001	0.60 [0.20–1.45]
Fibrinogen, mg/dl	320 [280–350]	<.001	420 [368–480]	<.001	370 [333–438]
Leptin, ng/ml	13.60 [5.66–18.93]	<.001	63.00 [42.85–101.35]	<.001	15.00 [6.70–28.85]
Adiponectin, µg/ml	22.66 [18.70–25.92]	<.001	9.58 [4.88–15.85]	<.001	17.12 [9.68–22.55]
sTNF-R1, ng/ml	0.24 [0.07–0.43]	<.001	1.50 [1.01–2.24]	<.001	0.89 [0.34–1.61]
IL-8, pg/ml	4.00 [4.00–5.56]	0.019	9.22 [4.00–25.02]	0.012	4.00 [0.98–13.66]
IL-10, pg/ml	3.50 [3.50–148.54]	0.72	3.50 [3.50–16.59]	0.46	3.50 [3.50–11.45]
8-isoprostane, pg/ml	40.24 [23.90–58.50]	<.001	162.25 [108.30–211.90]	0.26	163.30 [93.27–213.36]
Exhaled condensate markers					
Exhaled IL-8, pg/ml	0.60 [0.33–1.36]	<.001	4.77 [2.21–8.74]	0.019	3.83 [1.26–6.79]
Exhaled IL-10, pg/ml	4.71 [2.32–7.46]	<.013	8.84 [4.75–15.24]	0.004	6.13 [4.07–10.29]
Exhaled 8-isoprostane, pg/ml	350.91 [177.31–603.23]	0.018	231.80 [113.15–362.69]	0.86	216.00 [129.65–372.65]

ND: Not done; FRC: functional residual capacity; ERV: expiratory reserve volume; TLC: total lung capacity; RV: residual volume; SG_{aw}: specific conductance; 6MWT: 6-minute walking test; sTNF-R1: soluble tumor necrosis factor-receptor 1; IL: interleukin. * p-values for comparisons between controls individuals and obese subjects before bariatric surgery whereas † indicate p-values for comparisons between obese subjects before and after bariatric surgery.

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coexistence of OSAS and/or MS; *(2)* significantly reduced, albeit not fully normalized, after BS; and, *(3)* related to pulmonary inflammation.

Previous Studies

Several previous studies have shown that severe obesity is associated with systemic inflammation that is considerably reduced after BS [4;7]. Our findings confirm and expand these previous results by providing an integrated network approach of the interplay among the different inflammatory markers (inflammome) as well as the effects of potential confounders, such as sex, smoking, OSAS and/or MS, and BS. This approach has been used successfully in other diseases, such as COPD [8;32]. On the other hand, it is also worth noting that many previous papers have also investigated the effects of obesity on lung function [14–16;33], including a recent report by our group that used the multiple inert gases elimination technique (MIGET) to investigate the pulmonary and non-pulmonary factors governing gas exchange in a small subset of females [24].

Interpretation of Findings

Several observations of our study deserve specific discussion. First, our results confirm [3;34–35] that morbid obesity is associated with a notable systemic inflammation component (Figure 1), here illustrated for the first time as an inflammome (Figure 2). Adipose tissue is an active endocrine organ capable of producing cytokines and hormones that regulate metabolism and immune responses [36]. Hotamisligil *et al.* coined the term "*meta-inflammation*" (metabolically triggered inflammation) to describe a condition triggered by nutrients that engages a similar set of

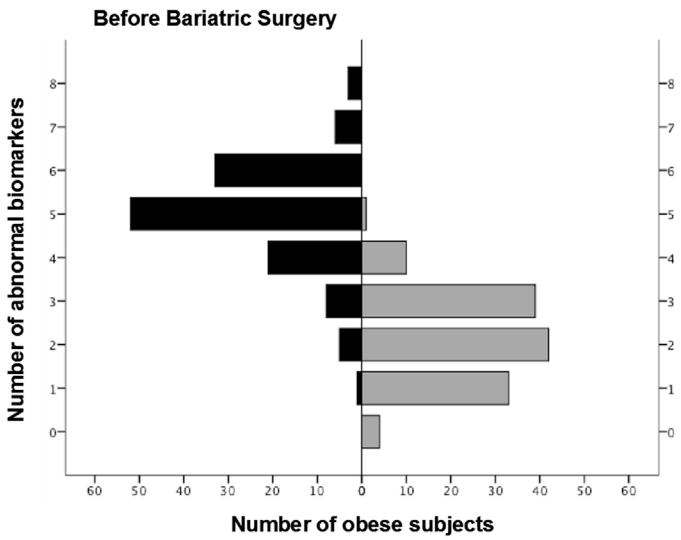

Figure 1. Frequency distribution of obese individuals according to the number of abnormal systemic (serum) biomarker values (>95th percentile of controls (or <5th percentile in the case of adiponectin), before and after BS.
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molecules and signaling pathways to those involved in other, more classical, forms of inflammation [37]. It is also known that, in obesity, the hypertrophic adipose tissue becomes infiltrated with pro-inflammatory macrophages, produces more pro-inflammatory cytokines and less adiponectin (an anti-inflammatory adipokine) and contributes to the systemic complications of obesity, including diabetes type 2 and MS, as well as to increased cardiovascular risk in these populations [37–38].

Second, contrary to our working hypothesis, we were not able to identify a clear effect of sex, smoking, OSAS or MS upon the systemic inflammome of morbidly obese individuals (Figures S1–S4), indicating that obesity *per se* is likely the main driving force of systemic inflammation in this clinical setting. By contrast, we observed a very significant effect of BS (Figure 2). Our findings confirm that BS is a safe and effective option for the treatment of very severe obesity but also showed that it has a profound effect on the systemic inflammome of these individuals (Figures 1 and 2). This may be related to the reduction of macrophage infiltration of adipose tissue, as well as to the change in the pro-inflammatory macrophage phenotype that has been reported after weight loss [39]. This further supports a key role of obesity in the pathobiology of systemic inflammation in these patients.

Finally, an important novel observation of our study relates to pulmonary inflammation in morbid obesity. In keeping with previous studies [30–31;40], we found evidence of airway inflammation in obese subjects (Table 2), but our results extend and complement these previous reports by showing that there was a significant interplay between systemic and pulmonary biomarkers and, notably, that BS not only reduced systemic inflammation but had a similar anti-inflammatory effect in the lungs as well. Most previous studies of pulmonary inflammation in obese subjects included individuals with OSAS [30–31;40–42], which was in fact believed to be the main pathogenic driver of the observed pulmonary inflammation. In contrast, we observed that airway inflammation was not different in obese patients with or without OSAS (and/or MS, or smoking). In keeping with these observations, a recent study in adults with obesity and OSAS has shown that the combined use of CPAP and weight-loss did not reduce serum CRP levels more than either intervention alone [43]. The fact that pulmonary inflammation was significantly reduced after BS in our study further supports the key role played by obesity in the pathogenesis of both systemic and pulmonary inflammation. In closing, exhaled 8-isoprostane, derived from free radical-catalyzed peroxidation of arachidonic acid, is a reliable biomarker of oxidative stress [44–45]. Pre-operative exhaled breath condensate levels of 8-isoprostane in obese patients were lower than in control participants (Table 2), suggesting that either oxidative stress does not play a key role in airway inflammation of morbidly obese subjects and/or that these individuals have developed a more efficient anti-oxidant capacity.

Strengths and Limitations

Our study has both strengths and limitations. As alluded to this is the first study to use a more comprehensive network approach to investigate the inflammatory pattern associated with morbid obesity, as well as the effects of potential confounding factors and BS, both in the systemic and pulmonary compartments. We acknowledge that we quantified a relatively low number of biomarkers and that we did not measure their levels in adipose or lung tissue.

Conclusion

Morbid obesity is associated with a significant systemic inflammome that is not influenced by sex, smoking status, presence of obstructive sleep apnea and/or metabolic syndrome, is related to pulmonary inflammation, and is significantly ameliorated after bariatric surgery.

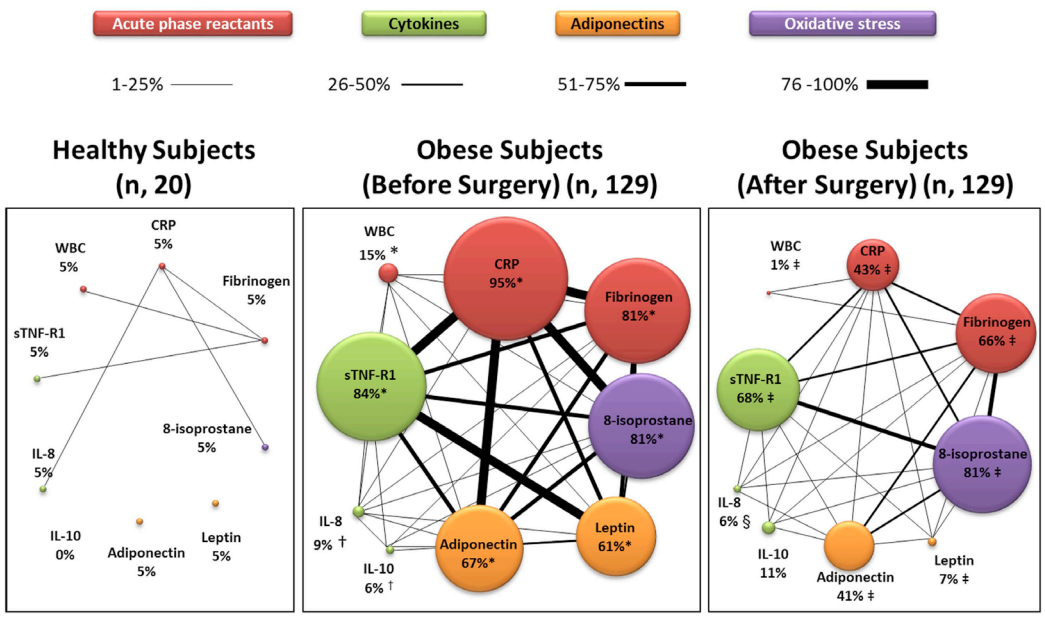

Figure 2. Systemic inflammome in healthy and obese individuals before and after BS. Each node represents one inflammatory marker and color indicates the type of inflammatory marker considered (acute phase reactants, cytokines, adipokines or oxidative stress). The node diameter is proportional to the prevalence of abnormal values (i.e.,>95th or <5th of controls) of that particular biomarker in the population under consideration (control or obese individuals) and the thickness of the edges linking pairs of nodes is proportional to the prevalence of co-occurrence of abnormal biomarkers of that particular pair of nodes.
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Supporting Information

Figure S1 Systemic inflammome in obese participants classified according to sex before BS (for further explanation, see legend to Figure 2).
(TIF)

Figure S2 Systemic inflammome in obese participants classified according to smoking habits before BS. Current smokers (≥10 pack-years); non- (<10 pack-years) or former (>1 year after cessation) smokers (for further explanation, see legend to Figure 2).
(TIF)

Figure S3 Systemic inflammome in obese participants classified according to the presence or absence of obstructive sleep apnea syndrome (OSAS) before BS. OSAS was define as apnea/hypopnea index>15 events/hour (for further explanation, see legend of Figure 2).
(TIF)

Figure S4 Systemic inflammome in obese participants classified according to the presence or absence of metabolic syndrome (MS) before BS (for further explanation, see legend to Figure 2).
(TIF)

Table S1 Demographic and clinical characteristics (mean ± SD or median [interquartile range]).
(DOC)

Table S2 Functional characteristics and serum and Exhaled Breath Condensate Biomarkers in Obese Individuals divided according to sex.
(DOC)

Methods S1 Additional information regarding methods.
(DOC)

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Author Contributions

Conceived and designed the experiments: RRR JV AA EA. Performed the experiments: EA ER EB. Analyzed the data: EA AA RRR JV JR. Contributed reagents/materials/analysis tools: EA AA RRR JV JR EB. Wrote the paper: EA AA RRR.

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ORIGINAL CONTRIBUTIONS

Airway Hyperresponsiveness to Mannitol in Obesity Before and After Bariatric Surgery

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Abstract
Background The relationship between airway hyperresponsiveness (AHR) and obesity, a low-grade systemic inflammatory condition, remains largely unknown. It is established that AHR to indirect stimuli is associated with active airway inflammation. The objectives were to investigate the rate of AHR to mannitol in obese subjects and its changes 1 year after bariatric surgery (BS).
Methods We enrolled 58 candidates to BS severely obese (33 nonsmokers and 25 smokers) without history of asthma and 20 healthy, nonobese participants and related AHR to functional findings and serum and exhaled biomarkers.
Results Before surgery, AHR was observed in 16 (28 %) obese with the provocation doses of mannitol to induce a 15 % fall in FEV_1 (PD_{15}) of (geometric mean [95 % CI]) 83 (24–145) mg. Compared to control participants, obese participants had lower spirometric values and higher serum and exhaled biomarkers ($p<0.05$ each). After surgery, AHR was abolished ($p<0.01$) in all but four obese subjects.
Conclusions Weight loss induced by BS was the key independent factor associated to AHR improvement. AHR to mannitol is highly prevalent in obesity, and it is largely abolished by BS.

Keywords Abdominal obesity · Airway inflammation · Bronchial hyperreactivity · Indirect bronchoconstrictor · Systemic inflammation

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Introduction

The concurrence of obesity and bronchial asthma has become an increasing worldwide major public health problem [1, 2]. Some studies show a clear evidence for a relation of airway hyperresponsiveness (AHR) to body mass index (BMI) [3]. However, the mechanisms of the relationship between obesity, AHR, and asthma are not sufficiently established [4, 5], and the impact of its underlying low-grade chronic systemic inflammation on AHR remains unsettled [6]. Obesity is characterized by an increased number of resident macrophages in adipose tissue that secrete a variety of inflammatory molecules, such as leptin and adiponectin [7, 8]. It is known that AHR to osmotic challenge agents is associated with the presence of airway inflammation as they increase airway

osmolarity and induce the release of inflammatory cells and of mediators that act on their specific receptors on the airway smooth muscle causing contraction [9, 10].

Bariatric surgery (BS) is the most efficacious therapeutic strategy to achieve major and sustained weight loss in morbidly obese subjects [11] and is associated with improved systemic inflammation [12]. There have been very few studies in obese subjects, mostly in asthmatics, to assess AHR using methacholine [13] before and after effective weight loss with discrepant results [14–17]. We investigated the prevalence of AHR to mannitol in obese candidates to BS before and 1 year after BS. Part of the results of this study has been previously reported in abstract form [18].

Methods

Subjects

This is a prospective, observational study in 58 morbidly obese individuals (44 females/14 males; aged 46±12 years) with BMI ≥40 kg/m^2 or ≥35 kg/m^2 (in patients with obesity-related diseases), candidates to BS in our center [18]. This sample of obese subjects was part of a study with a larger cohort of severe obese candidates to BS aimed to describe the systemic inflammome before and after BS [19]. Thirty-three obese individuals were nonsmokers (<10 pack-years) and 25 current (≥10 pack-years) or former (≥1 year after complete cessation) smokers (38±26 pack-years). Participants with cardiovascular and pulmonary (i.e., asthma, chronic obstructive pulmonary disease, and bronchiectasis) diseases were excluded. One year after BS, participants were also evaluated. A control group of 20 healthy nonsmokers (16 females/4 males), with normal weight and lung function, age-matched (46±7 years) was also recruited. Written informed consent (Protocol 2008/4015) was obtained from each participant according to the requirements of the Ethics Committee of the *Hospital Clínic*, *Universitat de Barcelona*.

BS including either Roux-en-Y gastric bypass or sleeve gastrectomy was carried out according to standard procedures [20]. The selection of the surgical technique was based on the presence of larger BMI, an estimated operative risk, or the presence of an enlarged liver [21]. Postoperative weight loss was expressed as a percentage of the presurgical excess weight (% excess weight loss=[100×[weight before surgery−weight at the time of evaluation]/[weight before surgery−weight corresponding to body mass index=25 kg/m^2]) [20]. BS was considered successful when excess weight loss was >50 % of presurgical excess weight.

Study Design

The 58 enrolled obese subjects visited the laboratory twice on two consecutive days, before (median, 5 weeks) and 1 year after BS (median, 13 months). At day 1, clinical evaluation, forced spirometry, and static lung volumes using our own predicted values [22–25] were carried out; at day 2, serum and exhaled samples were obtained, and the mannitol challenge was performed in all obese participants. At day 1, serum and exhaled samples and forced spirometry were determined in all control individuals; at day 2, eight subjects were challenged to mannitol alone. Control participants were explored once only.

Mannitol Challenge

Mannitol challenge was carried out using a commercially available kit (Pharmaxis Ltd, Burnham, UK) [26]. Bronchial challenge to mannitol was performed as previously reported by our group [27]. Pre-challenge baseline FEV_1 was used to calculate the maximum % fall FEV_1 after mannitol. The dose of mannitol that induced a fall of 15 % below baseline (i.e., PD_{15}) was expressed as geometric mean (GM) (GM PD_{15}) in milligrams. AHR to mannitol was defined as PD_{15}<635 mg. The response-dose ratio (RDR) was calculated as the % fall FEV_1 at the last dose, divided by the total cumulative dose of mannitol given (expressed as % fall/mg) in milligrams administered [26]. The test stopped when 15 % fall FEV_1 was achieved or the cumulative dose of 635 mg had been administered. Mannitol bronchoprovocation was completed by control and obese individuals without major adverse events.

Biomarker Measurements

Serum and exhaled samples were obtained and stored at −80 °C. Serum concentrations of C-reactive protein (CRP) were determined using an immunoturbidimetry method (ADVIA Chemistry, Siemens Tarrytown, NY, USA) and those of leptin using the specific enzyme-linked immunoabsorbent assay (ELISA) kit (Diagnostic Biochem Canada Inc., Ontario, Canada). Serum adiponectin, soluble tumor necrosis factor-receptor 1(sTNF-R1), and interleukin (IL)-8 levels were determined using a specific ELISA (US Biological Salem, MA, USA; IBL international Hamburg, Germany; and Anogen Ontario, Canada), respectively. Exhaled breath condensate samples of IL-8 were determined using an EcoScreen condenser (Jaeger, Würzburg, Germany) following current recommendations [28, 29] and specific ELISA kits (Cayman Chemical Company, Ann Arbor, MI, USA).

All biomarker measurements were performed in duplicate and the mean value used for analysis. Biomarker concentrations were below the lower limit of quantification (LLQ) in

some individuals. To avoid a downward bias of biomarkers, a nominal level of half of the LLQ value was used in the analysis in individuals with values below the LLQ [30].

Statistical Analysis

Data were expressed as mean±SD or median (interquartile range). Comparisons between obese and control individuals before BS were evaluated using one-way ANOVA with Bonferroni post hoc analysis (parametric) and Kruskal-Wallis (nonparametric) test. For comparisons between obese subjects before and after surgery, paired Student's for related samples (parametric) or Wilcoxon (nonparametric) tests for continuous variables were used. Unpaired Student's (parametric) and Mann-Whitney *U* (nonparametric) tests with Bonferroni-Holm correction were performed to compare obese individuals with and without AHR. Chi-square and McNemar tests for categorical variables and Spearman correlations were used as appropriate. An odds ratio calculated using generalized estimating equations (GEE) analysis [31], to account for non-independent evaluations from the same individuals before and after BS, was determined to assess the potential relationships of anthropometric data, cigarette smoking, and serum and exhaled biomarkers with AHR response. Statistical analysis was performed with specialized computer software (SPSS 20.0, Chicago, IL). Unless otherwise stated, significance was set at $p<0.05$.

Results

Baseline Findings (Before Bariatric Surgery)

The most relevant characteristics of obese and control individuals are displayed in Table 1. Obese participants had, compared to control, decreased spirometric values, higher FEV_1/FVC ratios, and higher serum and exhaled biomarkers but lower adiponectin levels. In obese subjects, comorbidities were common. Eight obese nonsmokers (14 % of total and 24 % of 33 nonsmokers) (median cumulative dose of inhaled mannitol, 176 mg [range, 5–629 mg]) and the same number of obese smokers (8 [14 % of total and 32 % of 25 smokers]) (121 mg [range, 5–475 mg]), respectively, were responsive to mannitol. Responsive obese nonsmokers had, compared to responsive smokers, lower mannitol-induced baseline FEV_1 (2.59±0.63 and 3.15±0.65 l) and final FEV_1 (1.88±0.56 and 2.57±0.64 l) values, respectively ($p<0.05$ each), without differences in the rates of AHR (chi-square test) (Table 2). The higher were the exhaled IL-8 levels in obese individuals, the greater were reactivity (RDR) (Rho, 0.35, $p<0.01$) and % fall FEV_1 (Rho, 0.32, $p<0.05$) to mannitol. AHR was not documented in any of the eight control subjects. There were no differences between obese nonsmokers and smokers who completed the study.

The logistic regression model fitted to data for all obese individuals did not yield interaction (all p values >0.4), without significant associations with anthropometric co-variables

Table 1 Demographic, clinical, and functional characteristics and serum and exhaled biomarkers in control and obese participants before and after bariatric surgery

	Control subjects (*n*, 20)	Obese subjects (*n*, 58)		*p* value
		Before	After	
Age, years	46±7	46±12	47±12	–
Female, *n* (%)	16 (80)	44 (76)	–	–
BMI, kg/m^2	22±3 *	45±5 *	30±4	<0.001
Waist circumference, cm	81±11 *	130±11 *	97±11	<0.001
OSA, *n* (%)	–	41 (71)	12 (21)	<0.001
MS, *n* (%)	0	44 (76)	10 (17)	<0.001
FVC, % pred	105±12*	90±13*	101±13	<0.001
FEV_1, % pred	103±13**	94±15**	102±13	<0.001
FEV_1/FVC, %	78±5*	83±5 *	80±5	<0.001
BD response, %	2±3	5±6	2±4	<0.01
FRC, % pred	ND	73±14	112±22	<0.001
ERV, % pred	ND	39±25	113±32	<0.001
C-reactive protein, mg/l	0.4 (0.1–0.5)*	6.4 (3.7–11.4)*	0.4 (0.2–0.8)	<0.001
Leptin, ng/ml	13.6 (5.6–19.7)*	46.3 (38.4–96.9)*	13.6 (6.9–22.1)	<0.001
Adiponectin, μg/ml	22.1 (18.1–25.9)*	13.4 (8.7–18.8)*	19.0 (12.0–25.2)	<0.001
sTNF-R1, ng/ml	0.4 (0.2–0.5)*	1.4 (1.0–1.9)*	0.9 (0.3–1.3)	<0.001
IL-8, pg/ml	2.0 (2.0–3.3)	9.4 (2.0–31.7)	3.4 (1.9–14.2)	0.16
Exhaled IL-8, pg/ml	0.9 (0.4–4.0)**	4.4 (1.5–6.6)**	4.0 (1.1–7.1)	0.62

Unless otherwise stated, data are expressed as mean±SD, *n* (%) or median (interquartile range)

BMI body mass index, *OSA* obstructive sleep apnea, *MS* metabolic syndrome, *pred* predicted, *BD* bronchodilator, *FVC* forced vital capacity, FEV_1 forced expiratory volume in the first second, *FRC* functional residual capacity, *ERV* expiratory reserve volume, *sTNF-R1* soluble tumor necrosis factor-receptor 1, *IL* interleukin

*$p<0.001$ for comparisons between control and obese subjects ?before surgery; **$p<0.05$ for comparisons between control ?and obese subjects before surgery

Table 2 Differences in outcomes between obese subjects with and without airway hyperresponsiveness (AHR) to mannitol before and after bariatric surgery

	Before		p value[a]	After		p value[b]
	With AHR	Without AHR		With AHR	Without AHR	
Patients, n	16	42	–	4	54	–
Age, years	49±7	46±12	0.29	60±4	46±10	0.009
BMI, kg/m^2	46±5	44±5	0.79	27±3	30±4	0.11
Waist, cm	134±9	128±11	0.052	97±11	97±11	0.95
Baseline FEV_1, % pred	89±15*	96±15	0.15	117±23**	99±13	0.014
Final FEV_1, % pred	70±18*	91±18	<0.001	97±17**	94±14	0.79
% Fall FEV_1	21±9	6±4	<0.001	16±1	3±6	<0.001
GM PD_{15}, mg	83 (24–145)	>635	NA	224 (156–322)	>635	NA
RDR, %/mg	0.11 (0.05–1.88)	0.007 (0.003–0.129)	<0.001	0.05 (0.05–0.17)	0.008 (0.003–0.011)	<0.001
Exhaled IL-8, pg/ml	6.23 (2.94–12.66)	3.97 (1.26–5.70)	0.99	7.10 (2.58–11.46)	3.90 (1.07–6.51)	0.44

Unless otherwise stated, data are expressed as mean±SD or median (interquartile range); baseline FEV_1, FEV_1 values measured after inhaling the 0-mg of mannitol capsule; final FEV_1, FEV_1 values measured at the end of mannitol test; % fall FEV_1, mannitol-induced percentage decrease of FEV_1 from baseline to final values; GM (geometric mean) of PD_{15}, provocation dose of mannitol to cause a 15 % fall FEV_1; RDR, response-dose ratio (% fall FEV_1 divided by cumulative dose given of mannitol). For other abbreviations, see Table 1

*$p<0.001$ for comparisons between baseline and final FEV_1 values before surgery; **$p<0.05$ for comparisons between baseline and final FEV_1 values after surgery

[a] p value for comparisons between obese subjects with and without AHR before surgery

[b] p value for comparisons between obese subjects with and without AHR after surgery

or cigarette smoking. The levels of exhaled IL-8 had a marginal association with AHR (odds ratio [OR], 1.10; 95 % confidence interval [CI], 0.97–1.22; $p=0.06$) only.

Findings after Bariatric Surgery

Sleeve gastrectomy (SG) was performed in 32 and Roux-en-Y gastric bypass (RYGBP) in 26 subjects, without differences in the allocation to the two procedures. The three subjects with <50 % excess weight loss were nonsmokers, two treated with SG, and one with RYGB. There was an excess weight loss of 76±17 %, and BS was successful (i.e., >50 % excess weight loss) in 55 participants (95 %). In obese individuals, clinical and spirometric findings and all but serum and exhaled IL-8 biomarkers significantly improved or normalized (Table 1). Two subjects ceased cigarette consumption only.

One year after BS, AHR was abolished in 15 out of the 16 responsive individuals. In addition, three preoperative nonresponsive subjects became now responsive to mannitol. A univariate analysis (McNemar's test) showed a significant reduction in AHR in obese individuals after BS ($p<0.001$).

The results from the models fitted to all pre- and postoperative data failed to detect any significant interaction (all p values >0.2). The GEE analysis [31] showed that the period of evaluation before and after BS was the key independent factor associated with AHR improvement (OR, 4.7; 95 % CI, 1.4–15.4; $p<0.01$). Cigarette smoking was not related to AHR. Likewise, postoperative changes in exhaled IL-8 and in AHR were not significant (OR, 1.09; 95 % CI, 1.0–1.2; $p=0.07$).

Discussion

This is the first prospective study that comprehensively assessed AHR to mannitol in morbidly obese, non-asthmatic adults, including smokers and nonsmokers. Two were the novel findings in our study. First, preoperative AHR to mannitol was abolished after BS in all but one individual and emerged in three new obese individuals. Second, the degrees of airway sensitivity (PD_{15}) and reactivity (RDR) to mannitol were considerably altered in obese participants akin to patients with currently active asthma [26, 32]. The prevalence of AHR in obese nonsmokers was high when compared to what has been previously observed in young normal weighted adults [33]. Similarly, our results extend and complement previous studies using direct stimuli in normal weighted individuals [34, 35], obese adults with and without asthma [5], and overweight Chinese families of asthmatic adults [36].

The agents that act indirectly to induce AHR are considered more specific for identifying the presence of airway inflammation and more consistent with the diagnosis of asthma than direct stimuli [26]. Numerous hypotheses have been suggested to explain the relationships between obesity, asthma, and AHR, from dietary lifestyle through mechanisms of

immunoregulation and inflammation [1, 37, 38]. Responsive obese nonsmokers had a higher likelihood of bronchial asthma and might therefore identify patients at high risk of asthma early before the expression of symptoms [10]. Notwithstanding, the mechanisms need to be probed further. From a clinical viewpoint, the significance of a positive response to mannitol is highly suggestive of active bronchial inflammation.

Before surgery, except for serum IL-8 values, obese individuals had higher biomarkers and lower adiponectin levels than control individuals, indicating more active systemic inflammation [39]. Serum adiponectin levels are reduced in obese subjects, and this is thought to induce systemic complications and augment airway inflammation [7, 19].

There were no differences in the rates of preoperative AHR between obese nonsmokers and smokers, and cigarette smoking was not related to AHR. Some smokers without clinical asthma can also have significant airway sensitivity to mannitol that is lost when smoking is stopped after a few weeks [40]. While the same mechanism of airway inflammation in obese nonsmokers could be invoked in smokers, it is known that smoking also recruits airway macrophages and neutrophils.

Effective weight loss promoted by BS was successfully achieved after surgery in the obese population and was associated to a practical abolition of AHR alone with lung function improvement, including that of most biomarkers and considerable reduction in comorbidities. Moreover, although the current study design did not preclude the role played by other factors that could influence postoperative evaluations potentially linked to changes in AHR, multivariate analysis suggested that BS was a key determinant of the significant AHR improvement observed in the obese cohort. The development of postoperative AHR in three new obese individuals (one nonsmoker and two smokers) and its persistence in one smoker suggest that aging and residual systemic inflammation may have also played an influential role.

The major strength of our study lies in its comprehensive design to assess clinical, functional, and biomarker outcomes in a cohort of asymptomatic, non-asthmatic, morbidly obese individuals with and without smoking habits, before and after surgery. There were, however, two shortcomings. First, the number of obese participants was not high due to the stringent design, and second, the indirect assessment of AHR in the current study was not compared with that made by direct stimuli because it was considered too aggressive in the setting of our study.

In conclusion, baseline preoperative AHR to mannitol in this cohort of asymptomatic morbidly obese candidates to BS is substantially high and associated to systemic inflammation; obese smokers had a similar rate of AHR but not associated to systemic inflammation. Our study provides new insights into the pathophysiology of AHR in obese individuals.

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A Statement of Informed Consent Informed consent was obtained from all individual participants included in the study.

A Statement of Human and Animal Rights "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

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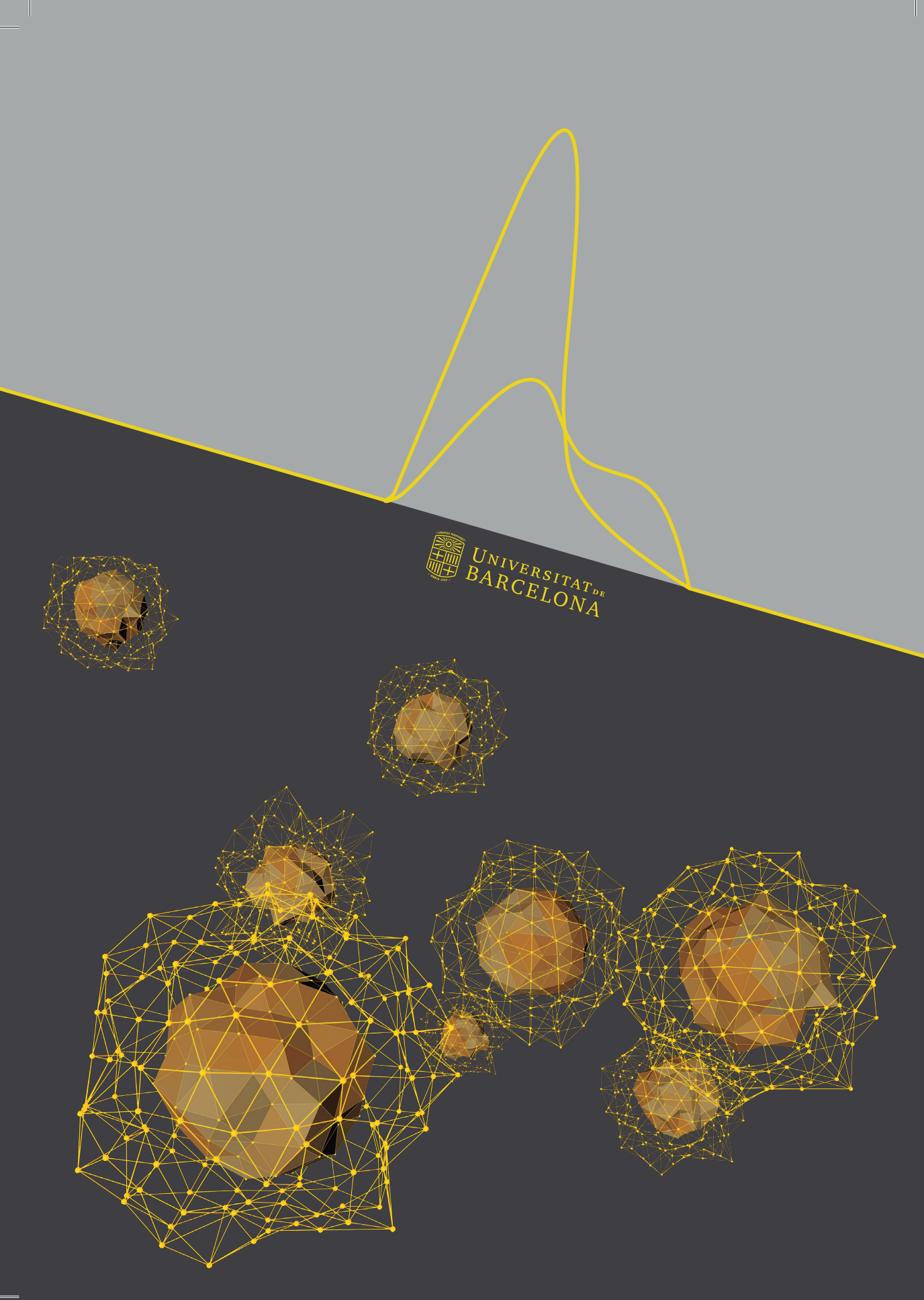
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