GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

A MULTIDISCIPLINARY APPROACH FOR UNDERSTANDING THE BIOLOGICAL PROFILE OF THE OBESE PATIENT – FOCUSING ON METABOLIC SURGERY

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CONTENTS

ABBREVIATIONS

AGB - adjustable gastric banding
AHA – American Heart Association
BMI – Body Mass Index
BPD – biliopancreatic diversion
BS – Bariatric surgery
CCL-18 – chemokine-ligand 18
CI – confidence intervals
CKD – chronic kidney disease
CVD – cardiovascular disease
DM II – type II diabetes
DMEM - Dulbecco's Modified Eagle Medium
%EBWL – preoperative body mass index – current BMI
%EWL – percentage of excess weight loss
ESRD – end-stage renal disease
EU – Europe
FFa – free fatty acids
FM – fat mass
FBS – fetal bovine serum
GFR – glomerular filtration rate
GI – gastrointestinal
HDL-C – high density lipoprotein cholesterol
HDL–C – HDL Cholesterol
IE – infective endocarditis
IR – Insulin resistance
LBM – lean body mass
LDL-C – low density lipoprotein cholesterol
LRYGB – laparoscopic Roux-en-Y gastric bypass
LSG - laparoscopic sleeve gastrectomy
MCP-1 – monocyte chemoattractant protein-1
MD – mean difference
MIF – migrationn inhibitory factor
MM – muscle mass
MS – metabolic syndrome
NAFLD – Nonalcoholic fatty liver disease
NIH - National Institutes of Health
OR – odd ratio
RR – risk ratio
RRT – renal replacement therapy
SG – sleeve gastrectomy
TC – total cholesterol
TG – triglyceride

THESIS SUMMARY

The present habilitation thesis presents, the main results of my postdoctoral scientific, didactic and professional activity. An academic profession is a complex profession, its success being based on perseverance and desire for self-refinement, receptivity to new ideas and concepts, flexibility, dynamism and critical reflection.

During my entire academic career, I was attracted by the research perspective. Thus, I concentrated my surgery on pancreatic malignant pathology and in the last 8 years upon obesity, two themes with major socio-economic impact. The research we have carried out so far is largely addressed by these two main topics of interest.

Also, didactic work was directed towards the same two major research directions, to stimulate and encourage future physicians to explore these themes.

The main goals of my professional career are to pursue research in the two major directions mentioned.

The efforts made in the complex study of bariatric surgery patient should materialize in the realization of the national registry of the obese patient that underwent surgery. This would be an extremely valuable tool in assessing the socio-economic impact of obesity in our country, in the management of these patients and implementation of propper medical-surgical protocols.

The starting point in the research activity was the graduation of my PhD, conducted between 2000 and 2007, under the coordination of Prof. Dr. Cristian Dragomir. The theme was "Monitoring Patients Operated for Tumor Formations of the Exocrine Pancreas" and was conducted in collaboration with the Karolinska Institute Hospital, Stockholm, Sweden.

Bariatric surgery has materialized as the second major area of interest as a natural evolution of my collaborations with DELTA (now Ponderas) Laparoscopic Surgery Center in Bucharest under the coordination of Prof. Dr. Catalin Copaescu and Harvard Medical School, Boston, USA (Professor Alan Scott Shikora).

In this direction we have produced and published a total of 15 scientific papers in extenso. Both research departments have allowed me to participate in a number of 7 grant research projects.

The habilitation thesis is structured in three major sections, according to the CNATDCU recommended and approved criteria. The paper presents the overview of my concerns in the fields of general surgery reflected in the activities performed in the Ist Department of Surgery of the 'Grigore T Popa' University of Medicine and Pharmacy in Iaşi.

The first section - entitled SECTION I - includes an overview of personal, professional, academic and scientific achievements. This section is divided in two main parts: an introduction and a second part formed by other two chapters.

The introduction contains a register of the most significant features of my professional achievements, research and academic activity. These add strength and value to my empowerment in achieving the neccesary skills for top academic career.

My entire medical career mirrors into the academic one and research projects under the topic of the complexity of obese patient treatment.

All my personal research projects have a compulsory support consisting in a series of attestations obtained from the continuous medical training courses that I have followed in the

fields of diagnostic digestive endoscopy, general ultrasound, health management and services and therapeutic digestive endoscopy.

The scientific achievements so far have been materialized in book and book chapter publications, ISI articles in journal with impact factor and communications at congress conferences. All of these are the results of my teamwork and would not have been possible without the help of the medical school trainers from whom I learned general surgery and, especially bariatric surgery.

The results of our work had as a implicitly consequence the enhancement of our international visibility and the repute of the university I represent.

The topic chosen for the doctoral study - monitoring patients operated for pancreatic exocrine tumor formation - have opened the way for my research career. During the PhD study I learned the skills of a researcher for my future career.

In Chapter I, entitled "The adipose tissue: from metabolic surgery to fundamental research" I present our expertise in terms of pre-operative and post-operative particularities of obese patients. The main part of this chapter consists in relating the results of our work in the field of methabolic surgery.

Chapter II describes our results in studying the systemic impact of the obesity. Methabolic surgery has a major impact on renal and liver functions and also a great psychological impact on the patients.

Section II details our research perspectives on short, medium and long-term plans.

In Section III I added to the manuscript the complete list of the most significant refferences from the fundamentum of my scientific, professional and academic career.

REZUMATUL TEZEI

Prezenta teză de abilitare prezintă principalele rezultate ale activității mele științifice, didactice și profesionale de după finalizarea studiului doctoral. O profesie academică este o profesie complexă, succesul său fiind bazat pe perseverență și dorință de auto-rafinament, receptivitate la idei și concepte noi, flexibilitate, dinamism și reflecție critică.

În toată cariera mea academică, am fost atras de perspectiva cercetării. Astfel, mi-am concentrat inițial practica chirurgicală asupra patologiei maligne pancreatice urmând ca în ultimii 8 ani să mă ocup în special de chirurgia obezității, două teme cu un impact socio-economic major. Cercetarea pe care am realizat-o până acum abordează în mare parte aceste două subiecte principale de interes.

De asemenea, cariera didactică a fost îndreptată spre aceleași două direcții principale de cercetare, pentru a stimula și a încuraja viitorii medici să exploreze aceste teme.

Eforturile depuse în vederea studierii complexității pacienților supuși chirurgiei bariatrice s-au materializat în realizarea registrului național al pacienților obezi supuși unei intervenții chirurgicale. Acesta ar fi un instrument extrem de valoros în evaluarea impactului socio-economic al obezității în țara noastră, în gestionarea acestor pacienți și implementarea protocoalelor medicalo-chirurgicale specifice.

Punctul de plecare în activitatea de cercetare a fost absolvirea studiului meu doctoral doctoral, desfășurat între 2000 și 2007, sub coordonarea prof. Dr. Cristian Dragomir. Tema a fost "Monitorizarea pacienților operați pentru formarea tumorilor pancreasului exocrin" și a fost realizată în colaborare cu Spitalul Institutului Karolinska, Stockholm, Suedia.

Chirurgia bariatrică s-a materializat ca a doua arie importantă a evoluției naturale a colaborărilor mele cu Centrul de chirurgie laparoscopică DELTA (acum Ponderas) din Bucuresti sub coordonarea Prof. Dr. Cătălin Copăescu și Harvard Medical School, Boston, SUA (Profesor Alan Scott Shikora).

În această direcție am publicat un total de 15 lucrări științifice în extenso. Ambele direcții de cercetare mi-au permis să particip la 7 proiecte de cercetare de tip grant.

Teza de abilitare este structurată în trei secțiuni majore, conform criteriilor recomandate și aprobate de către CNATDCU. Lucrarea prezintă o privire de ansamblu asupra preocupărilor mele în domeniile și subdomeniile chirurgiei generale, reflectate în activitățile desfășurate în cadrul Departamentului de Chirurgie I al Universității de Medicină și Farmacie "Grigore T Popa" din Iași.

Prima secțiune - intitulată SECTION I - conține o prezentare generală a realizărilor personale, profesionale, academice și științifice. Această secțiune este împărțită în două părți principale: o introducere și o a doua parte formată din alte două capitole.

Introducerea conține un registru cu cele mai importante caracteristici ale realizărilor mele profesionale, ale activității de cercetare și ale activității academice. Acestea adaugă un plus de valoare în îndeplinirea abilităților necesare pentru o carieră academică de top.

Întreaga mea carieră medicală se oglindește în cea academică și în proiectele de cercetare, respectiv subiectul complexității tratamentului pacienților obezi.

Toate proiectele mele de cercetare personale au un suport specific, constând într-o serie de atestate obținute prin cursurile de perfecționare continuă pe care le-am urmat în

domeniul endoscopiei digestive diagnostice, ultrasonografiei generale, managementului sănătății și al serviciilor de sănătate precum și în cel al endoscopiei digestive terapeutice.

Realizările științifice de până în prezent s-au concretizat în publicații de cărți și capitole de carte, articole ISI în reviste cu factor de impact și comunicări la conferințe și congrese de profil. Toate acestea sunt rezultatele muncii mele în echipă și nu ar fi fost posibile fără ajutorul formatorilor de școală medicală de la care am învățat bazele chirurgiei generale și, în special, ale chirurgiei bariatrice.

Rezultatele activității noastre au avut drept consecință implicit îmbunătățirea vizibilității pe plan internațional și a reputației universității pe care o reprezint.

Tema aleasă pentru studiul de doctorat - monitorizarea pacienților operați pentru formațiuni tumorale ale pancreasului exocrin - a deschis calea în cariera mea de cercetare. În timpul studiului doctoral am dobândit abilitățile de cercetător necesare pentru cariera mea viitoare.

În capitolul I, intitulat "Țesutul adipos: de la chirurgia metabolică până la cercetarea fundamentală", prezint experiența noastră în ceea ce privesc particularitățile preoperatorii și postoperatorii ale pacienților obezi. Partea principală a acestui capitol constă în corelarea rezultatelor activității noastre în domeniul chirurgiei metabolice.

Capitolul II descrie rezultatele noastre în studierea impactului sistemic al obezității. Chirurgia metabolică are o influență majoră asupra funcțiilor renale și hepatice, precum și un mare impact psihologic asupra pacienților.

Secțiunea II detaliază perspectivele noastre de cercetare pe termen scurt, mediu și lung, ca planuri de studiu detaliate.

În secțiunea a III-a am adăugat acestui manuscris lista completă a celor mai semnificative referințe care stau la baza formării carierei mele științifice, profesionale și academice.

SECTION I. PROFESSIONAL, SCIENTIFIC AND ACADEMIC ACHIEVEMENTS

INTRODUCTION

The professional development of a person is the cumulative, objective and appreciative result of his life experiences. Achievement of a high performance and an academic recognition represent the successful conquest of the efforts made until that moment.

The academic career is integrating in its complex requests and exigences the medical activity and, firstly, the research one. In this respect, during the 16 years of academic activity, I have gained a wealth of experience in student class management, work with residents, and surgical practice as well.

During my entire academic career, I was attracted by the research perspective. Thus, I concentrated my surgery on pancreatic malignant pathology and in the last 8 years of obesity, two themes with major socio-economic impact. The research we have carried out so far is largely addressed by these two main topics of interest.

Also, didactic work was directed it towards the same two major research directions, to stimulate and encourage future physicians to explore these themes.

The main goals of my professional career are to pursue research in the two major directions mentioned.

As a short- and medium-term perspective, priority is given to setting new research opportunities and topics such as breast cancer surgery and the psycho-therapeutic management of the pre- and post-interventional surgical patient.

Also, the efforts made in the complex study of the patient of bariatric surgery should materialize in the realization of the national registry of the obese patient operated. This would be an extremely valuable tool in assessing the socio-economic impact of obesity in our country, in the management of these patients and the implementation of medical-surgical protocols in this respect.

I intend to apply for a multi-institutional grant in a public-private partnership on the socio-economic impact of metabolic surgery on active people and the writing of a book on the treatment of obesity.

All these efforts and efforts could not materialize without the corresponding human resource. I hope to involve as many colleagues as possible in this national health issue in order to coagulate several elite, multidisciplinary medical centers and teams that are also medical school trainers in this direction both in medical and research.

Professional achievements

My academic career has been built, first and foremost on hard work, on the awareness of the need for research and the opportunities offered by training sessions in the country and abroad.

The co-ordination by the elites of Romanian and international surgery, coupled with access to cutting-edge information and technology, has allowed me to develop this side of my career.

Equally important was and is, in this respect, the opportunity that I am offered in correlating the three major aspects of my career - didactic, surgical and research - around the main themes of interest.

The experience we have gained in medical and research practice comes from the work carried out in these areas under the coordination of reference personalities and internationally recognized centers on those research directions.

Thus, we have attended 25 specialized courses and internships in the country and 10 abroad. Among the latter, I would like to mention: Queens Medical Center, Nottingham, UK, Karolinska Institute, Stockholm, Sweden, Norway, Milan, Italy, St. Gallen, Switzerland, UK London, Boston, USA, Harvard Medical School.

The current professional level we have reached would not have been possible without having some professional skills: diagnostic digestive endoscopy, general ultrasound, management of health services and therapeutic digestive endoscopy.

Research activity

The starting point in the research activity was the conduct of the doctoral study, conducted between 2000 and 2007, under the close coordination of Prof. Dr. Cristian Dragomir. The theme of this study is "Monitoring Patients Operated for Tumor Formations of the Exocrine Pancreas" and was conducted in collaboration with the Karolinska Institute Hospital, Stockholm, Sweden. The work carried out in this university center, under the coordination of Prof. Ake Andren Sandberg, has made a mark on my entire surgical and research careers, materializing by writing a book in this area: "The follow-up of patients radically operated for pancreatic cancer".

Starting from this topic, I published a number of 10 scientific papers in ISI journals and indexed in international databases.

All this research on pancreatology is also my first study direction.

Bariatric surgery has materialized as the second major area of interest as a natural evolution of my collaborations with DELTA (now Ponderas) Laparoscopic Surgery Center in Bucharest under the coordination of Prof. Dr. Catalin Copaescu and Harvard Medical School, Boston, USA (Professor Alan Scott Shikora).

In this direction we have produced and published a total of 15 scientific papers in extenso.

Both research departments have allowed me to participate in a number of 7 grant research projects.

During my scientific activity I was the author of some book chapters from which the following appeared:

- D. Timofte, Ake A. Sandberg Pancreatic cancer-an everlasting surgical challenge, Publishing 'Gr.T. Popa' Iasi 2018, ISBN 978-606-544-317-4

- Timofte D., 5 book chapters: "From clinical sign to diagnosis in surgical pathology" Under Diaconu Corneliu, Publishing 'Gr.T. Popa' Iasi, 2013, ISBN 978-606-544-177-4

- Timofte D., Eva L., Vasincu D. 1 chapter "Implications of the" subquantum level "in carcinogenesis and tumor progression through scale relativity theory", in the book "Quantum Mechanics", ISBN 978-953-51-4131, InTech, Rijeka, Croatia;

• "Pancreatology - the next decade" book published at the Pancreatica Symposium of Surgery with International Participation, organized by Prof. Ake Andren Sandberg at Karolinska University in November 2014, where I was invited to the lecturers' group. In the book I had published 2 chapters as main author and 1 co-author chapter;

• Scientific advisor for the book: Sebastian Popescu, Physics and Acoustics Mechanics - Biomechanics, Ed. Tehnopress, Iasi 2005, ISBN 973-702-117-7 (222 pages);

Since 2011 we have developed a major concern for advanced metabolic and laparoscopic surgery, which is why I have participated in numerous conferences, courses and training sessions. From the current position of expert trainer, we have developed the largest database of more than 75 operated patients, which are prospectively tracked.

My concerns extend to the other areas of surgery and have materialized in awards in the areas of breast cancer surgery, unconsciousness, and gastroenterology.

Academic activity

Teaching is an essential part of my university career and a cornerstone of scientific research. In this respect, my work has focused on raising students 'and residents' interest in the subject matter they teach through extracurricular activities and in implementing new teaching and management techniques for the student class.

Collaboration with the rest of the department's team and with the leadership of the university was materialized in the elaboration of a practical work program for students, books and textbook editing, elaboration of an electronic study support for practical works, with a view to introducing extracurricular themes such as surgery obesity

Collaboration with students, concretized by case presentations, transmission from the operating room, co-opting students in operations bariatric and pancreatic disorders.

The activity with the residents has materialized in guiding and encouraging them in the practice of surgical techniques and in conducting scientific works for publishing / presenting at conferences and encouraging them by co-opting in the team of authors.

The main themes of my career research have also been found in this activity, guiding and encouraging young colleagues to deepen them.

Personally, I think I have managed to focus my career on two major research areas: pancreatology and metabolic surgery. This makes it easier to continue and deepen them, to open new collaborations and experience exchanges with international reference medical research centers.

The new research themes I have proposed to address are derived from the needs dictated by the previous ones.

The issue of obesity is an extremely topical and international concern, and it is a major health problem, especially in developed countries.

Working in this direction aim both in adopting a personalized management of patients and raising awareness of the population in order to reduce the socio-economic impact that obesity has in our country, for the benefit of the public health.

CHAPTER I. THE ADIPOSE TISSUE: FROM FUNDAMENTAL RESEARCH TO METABOLIC SURGERY

I.1. Obesity up to date - State of the Art

Obesity has become pandemic, with billions of people around the world today being overweight or obese. This affects not only the health and longevity of every overweight person, but also has a massive impact on the economy and health system of each affected country, which is one of the biggest challenges that the health systems faces around the world (Hruby and Hu, 2015).

According to the Romanian Endocrinology Society, in Romania about 21.3% of the population suffers from obesity, 31.1% is overweight and the number of overweight children has increased by 18%, reaching a prevalence of 40% among children and adolescents. The most affected are people with age between 15 – 64 years. (Asociaţia Română pentru Studiul Obezităţii, 2015) 60% of adults in Romania are overweight and 30% are obese, of which 23% are men and 20.3% are women according to the Healthcare Ministry. The report published in October 2017 by the World Health Organization shows alarming statistics: the number of people with obesity has tripled compared to the number reported in 1975, in 2016 1.9 billion of people are overweight (39% of the adult population), over 650 million of whom suffer from obesity (13% of the adult population) (The GBD 2015 Obesity Collaborators, 2017).

The pathophysiological mechanisms of this pathology are incompletely elucidated, but two elements have been shown to be involved in the etiology of the disease: the consumption of foods with high caloric value and the lack of physical activity. Environmental factors are likely to contribute significantly to this epidemic of obesity along with biological predisposition. It is certain that obesity develops when there is a positive imbalance between intake and consumption of energy, but the relative contribution of these factors is poorly understood (Lenzi, 2015).

In countries that are heavily industrialized, it is noticed that obesity occurs since childhood and, according to WHO, every third child in these countries is obese.

Dozens of years of research show that there is a clear link between obesity and genetics. If one of the parents is obese, the risk of becoming an obese adult increases significantly. Moreover, if obesity is present from early childhood, the chances of being obese are higher than in another patient who becomes obese later. A BMI can be genetically influenced by about 75% if both parents are obese and 25-50% if only one parent is obese. It is important to know that low birth weight is associated with an increased risk of visceral obesity, glucose intolerance, hypertension, diabetes, X syndrome and cardiovascular mortality (Gould, 2009).

Family aggregation of cases of obesity can be explained by the induction of an obesogenic behavioral model, such as wrong quantitative eating habits, qualitatively, or as a modality or frequency of eating, plus the restrictive attitude towards physical effort, ie excessive sedentarism. Some genetic factors that do not intervene in a monogenic Mendelian manner may be involved in the development of obesity, rather than in certain syndromes

such as Morgagni-Stewart-Morel, Lawrence-Biedl-Moon, Prader-Labhart-Willi, rather, by polygenic inducement (Buchwald et al., 2004).

Another mechanism act through the genetic predisposition to which environmental factors act, determining obesity. Genetics have discovered an FTO gene (fat mass and gene associated with obesity), which is closely related to obese traits in a population. Different alleles of the FTO gene are quite common (16% of the population has an allele variant). On average, the body weight of the person presenting this allele increases by approximately 1.5 kg (Beales, 2009).

The pathogenic mechanisms of obesity are very complex, some even unknown, but the implication of etiological factors is clearly demonstrated.

Of the hormones that are involved in the etiology of obesity, we mention: **Norepinephrine** – increases energy consumption; **Serotonin** – decreases food intake; **Neuropeptide Y** – anabolic promoter – increases food intake and promotes energy storage; **CRH** – decreases intake of food; **Leptin**, which is a satiety factor, lowers the expression of neuropeptide Y in the hypothalamus, the main physiological role being the sign of starvation to the brain, because it rapidly falls to the food restriction, the circulating level correlating with the body's fat reserves (Agrawal, 2016).

Excessive caloric intake on copious meals, including alcohol; preferential abuse of fat and/or carbohydrates, especially concentrated sugars, rapidly resorbable, stimulating hyperinsulinism, i.e. appetite, and lipogenesis; fast food, greedy food, in rare and abundant outlets; increasing availability of food as a consequence of material welfare or social success plays one of the greatest roles in the etiology of obesity. Excessive sedentarism reduces energy loss, and decreasing muscle mass causes obesity. Nicotine and caffeine increase thermogenesis, so withdrawal results in weight gain; moreover, nervousness and smoking habits may favor the appearance of gritty (snacking of candies, cookies, peanuts, etc.). Anxiety, psychic trauma, conflicts or even lack of culture can promote bulimia, feeding has a tranquilizing role (Agrawal, 2016).

Some medications such as sleeping pills, antidepressants or tranquilizers can also cause increased appetite (Buchwald et al., 2004).

Hormonal pathology associated with increased appetite – hyperinsulinism, hypercorticism, pregnancy, menopause – as well as some nervous system diseases associated with dysregulation of hypothalamic hunger and satiety centers are involved in the development of obesity (Sidhu et al., 2017).

In order to treat severe obesity in non-responsive patients in the therapeutic diet, physical exercise and drug therapy, surgery is finally performed. Bariatric surgery includes a series of procedures designed to help obese patients reduce weight and lower the rate of comorbidities.

Bariatric procedures can be divided into: restrictive procedures that reduce the size of the stomach and thus reduce the amount of food that can be stored at its level but does not interfere in any way with normal digestion - VBG, LAGB; malabsorption procedures that shorten digestive tract to limit calories and nutrients that can be absorbed - BPD, BPD / DS; combined procedures - restrictive and malabsorption, including RYGB and more recently GS. For other surgical techniques and procedures for the treatment of morbid obesity, such as gastric balloon or gastric stimulation, no remarkable results have yet been reported. The vast

majority of bariatric surgery techniques can be performed laparoscopically, resulting in less affected tissues, shorter hospitalization and fewer complications (Angrisani, 2017).

For any of the surgical procedures used to treat obesity, patients should be carefully selected. The indications for surgical management for extreme obesity were established in 1991 by the National Institutes of Health, criteria that are still up to date. The selection criteria for bariatric surgery begin with a diagnosis based on a BMI greater than 40 kg / m^2 or more and equal to 35 kg / m^2 for patients with comorbidities such as apnea, cardiomyopathy, hypoventilation syndrome, diabetes or osteoarthritis severe (Agrawal, 2016).

Contraindications include patients with severe psychiatric illness, substance abuse, non-compliance with previous medical treatments or pre-existing severe medical conditions. Diarrheal disorders should be treated carefully before surgical treatment is considered because bariatric procedures, especially those involving diminishing the size of the stomach, may exacerbate bulimia or anorexia nervosa. Before undergoing surgery, patients need to know the risks, expected benefits and life changes that such treatment requires (Agrawal, 2016).

Bariatric techniques are not recommended for patients with endocrine disorders or other pathologies that can lead to obesity, such as Cushing's syndrome or hypothalamic obesity syndrome (Blackstone, 2016).

General contraindications for bariatric surgery are the same as with other abdominal surgery. For example, the presence of unstable coronary artery disease or advanced portal hypertension pathology will significantly increase operator risks. The pregnancy should be postponed until the weight stabilizes, generally 12-24 months after surgery; therefore contraception is recommended (Waine, 2002).

Bariatric surgery is the surgical treatment of obesity and the complications it generates. Generally, the gastric sleeve is considered the gold standard in the surgical treatment of obesity.

The gastric sleeve is indicated for the vast majority of patients who have morbid obesity and who meet the criteria for bariatric surgery. This is a safe technique for high risk patients and has few contraindications including Barrett's esophagus, neoplasms, liver cirrhosis with severe portal hypertension, severe heart and lung dysfunction (Christopher et al., 2004).

As with other surgical procedures, the patient must understand the nature of surgery and accept the risks of surgery. At the same time, it must be sufficiently motivated to comply with changing lifestyle, diet and long-term behavior. The purpose of this procedure is to reduce the size of the stomach by cutting it vertically parallel along the small gastric curves and to reduce gherlin production by completely removing the gastric fundus (Christopher et al., 2004).

Sleeve Gastrectomy, also known as the gastric sleeve, restricts the amount of food you eat by reducing the size of the stomach. The minimally invasive procedure removes a portion of the stomach, making the stomach roughly the size and shape of a banana. Patients who have a sleeve gastrectomy feel full after eating much less. In addition, the surgery removes the portion of the stomach that produces a hormone that can make you feel hungry, so you won't want to eat as much. This procedure can be an excellent alternative to gastric bypass or gastric banding. Sleeve gastrectomy is a simpler operation than the gastric bypass

procedure because it doesn't involve rerouting or reconnecting the intestines (Christopher et al., 2004).

Advantages of Sleeve Gastrectomy: fewer food intolerances than with gastric banding; weight loss generally is faster with the sleeve than with gastric banding. There is no implantable band device, so slippage and erosion are not a risk. The surgical risk is lower than with gastric bypass procedures, but the weight loss is similar. No device that needs adjustment is inserted, so the follow-up regimen is not as intense as it is with gastric banding. Sleeve Gastrectomy is not adjustable or reversible (Christopher et al., 2004).

Complication risks are slightly higher than with the band. Standard risks associated with surgery: leakage at the suture site; blood clots.

With **gastric banding**, an inflatable band is placed around the upper part of the stomach. The band creates a smaller stomach pouch, restricting the amount of food that can be consumed at one time. The band also increases the time it takes for the stomach to empty. There is no cutting or stapling needed to separate the upper stomach pouch from the lower stomach. Unlike stomach stapling, the gastric band can be adjusted to suit your needs. As a result, patients achieve sustained weight loss by limiting food intake, reducing appetite and slowing digestion. During gastric banding surgery, the surgeon makes a few small incisions in the abdominal wall. Using laparoscopic techniques, a silicone adjustable band is secured around the upper part of the stomach, creating a small stomach pouch. The band is connected to tubing which attaches to an access port fixed beneath the skin of the abdomen. The stomach pouch created by the adjustable band controls the amount of food taken in. It allows a small amount of food to pass through, delaying the emptying of the stomach into the intestines. This process creates the sensation of fullness sooner and over time, hunger decreases. The procedure takes about 40 minutes and can be performed on an outpatient basis with no required hospital stay. Recovery times may vary, but patients can generally return to work and normal activities within four to seven days. Because the surgery uses laparoscopic techniques, patients experience less postoperative pain, recover quicker and are able to return to normal activities sooner, compared to other forms of bariatric (Christopher et al., 2004).

Gastric bypass limits the amount of food that you can eat and digest. This minimally invasive, laparoscopic method allows for less time spent in the hospital and faster recovery and healing time. In a Roux-en-Y gastric bypass, the stomach is made smaller by creating a small pouch at the top of the stomach using surgical staples or a plastic band. The resulting pouch is only about the size of a walnut and can hold about one ounce of food. After the pouch has been created, most of the stomach and part of the intestines are bypassed by attaching (usually stapling) part of the intestine to the small stomach pouch. As a result, a gastric bypass patient cannot eat as much and absorbs fewer nutrients and calories (Christopher et al., 2004).

Advantages of Gastric Bypass: initial weight loss is rapid; approach is minimally invasive; slightly higher total average weight loss reported than with purely restrictive procedures; rapid improvement or resolution of type 2 diabetes and metabolic syndrome. Disadvantages of Gastric Bypass: cutting and stapling of stomach and bowel are required. More potential operative complications: portion of digestive tract is bypassed, reducing absorption of essential nutrients; "Dumping syndrome" can occur. Procedure is not adjustable and difficult to reverse. Also has a higher mortality rate. Standard risks associated with major

surgery are associated with gastric by-pass: nausea and vomiting; separation of stapled areas (requires major revisional surgery); leaks from staple lines (requires major revisional surgery); nutritional deficiencies (Christopher et al., 2004).

Laparoscopic gastric plication is a newer minimally invasive weight-loss surgery technique that reduces the size of the stomach capacity to approximately 200 ml. Gastric plication procedure folds the stomach in on itself to reduce its size. The procedure does not involve the use of an implanted device (such as gastric banding). Also, unlike the gastric sleeve procedure, gastric plication may be reversible because a portion of the stomach is not removed. In addition, unlike gastric bypass, gastric plication does not involve rerouting and reconnecting the intestines. It is a restrictive weight-loss surgery, meaning that it restricts the amount of food the stomach can hold. You will feel full sooner so you won't want to eat as much. Advantages: no re-routing of intestines as with gastric bypass; does not involve implanting a banding device around a portion of the stomach; no adjustments are needed as with gastric banding. Procedure may be reversible, unlike sleeve gastrectomy or gastric bypass. Gastric plication is a newer procedure and hasn't been tested as long as sleeve gastrectomy, gastric banding or gastric bypass (Christopher et al., 2004).

In 2007, in the SOS study, it was first demonstrated that weight loss deliberately led to a clear improvement in the vital and functional prognosis of obese people, but still remains a series of questions about the choice of the right technique or the weight loss level to be reached in correlation with the importance of obesity and the presence of comorbidities. Bariatric surgery demonstrates its effectiveness primarily by treating comorbidities, and it is therefore desirable to prioritize patients who will benefit most from this intervention (Karlsson et al., 2007).

Following the 10-year observation of patients undergoing surgery to treat obesity, the main improvements were linked to the extent of weight loss, with the exception of anxiety. The immediate effects of bariatric surgery were considered temporary. After reaching the maximum weight loss level, it has been noticed that large groups of patients have begun to gain weight again, which has contributed to a gradual regression of quality of life and mental well-being. However, the long-term outcomes of the study suggest that a sustained weight loss is sufficient to produce positive effects on the quality of life, which was seen in more than a third of patients (Karlsson et al., 2007).

Thus, bariatric surgery is a favorable option for the treatment of severe obesity, but collaboration between the patient and the surgeon must be constantly maintained and flanked by continuous behavioral support. Study data has also revealed that patients who have had bariatric surgery have difficulty in having an optimal postoperative diet. Another crucial factor for controlling long-term weight is regular physical activity that also has a strong positive effect on the entire health state. There is, in particular, strong evidence that regular exercise is an essential component for the primary and secondary prevention of many chronic conditions associated with overweight such as cardiovascular disease, diabetes mellitus, hypertension, cancer, etc. (Christopher et al., 2004).

Thus, it is important to implement a series of therapeutic strategies that stimulate and facilitate the adoption and maintenance of normal physical activity among patients with bariatric surgery (Karlsson et al., 2007).

The main preoccupations that I had in this direction of research are reflected in the next articles and projects:

1. Hristov I, Mocanu V, Zugun-Eloae F, Lăbușcă L, Crețu-Silvestru I, Oboroceanu T, Tiron C, Tiron A, Burlacu A, Pinzariu AC, Armașu I, Neagoe RM, Covic A, Scripcariu V, **Timofte DV**. Association of intracellular lipid accumulation in subcutaneous adipocyte precursors and plasma adipokines in bariatric surgery candidates. Lipids in Health and Disease, 2019, 18:141.
2. **Timofte D**, Pantea Stoian A, Hainarosie R, Diaconu C, Bulgaru Iliescu D, Bălan G, Ciuntu B, Neagoe RM. A review on the advantages and disadvantages of using administrative data in surgery outcome studies. J Surgery 2018, 14(3) :97-99.
3. Pricope-Veselin A E, Mocanu V, **Timofte D.** Open surgical and needle biopsy to study abdominal subcutaneous adipose tissue in obesity. J Surgery 2018, 14 (3):111-113.
4. **Timofte D**, Ochiuz L, Ursaru M, Ciuntu B, Hristov I, Puia I, Calu V, Mocanu V. The biochemical effect of laparoscopic sleeve gastrectomy on serum magnesium levels. Rev Chim (Bucharest) 2017, 68(9): 1997 - 2001.
5. **Timofte D**, Ochiuz L, Ursaru M, Ciuntu B, Ionescu L, Calu V, Mocanu V, Puia IC. The impact of laparoscopic sleeve gastrectomy on serum zinc or copper and body composition. Rev Chim (Bucharest) 2017, 68(11): 2628- 2634.
6. **Timofte D**, Hristov I, Zugun-Eloae F, Ungureanu MC, Galeșanu C, Mocanu V. Middle term impact of sleeve gastectomy on major cardiovascular risk facors in a group of romanian obese patients. Acta Endocrinologica (Buc), 2017, 12(4): 454 – 460.
7. Livadariu RM, **Timofte D**, Dănilă R, Sângeap AM, Constantinescu D, Trifan A. Obesity is linked with inflammation-evaluation of subclinical inflammatory status in obese patients. J Surgery, 2017, 13(4): 127-131.
8. **Timofte D**, Ochiuz L, Ursaru M, Ciuntu B, Ionescu L, Calu V, Mocanu V, Puia I. Biochemical modifications related to Calcium deficiencies in obesity and after laparoscopic sleeve gastrectomy. Rev Chim (Bucharest), 2017, 68(10): 2341 - 2345.
9. Livadariu R, **Timofte D**, Ionescu L, Dănilă R, Drug V, Trifan A. Upper digestive endoscopy prior to bariatric surgery in morbidly obese patients – a retrospective analysis. J Surgery, 2015, 12(1):19-21.

Research projects:

1. Adipose tissue biomarkers in short-term remission of metabolic syndrome after bariatric surgery. Contract no. 30340/2017, 2018-2019. Coordonator ”Grigore T. Popa” University of Medicine and Pharmacy Iasi. Daniel Timofte- Project Director.
2. Expression of the ghrelin receptor (GHSR-1a) in subcutaneous adipose tissue and the effect on proliferation and differentiation of preadipocytes. Contract no. 29032/2016, 2017-2018. Coordonator ”Grigore T. Popa” University of Medicine and Pharmacy. Veronica Mocanu – project director; Daniel Timofte – member in research team

I.2. Commune practices before the metabolic surgery

I.2.1. Introduction

As already mentioned, obesity has become one of the world's major health issues due to its endemic progression and associated comorbidities. The International Association for the Study of Obesity reported that approximately 40-50% of men and 25-35% of women in the EU were overweight (defined as a BMI between 25.0 and 29.9 kg/m^2), and an additional 15-25% of men and 15-25% of women were obese (BMI $\geq$ 30.0 kg/m^2) (James, 2008). Obesity is strongly associated with hypertension, type 2 diabetes or insulin resistance, dyslipidemia, coronary heart disease, nonalcoholic fatty liver disease (ranging from simple steatosis to steatohepatitis, hepatic cirrhosis and end-stage liver disease), hepatocellular carcinoma and multiple other types of cancer, including colonic and gynecological cancers. This is why the treatment of obesity has become one of the major concerns of health systems in the affected areas, including primarily economically developed countries. To date, bariatric surgery is the only treatment that has shown long-term usefulness. Despite the existing guidelines for the preoperative evaluation of the morbidly obese patient's candidates for bariatric surgery, the routine preoperative upper GI endoscopy is still a matter of debate. By analyzing the impact of endoscopic findings on the bariatric surgical management, some authors found that routine preoperative upper endoscopy is not required (Gomez et al., 2014); however, a study on 212 morbidly obese patients who underwent bariatric procedures showed a high prevalence of gastrointestinal diseases with a significant impact on perioperative management in two thirds of the cases of bariatric patients who underwent preoperative upper gastrointestinal (GI) endoscopy and therefore they recommend routine gastroscopy about 2 – 4 weeks prior to surgery (Wiltberger et al., 2015).

Preoperative evaluation and preparation of the morbidly obese patient

In the complex management of the patient who will benefit from the Bariatric procedure, the following steps will be taken: (a) establish Bariatric indication for the patient to benefit from the Bariatric procedure and recommended types of Bariatric procedures; (b) Preparing the Bariatric patient. All patients should undergo a pre-operative evaluation of comorbidities related to obesity with particular attention to those factors that may affect a recommendation for Bariatric surgery (Christopher et al., 2004).

Preoperative assessment shall include a complete medical history: obesity comorbidities, causes of obesity, weight / BMI, history of weight gain and weight loss, psychosocial history, physical exam, appropriate laboratory tests to assess surgical risk (**TABLE 1)**. It is important that the medical need for Bariatric surgery should be documented (Pelosi and Gregoretti, 2010).

Because informed consent is a dynamic process, there should be a thorough discussion with the patient about the risks and benefits, procedural options, the choice of the surgeon and the medical institution, as well as highlighting the need for long-term follow-up and supplementation with vitamins (including the costs necessary to maintain a proper follow-up). Patients should be provided with educational materials and access to preoperative education sessions. Also the patient must be provided with financial information and the

Bariatric surgery program should provide all the necessary clinical materials for documentation so that the reimbursement criteria are met (O'Leary et al., 2007).

The preoperative weight loss may reduce the patient's liver volume and may help improve the technical aspects of surgery in patients with a fatty liver or extensive liver disease, and therefore weight loss should be encouraged prior to Bariatric surgery. Preoperative weight loss or medical nutrition therapy can also be used in selected cases to improve the condition of comorbidities, such as achieving reasonable pre-operative glycemic values (McGlinch, et al. 2006).

Medical clearance for Bariatric surgery includes preoperative glycemic control optimized with a comprehensive diabetes care plan, including healthy eating habits, nutritional medical therapy, physical activity, and pharmacotherapy as appropriate. Reasonable goals for preoperative glycemic control that may be associated with an improvement in Bariatric surgery results include:

- Hemoglobin A1c value of 6.5% - 7.0% or less;
- Blood glucose level of ≤ 110 mg / dL;
- Postprandial blood sugar at 2 hours with a concentration of less than 140 mg / dL. (Al-Benna, 2011).

Lighter preoperative targets, such as 7% - 8% HbA1c, should be considered in patients with advanced microvascular or macrovascular complications, extensive comorbid conditions, or long-term diabetes in which the overall objective was difficult to achieve, despite intense efforts. In patients with HbA1c> 8% or with uncontrolled diabetes by other means, the need for Bariatric intervention will be appreciated by the clinical judgment applied to each case (DeMaria and Carmody, 2005).

Routine screening for primary hypothyroidism before Bariatric surgery can be performed, but is not strongly recommended. Patients at risk of primary hypothyroidism should be screening the serum thyroid stimulating hormone (TSH). Also, patients with hypothyroidism should be treated with L-tyrosine monotherapy (Cullen and Ferguson, 2012).

An obese lipid profile should be obtained in all obese patients. Treatment should be initiated in accordance with the recommendations in force (Cullen and Ferguson 2012).

Candidates for Bariatric surgery should avoid preoperative and postoperative pregnancy for 12 to 18 months. Women who become pregnant after Bariatric surgery should be counseled and monitored for adequate weight gain, nutritional supplementation, and fetal health. All reproductive age women should be informed about contraceptive options after Bariatric surgery. Patients with RYGB or malabsorption should be informed about non-oral contraceptive therapies. The patients who become pregnant after Bariatric surgery should have nutritional surveillance and laboratory screening for deficits every quarter, including iron, folic acid and vitamin B12, calcium, and liposoluble vitamins. Post-LAGB pregnant patients should have adequate and necessary adjustments of the stomach ring to have an appropriate weight gain for fetal health (O'Leary et al., 2007).

Estrogen therapy should be discontinued prior to Bariatric surgery (1 cycle of oral contraceptives in premenopausal women, 3 weeks of hormone replacement therapy in postmenopausal women) to reduce the risk of post-operative thromboembolic phenomena. Patients with polycystic ovary syndrome (PCOS) should be informed that their fertility status may improve postoperative (O'Leary et al., 2007).

The screening decision for rare causes of obesity will be taken on a case-by-case basis and should be based on specific findings in the patient's history and physical examination (McGlinch et al. 2006).

Noninvasive cardiac testing, apart from the electrocardiogram, will be performed on the basis of individual risk factors and on the basis of conclusions regarding the history and physical examination of patients. Patients with known heart disease require an official cardiology consultation before Bariatric surgery. Patients at risk for heart disease should be evaluated for perioperative beta-adrenergic blocking routine eco-cardiothoracic routine, cardiac and vascular indexing, and risk stratification associated with cardiac surgery are also recommended (Poirier et al., 2009).

Thoracic X-ray and standard screening for obstructive sleep apnea (with confirmation polysomnography if screening tests are positive) should be considered in patients considered for bariatric surgery (Mickelson, 2007).

Patients with intrinsic lung disease or disordered sleep rhythm must have a formal lung assessment, including blood, blood count, when knowing these results would alter patient care (Kaw et al., 2008).

Tobacco consumption should be avoided at all times by all patients. In particular, patients who smoke cigarettes should preferably stop at least 6 weeks before Bariatric surgery. Also, tobacco use should be avoided after Bariatric surgery, given the increased risk of wound healing, anastomotic ulcer, and overall health damage (Levin and Weissman, 2009).

Patients with a history of deep vein thrombosis (DVT) or pulmonary cord should undergo a suitable diagnostic evaluation for DVT. The prophylactic care may be higher than a benefit in patients with a history of PE or DVT, as a result of the risk of complications related to the filter, including thrombosis (Ramchandani and Belani, 2007).

Significant gastrointestinal symptoms should be evaluated before Bariatric surgery through imaging studies, upper gastrointestinal (UGI) series, or endoscopy (Kuruba et al., 2007).

Abdominal ultrasound is not recommended as a routine screening test for liver disease. Abdominal ultrasound is indicated to evaluate symptomatic biliary disorders and elevated liver function tests. In patients with elevated liver function tests (2 to 3 times the upper limit of normal), abdominal ultrasound and a viral hepatitis (Grade D) test may be performed. A liver biopsy can be considered during the operation to document steatohepatitis and / or cirrhosis that might otherwise be unknown due to normal liver appearance and / or normal liver function tests. Routine screening for the presence of Helicobacter pylori before Bariatric surgery will be performed, especially in areas with high prevalence (Cullen and Ferguson, 2012).

In patients with a history of gout prior to Bariatric surgery, the prophylactic treatment of gout attacks should be considered. Bone mineral density - there is insufficient data in the literature to justify the preoperative bone mineral X-ray (DXA) assessment beyond the official recommendations of the guidelines for osteoporosis (Guss and Bhattacharyya, 2006). For all patients, prior to Bariatric surgery, a psycho-behavioral assessment is required to assess environmental, family, and behavioral factors. Any patient considered for Bariatric surgery with a psychiatric illness known or suspected of a psychiatric illness or substance

abuse or addiction must be subjected to a formal mental health assessment before performing the surgical procedure (Apovian et al., 2009).

In the case of RYGB, high risk groups should eliminate alcohol consumption due to metabolic damage (Apovian et al., 2009).

Pre-operative endoscopy

Upper-gastrointestinal endoscopy is the diagnostic, prognostic and often therapeutic reference examination of the main digestive pathologies. This procedure must be performed by a trained specialist. Minimum procedural thresholds have recently been defined by the American Endoscopy Society (ASGE) (Guidelines for privileging, credentialing, and proctoring to perform GI endoscopy, 2017).

Selective preoperative endoscopy prior to bariatric surgery is recommended. Patients with symptoms of GERD, such as heartburn, regurgitation, dysphagia, or any postprandial symptoms that suggest a foregut pathology and/or who chronically use antisecretory medications, should have an upper GI endoscopic evaluation before bariatric surgery. Routine endoscopy before surgery can identify a variety of conditions including hiatal hernia, esophagitis, ulcers, Barrett's esophagus, Helicobacter pylori infection, polyps and tumors, non-alcoholic fatty liver disease (NAFLD), cirrhosis (Angrisani, 2017). These diseases are more frequently in obese patients than in normal weight individuals (De Palma and Forestieri, 2014).

In a study by Gomez et al., more than 60% of the cohort had abnormal findings on EGD of varying severity, but the majority of these findings are of little clinical consequence, rarely change the surgical management (Gomez et al., 2014). In a study by Schirmer et al., 4.9% of the patients had endoscopic findings (esophagitis, gastroduodenal ulcers, hiatal hernia, and gastric polyps) that changed or altered the operative procedure. These findings did not lead to cancellation of any procedure (Schirmer et al., 2002).

Patients with morbid obesity might have a higher rate of endoscopic abnormalities, however the majority of abnormal endoscopic do not affect operative management. Performance of preoperative EGD is recommended in patients with upper gastrointestinal symptoms (ASMBS, 2015).

Radiographic UGI evaluation should be reserved for symptomatic patients or those with history of prior gastric surgery. A barium contrast study may be a useful alternative as it can provide information complementary to endoscopy. The presence of a hiatal hernia and endoscopic signs of reflux esophagitis represent a relative contraindication to sleeve gastrectomy because of an increased risk of the development of de novo GERD-type symptoms and esophageal mucosa injury after SG (ASMBS, 2015).

The published guidelines of the European Association for Endoscopic Surgery state that esophagogastroduodenoscopy is advisable for all bariatric procedures and strongly recommended for gastric bypass patients (Guidelines for privileging, credentialing, and proctoring to perform GI endoscopy, 2017).

Personal contribution – published paper:

Livadariu R, **Timofte D**, Ionescu L, Dănilă R, Drug V, Trifan A. Upper digestive endoscopy prior to bariatric surgery in morbidly obese patients – a retrospective analysis. J Surgery, 2015, 12(1):19-21.

The aim of our study was to evaluate the importance of routine upper gastrointestinal endoscopy before bariatric surgery.

TABLE 1 *Preoperative assessment*

Preoperatively check	Relevant information for risk / benefit assessment
Complete medical history with personal physiological, pathological and heredo-collateral history	- comorbidities related to obesity, - causes of obesity, - weight, height, BMI, - Abdome Circumference ratio / Hip Circumference, - history of weight gain and weight loss with weight loss attempts, - involving the patient in the process of weight loose, - Exclusions related to surgical risk
Laboratory Exam	- blood count, - the blood group and Rh, - blood glucose and lipid profile, oral glucose tolerance test, - Complete lipid profile (total triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol), - kidney function: urea, creatinine, - hepatic function: AST, ALT, total bilirubin (direct and indirect); alkaline phosphatase, - summary of urine and uroculture, - coagulation times: prothrombin, INR - ionogram: sodium, potassium, calcium, magnesium, chlorine, - sideremia, ferritin, - HBV / VHD testing, HCV, HIV,
Nutritional tests	- levels of iron, vitamin B12, folic acid (erythrocytic folic acid, homocysteine levels, methyl-malonic acid), - levels of vitamin D (optional vitamin A and - E) - if malabsorptive procedures are performed, testing will be performed - extensive according to symptoms and risks
Cardiopulmonary evaluation	- ECG, cardio-pulmonary radiography, ecocardiography if cardiac or HTP is suspected, - Deep vein thrombosis risk assessment if clinically indicated: pelvic venous Doppler ultrasound, protein C, S, other coagulopathy
Gastrointestinal evaluation	- Helicobacter pylori test, - cholecist assessment, - echographic liver evaluation, - endoscopy/ colonoscopy,

Endocrinological evaluation	- HbA1c evaluation if diabetes is suspected or diabetes or pre-diabetic status is diagnosed, - TSH, T3, free T4 if there are symptoms or a risk of thyroid disease, - Androgens if there is suspicion of Polycystic Ovary Syndrome (total testosterone available, D4-androstenedione), - Screening Cushing Disease when Suspected (Dexamethasone Test, Cortisol 24 hours free urine, salivary cortisol)
Clinical evaluation by nutritionist	
Psychosocial assessment	
Documenting the medical need for bariatric surgery	
Consent informed	
Providing relevant financial elements	
Continue the preoperative weight loss efforts	
Optimizing glycemic control	
Counseling in case of pregnancy	
Smoking cessation counseling	
Screening for cancer	

I.2.2. Material and methods

A clinical prospective study was carried out on a series of 77 patients referred for bariatric surgery between 2012 and 2015 at the III-rd Surgical Unit, "St Spiridon" Hospital, Iași. We reviewed all medical records focused on BMI, gastrointestinal symptoms, preoperative endoscopy diagnosis, histopathological reports on gastric endoscopic biopsies and the colonization with *Helicobacter pylori*.

The patients in our study had a median age of 39.25 years ranging from 22 to 63 years old, and a BMI ranging from 33.3 to 60.5 kg/m^2 (median of 44.66 kg/m^2). Our patients were offered to bariatric surgery under conditions of failing weight loss or inadequate losing weight after nutritional therapy. The chosen bariatric procedure for all of them was sleeve gastrectomy. In terms of symptomatology, most patients had postprandial mild epigastric pain (43%) and dyspepsia perceived as postprandial abdominal fullness (39% of cases) with 38% of these patients having confounding symptoms; 18% of the patients complained of postprandial regurgitation of whom 22% also complained of postprandial abdominal fullness and mild epigastric pain. Upper GI endoscopy was performed routinely before bariatric surgery; there were noted different pathological aspects: chronic gastritis in 36.36% of cases and Helicobacter pylori infection in 26% of cases. 6.5% of the patients were diagnosed with small hiatal hernia, a hyperplastic gastric polyp, a case of pancreatic ectopic tissue into the mucosa of the stomach and a case of gastric cancer were also detected (**Fig. 1**).

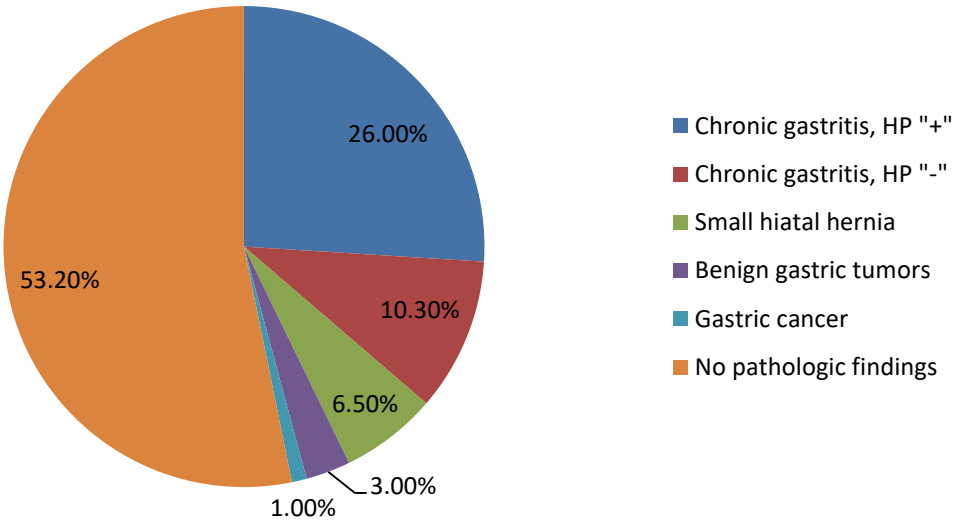

Fig. 1. *Pathological findings in upper GI endoscopy*

I.2.3. Results

The patient diagnosed with gastric cancer did not present any other complaints besides the described common symptoms and a slight recent weight loss attributed to nutritional regimen. The most important change in terms of treatment management regarded the patient with gastric cancer to whom bariatric surgery was contraindicated and consequently underwent complete oncologic evaluation and afterwards, total gastrectomy with Roux-en-Y esophago-jejunal anastomosis. Surgical intervention was delayed only for the patient with pancreatic tissue ectopy who required additional preoperative investigations to rule out other pathologic findings; regarding patients diagnosed with small hiatal hernia on GI endoscopy prior to bariatric surgery, there were mild changes in surgical technique, consisting in additional recalibration of the hiatal orifice. As seen in our results, digestive symptoms seem not to be consistent with the observed changes on endoscopy (**TABLE 2**). The patients diagnosed with H. pylori infection received sequential therapy with 5 days of pantoprazole and amoxicillin followed by 5 days of pantoprazole, clarithromycin, and metronidazole.

I.2.4. Discussion

While the role of upper GI endoscopy in the management of postoperative complications of bariatric surgery is well known and established (De Palma and Forestieri, 2014), the usefulness of preoperative routine upper GI endoscopy – regardless of gastrointestinal symptomatology – remains controversial. The patient symptoms and endoscopic findings are not always correlated in morbidly obese patients. Agreeing with other authors (Carabotti et al., 2014) and having our study as support, we believe that the presence of gastrointestinal symptoms in morbid obese patients may be a misleading marker to indicate endoscopy prior to bariatric surgery. On a study of 69 consecutive diagnostic upper GI endoscopies in morbidly obese patients before bariatric procedures, 80% of the patients with pathological findings were asymptomatic, authors concluding that every morbidly obese patient should undergo endoscopy before bariatric surgery (Kuper et al.,

2010). There are many other authors suggesting that preoperative endoscopy should be performed to all patients prior to bariatric surgery because it is useful in detecting both lesions and inflammation and the prevalence of gastrointestinal diseases with a significant impact on perioperative management is high (Wiltberger et al., 2015) (Munoz et al., 2009) (Csendes et al., 2007).

The controversy occurs with other studies on large serial cases, claiming that while abnormalities on preoperative GI endoscopy are often found in patients undergoing bariatric surgery evaluation, rarely do the findings change surgical management (Gomez et al., 2014). A large European retrospective study on 412 patients undergoing bariatric surgery does not support the performance of routine preoperative GI endoscopy prior to gastric by-pass by comparing the required resources for this investigation and the influence of the findings on the operative plan (Peromaa-Haavisto and Victorzon, 2013). Withal, the only study in literature considering also the estimative general cost for a patient undergoing upper GI endoscopy with biopsy under conscious sedation in an ambulatory surgery center concluded that due to rarely changes in surgical management related to endoscopic findings, alternative methods for screening for common GI conditions should be considered in appropriate patients (Gomez, et al., 2014). What do the guidelines say? According to American Society for Metabolic and Bariatric Surgery (ASMBS) guidelines, clinically significant gastrointestinal symptoms should be evaluated before bariatric surgery with imaging studies, upper gastrointestinal series, or endoscopy and routine screening for the presence of Helicobacter pylori before bariatric surgery may be considered in high-prevalence areas (Mechanick et al., 2013). The evidence-based guidelines of the European Association for Endoscopic Surgery concluded that upper gastrointestinal endoscopy or upper GI series is advisable for all bariatric procedures, but is strongly recommended for gastric bypass patients (Sauerland et al., 2005). Concerning Helicobacter pylori infection, bariatric patients are affected in 23 to 70% of cases (Erim et al., 2008) (Verma et al., 2013). The preoperative management of positive H. pylori morbidly obese patients is also a matter of debate. In a retrospective study of 560 patients who underwent laparoscopic Roux-en-Y gastric bypass (LRYGB), the incidence of postoperative marginal ulcers was higher (6.8%) in patients who did not benefit of Helicobacter pylori screening and treatment prior to bariatric surgery comparing to the incidence of these complications (2.4%) in patients who were treated for H. pylori infection before surgery (Schirmer et al., 2002). A more recent study showed the opposite, pointing out that there is no effect of H. pylori infection on the rates of marginal ulcer or stomal stenosis in patients undergoing LRYGB (Rawlins et al., 2013). Discussing sleeve gastrectomy as an alternative procedure in bariatric surgery, a study on 184 bariatric patients concluded that Helicobacter pylori infection seems not to influence postoperative outcome of patients benefiting of laparoscopic sleeve gastrectomy (Rossetti et al., 2014). Because in our group of patients there were no postoperative complications, we can say that Helicobacter pylori infection did not affect in any way the immediate postoperative evolution of patients undergoing sleeve gastrectomy.

Obesity is an important risk factor for multiple types of cancer, such as gynecological, renal and digestive malignancies (gallbladder, pancreas, esophagus, stomach and colon) (Bergstrom et al., 2001) (Chow et al., 1998) (Lin et al., 2014) (Calle et al., 2003). Thus, although obesity favors malignancy in different sites, this is the first case of gastric

cancer reported in literature as diagnosed by upper GI endoscopy during the preoperative evaluation for patients undergoing bariatric surgery. Even more, although preoperative endoscopy rarely diagnoses pathological conditions that may change the surgical approach, we believe that, for a complete work-out of these patients, gastroenterology consultation and upper GI endoscopy should be mandatory prior to bariatric surgery.

TABLE 2 *Digestive symptoms and endoscopic findings in bariatric patients*.

Postprandial symptoms	**Dyspepsia - abdominal fullness**	**Epigastric pain**	**Dyspepsia and epigastric pain**	**Regurgitations**	**Regurgitations, dyspepsia and pain**	**No symptoms**	**Number of patients**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Number of patients	6 (7.79)	9 (11.68)	24 (31.16)	10 (12.98)	4 (5.19)	24 (31.16)	77 (100)
Endoscopy findings: chronic gastritis. HP "+"	4 (20)	2 (10)	7 (35)	2 (10)	0 (0)	5 (25)	20 (100)
Endoscopy findings: chronic gastritis. HP "-"	1 (12.5)	2 (25)	3 (37.5)	0 (0)	1 (12.5)	1 (12.5)	8 (100)
Endoscopy findings: small hiatal hernia	0 (0)	0 (0)	1 (20)	1 (20)	2 (40)	1 (20)	5 (100)
Endoscopy findings: benign tumors	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	1 (50)	2 (100)
Endoscopy findings: gastric cancer	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
No pathological findings on endoscopy	0 (0)	5 (12.19)	13 (31.70)	7 (17.07)	0 (0)	16 (39.02)	41 (100)

I.3. Metabolic Surgery and the Metabolic Syndrome

I.3.1. Introduction

Obesity, defined by a body mass index (BMI) $\geq$ 30 kg/m^2, often coexists with the metabolic syndrome, which is associated with increased risk for cardiovascular diseases (CVD). The risk of serious health consequences is associated with an increase in BMI (Robins et al., 2003) but it is an excess of body fat in the abdomen measured simply by waist circumference that is more indicative of the metabolic syndrome profile than BMI. Excess lipid accumulation in several organs, including adipose tissue, liver, muscle, heart, and blood vessels, results in insulin resistance and triggers metabolic inflammation, a low-grade and chronic inflammatory response (Samuel et al., 2010) (Samuel and Shulman, 2012).

Obesity is associated with hypertension, diabetes, elevated triglyceride, and decreased HDL-cholesterol levels, all acknowledged as independent CVD risk factors by the American Heart Association (Poirier et al., 2006). Most patients with obesity present with lipid abnormalities; however, only 20% of the obese patients' population are not showing classical metabolic lipid changes (Karelis et al., 2004). Hyperlipidemia is widely recognized as one of the main co-morbidities in severe obesity. It is therefore not surprising that research and

treatment are increasingly focused on lipid profiles in the drive to potentially reduce cardiovascular related diseases (Vila et al., 2009). As the metabolic syndrome correlation with CVD risk and overall mortality was demonstrated for both lean and obese subjects (Hinnouho et al., 2013) (Malik et al., 2004), the accurate evaluation of the metabolic syndrome parameters is a more valuable follow-up parameter for bariatric patients than BMI and weight loss (Fica and Sirbu, 2015).

Bariatric surgery, designed to achieve and sustain substantial weight loss, was demonstrated by numerous studies to improve obesity-related comorbidities with still few long term follow-up data to confirm the stable effect. The control mechanisms of metabolic surgery are unclear, but it is likely that the surgery resets metabolic parameters in a balanced way, such that energy intake and expenditure are optimized. Among the various surgical procedures laparoscopic sleeve gastrectomy (LSG) was proven to obtain significant reduction in glucose, triglyceride levels, triglycerides/HDL ratio and increased HDL levels and these changes were maintained under normal ranges for at least two years after surgery (Ruiz Tovar et al., 2012).

Personal contribution – published paper:

Timofte D, Hristov I, Zugun-Eloae F, Ungureanu MC, Galeșanu C, Mocanu V. Middle term impact of sleeve gastectomy on major cardiovascular risk facors in a group of romanian obese patients. Acta Endocrinologica (Buc), 2017, 12(4): 454 – 460.

The aim of our study was to evaluate the impact of bariatric surgery procedure (LSG) on the main metabolic parameters.

I.3.2. Material and methods

This prospective study was conducted between June 2012 and January 2016 on obese patients hospitalized for bariatric surgery in the Surgery Service, "Sf. Spiridon" Clinical Emergency Hospital in Iasi (Romania). The study included 85 obese patients, proposed for bariatric surgery according to the recommendations of the National Institutes of Health (NIH) consensus on gastrointestinal surgery for severe obesity (NIH conference. Gastrointestinal surgery for severe obesity (Consensus Development Conference Panel, 1991): BMI≥ 40 kg/m^2 or BMI≥ 35 kg/m^2 with obesity-related morbidities including: type 2 diabetes mellitus, arterial hypertension, sleep apnea or dyslipidemia.

The study was approved the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (Romania) and all patients signed an informed consent

From the study group, 33 were males (38.8%) and 52 were females (61.2 %), with a mean age of 40.2±10.2 years. The mean BMI for the male's subgroup was: 44.5±5.3 kg/m^2 as for the female's subgroup was 43.4 ± 8.0 kg/m^2 and 37.6% (32 patients of 85) were morbidly obese (BMI>45 kg/m^2). (**TABLE 3, TABLE 4**). The bariatric surgery procedure was Laparoscopic Sleeve Gastrectomy (LSG). From the initial study group of 85 patients, we recorded 32 patients with complete lipid profile and serum glucose follow-up at 12 months in order to evaluate the paired statistical significance of the post bariatric surgery impact.

Anthropometric data were collected at three different points: preoperatively, at 6 months and at 12 months after surgery. Anthropometric evaluation was based on the

determination of weight (kg), height and BMI (kg/m^2). The weight change was reported as the percentage of excess weight loss (%EWL). This widely used equation was estimated as the percent excess weight loss (using the initial excess weight) or %EBWL = (preoperative body mass index – current BMI) x100)/(preoperative BMI – 25kg/m^2) (Ruiz-Tovar et al., 2012).

TABLE 3 *Characteristics of patients included in the study*

Parameter	Men (N=33)	Women (N=52)	Total (N=85)
Age, years (mean ± SD)	41.8 ± 8.4	39.2 ± 11.1	40.2 ± 10.2
BMI>45kg/m2, N (%)	14 (39.4%)	19 (36.5%)	32 (37.6%)
Arterial hypertension N(%)	16 (38.5%)	15 (28.8%)	31 (36.5%)
T2DM, N(%)	17 (51.5%)	15 (28.8%)	32 (37.6%)
Any dyslipidemia, N(%)	29 (87.8%)	45 (86.5%)	74 (87.1%)
Liver steatosis, N(%)	29 (87.8%)	19 (36.5%)	48 (56.5%)
MS, N(%)	29 (87.8%)	30 (57.7%)	59 (69.4%)

Mean values ± standard deviation. 2P-values were assessed between at baseline and at 12 months after bariatric surgery by paried Student's t-test. Abbreviations: BMI: body mass index, T2DM: Type 2 Diabetes mellitus, MS: Metabolic syndrome

The blood samples were collected after a 12 hour fast. We evaluated the serum lipid profile and glucose in obese patients before bariatric surgery (baseline levels) and at 6 and 12 months post-operative follow-up. Serum levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) were measured. The hexokinase method was used to measure serum glucose, and photometric method (Abbott Architect c16000 analyzer) was used to measure serum concentrations of total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-cholesterol), and low-density lipoprotein cholesterol (LDL-cholesterol). We evaluated the metabolic syndrome parameters using the reference values of the IDF definition of the metabolic syndrome criteria (2006), including the lipid profile with the following cutoffs for dyslipidemia: TG > 150 mg/dL, HDL-C < 40 mg/dL (male patients)/ HDL-C < 50 mg/dL (female patients), blood pressure measurement (systolic value ≥ 130 mmHg or diastolic value ≥ 85 mmHg) or arterial hypertension previous diagnosis criteria, and high fasting plasma glucose ≥ 100 mg/dL or previous diabetes mellitus diagnosis criteria. For obese patients BMI ≥ 30 kg/m^2, IDF criteria do not include waist circumference criteria. Additional data included the prevalence of hepatic steatosis evaluated by abdominal ultrasound as a serious metabolic consequence of obesity (**TABLE 3**).

I.3.3. Results

Regarding the results we obtained, the baseline lipid profile for our study group before bariatric surgery and it was characterized by atherogenic dyslipidemia (high serum concentrations of LDL-cholesterol/non-HDL-cholesterol and low levels of cardio-vascular protective fraction HDL cholesterol), high triglycerides and hyperglycemia (**TABLE 4**). The bariatric surgery outcomes followed the BMI reduction at 1 month, 3 months, 6 months and 12 months after bariatric surgery, when a mean BMI of 29 kg/m^2 was obtained for the study group (**Fig. 2**). The mean excess weight loss (EWL%) was 58% at 6 months and 72 % at 12 months after the intervention, the overall success rate for LSG defined by EWL% is > 50% (**Fig. 3**). When we analyzed EWL% according to age and BMI categories we obtained higher

EWL% rates for the subgroup of patients younger than 40 years old, and also in terms of BMI the subgroup of patients < 45 kg/m2 obtained better weight loss results (**Fig. 4, Fig. 5**)
At twelve months after the intervention, we found statistically significant improvements in triglycerides, HDL-cholesterol, total cholesterol and glucose in both female and male subgroups after bariatric surgery. The evidence showed a more important decrease in triglycerides level and total cholesterol for males (-40.6% and -18.9%) compared to females (-29.9% respectively - 2.8%). We could not find a statistically significant reduction of LDL cholesterol (**TABLE 4**).

TABLE 4 *Characteristics of patients included in the study*

PARAMETER		Before surgery (N=85) (M=33, F=52)	At 6 months (N=40) (M=12, F=28)	At 12 months (N=32) (M=10, F=22)	Change at 12 months (%)	P-value
BMI (kg/m^2)	M	44.5±5.3	33.9±3.7	30.2±2.2	-28.5	<0.001
	F	43.4±8.0	32.3±5.3	30.4±4.9	-30.3	<0.001
TC (mg/dL)	M	208.4±38.3	194.1±34.0	190.6±34.9	-18.9	0.004
	F	217.7±40.4	118.3±24.4	197.3±35.8	-2.8	0.004
LDL-C (mg/dL)	M	127.9±34.9	118.3±24.4	113.7±9.8	-16.5	0.253
	F	138.5±36.5	123.3±30.4	120.7±16.7	+0.8	0.929
HDL-C (mg/dL)	M	36.8±10.5	41.3±11.9	46.9±8.6	+26.2	0.006
	F	46.1±7.5	48.3±1.6	58.4±13.0	+29.0	0.004
TG (mg/dL)	M	231.9±78.1	133.7±50.6	130.0±10.6	-40.6	0.001
	F	132.9±54.2	109.8±31.9	78.7±8.7	-29.9	0.001
GLU (mg/dL)	M	113.5±31.8	87.8±4.8	88.3±3.3	-9.2±0.1	0.009
	F	107.1±41.0	86.9±3.5	92±0.9	-16.7±0.4	<0.001

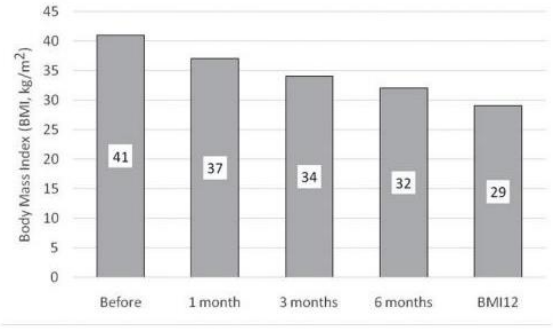

Fig. 2. *Mean BMI dynamics at 1 month, 3 months, 6 months and 12 months in post bariatric surgery obese patients*

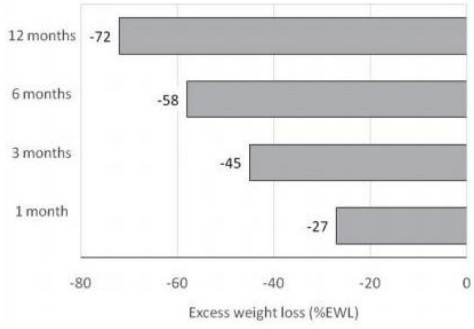

Fig. 3. *Excess weight loss precentage (%EWL) after bariatric surgery procedure (LSG) – follow-up at 3 months, 6 months and 12 months*

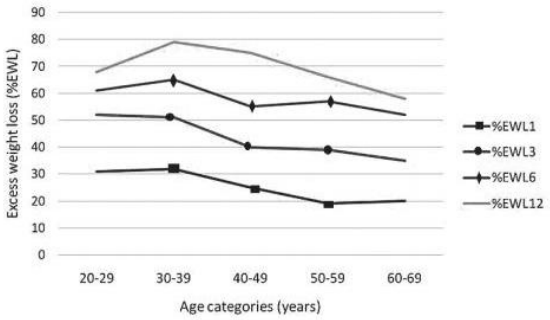

Fig. 4. *Excess weight loss percentaje (%EWL) according to patients age category*

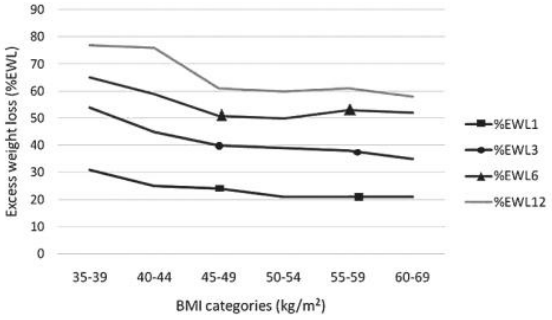

Fig. 5. *Excess weight loss percetage (5EWL) accroding to preoperative BMI category*

The metabolic syndrome prevalence was reduced progressively from 69% before the bariatric surgery intervention to 30% at 6 months after surgery and at 12 months after the intervention only 17% of our study group had persistent metabolic syndrome criteria. (**Fig. 6**). The evolution in post-bariatric surgery period for the metabolic syndrome parameters was subject for a detailed data analysis regarding glucose and lipid metabolism changes in post bariatric patients. Lipid fraction follow-up at 3 months, 6 months and 12 months after bariatric surgery shows a significant reduction for serum triglycerides (-30% at 12 months) and total cholesterol (-12% at 12 months) together with an augmentation of HDL fraction of cholesterol by 26% which contributes to an optimized, non-atherogenic lipid profile (**Figs. 7, 8 and 9**).

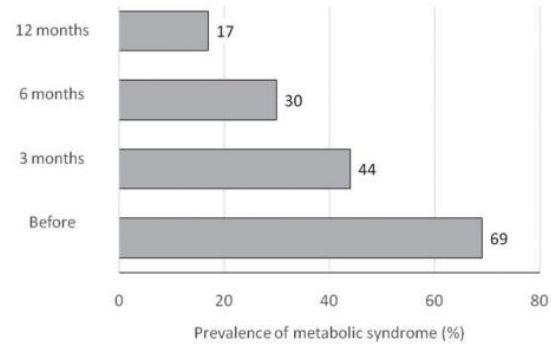

Fig. 6. *Metabolic syndrome (IDF 2006 definition) prevalence at baseline and after bariatric surgery procedure (LSG) at 3 months, 6 months and 12 months follow-up*

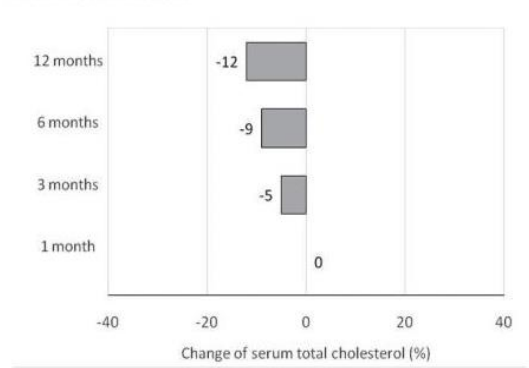

Fig. 7. *Total cholesterol reduction percentage after bariatric sugery procedure (LSG) follow-up at 3 months, 6 months and 12 months*

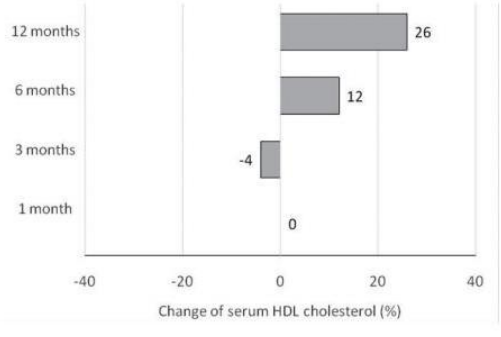

Fig. 8. *Changes in High density lipoprotein serum levels after bariatric surgery*

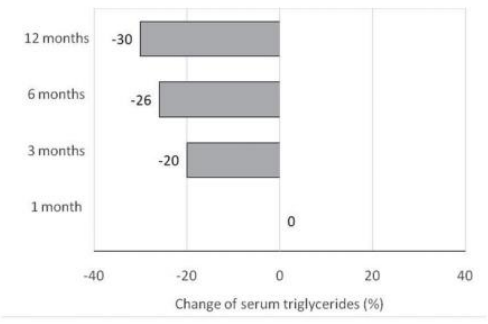

Fig. 9. *Serum triglycerides reduction percentage after bariatric surgery*

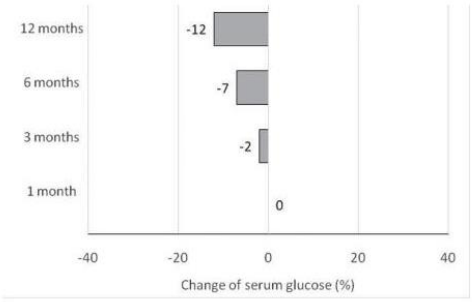

Fig. 10. *Changes in fasting glucose levels after LSG (%from pre-operative values)*

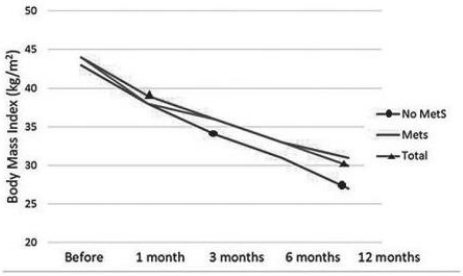

Fig. 11. *Body mass index (kg/m2) descendent curve after LSG according to the metabolic syndrome status*

Another metabolic syndrome parameter according to IDF definition we evaluated is fasting glucose that was also improved after the bariatric surgery procedure (mean decrease of 12% towards preoperative values) (**Fig. 10**).

The metabolic syndrome criteria in obese patients seem to have an influence also on weight loss velocity, as we obtained different descending curves for BMI after bariatric surgery, with a flatter descending curve in patients with associated metabolic syndrome criteria, translated as a tendency for weight loss resistance, compared to "metabolic healthy" obese subgroup (**Fig. 11**).

I.3.4. Discussion

In this study, we reported significant improvement in serum glucose, TC, HDL cholesterol and TG one year after LSG intervention in Romanian patients. This suggests that LSG surgery associated with appropriate weight loss has a positive effect on obesity comorbidities like atherogenic dyslipidemia and serum glucose levels. These positive results of LSG on metabolic parameters are obtained during the first 6 months after surgery and maintained at 12 months follow-up.

Similar studies following LSG effects on a Romanian group of patients also demonstrated a significant improvement on lipid fractions, including a decrease in total cholesterol, LDL cholesterol and TG levels as well as an increase in HDL levels (Sirbu et al., 2012) and also a major effect on type 2 diabetes remission, with normal fasting glucose levels in all diabetic patients at one-year follow-up (Iordache et al., 2008).

Bariatric surgery has been shown to resolve or improve cardiovascular risk factors such as diabetes, hypertension and dyslipidemia. The meta-analysis by Buchwald et al. (Buchwald et al., 2004) followed the bariatric surgery effect on type 2 diabetes, including 621 studies with 135,246 184 patients (all types of bariatric surgery) and reported that 78% of diabetic patients had complete resolution (HbA1C <6.5% and no anti-diabetic drugs) and 86.6% had diabetes improvement. Also, the Stampede Trial, a reference study evaluating long term bariatric surgery effects, showed that not only there was a significant reduction in overall oral hypoglycemic use, but at 1-year follow-up only 8% of Sleeve Gastrectomy patients required insulin (Schauer et al., 2012). Although the effect of bariatric surgery on the glucose metabolism is widely demonstrated, with significant improvement of overall glycemic control in diabetic patients, correlated with the duration of type 2 diabetes mellitus (Capoccia et al., 2015), there are data that show a tendency for increased fasting plasma

glucose in patients suffering gastrectomy intervention for malignancy (Jin et al., 2016). Our data regarding the fasting glucose levels follow-up at 12 months after LSG showed a significant decrease (12%) for serum glucose compared to pre-operative values.

Literature evidence are even less conclusive concerning the lipid metabolism with heterogeneous data regarding the effect on lipid fractions. Weight loss surgery promotes substantial decreases in triglycerides levels as well as improvements in cholesterol profiles. Buchwald et al. (Buchwald et al., 2009) showed a 41% reduction in serum triglycerides one year after RYGB, an elevation in HDL cholesterol by 23%, and lowering of LDL cholesterol by 19%, with sustained beneficial effects on cholesterol profiles up to 2 years follow-up. A long-term follow-up study of morbidly obese patients with T2DM (n=219) reported a 40% decrease in triglyceride levels and 20% increase in HDL-C levels. These improvements were maintained 2 to 4 years after surgery (Kim and Richards, 2010).

Valezi et al. (Valezi et al., 2004) reported normalization of serum TC concentrations in 51.7% of the patients, an improvement in 44.8%, and no changes in 3.5% Courcoulas et al. (Courcoulas et al., 2013) observed remission of all types of dyslipidemia in 61.9% of their patients after RYGB and in 27.1% after another surgical technique: laparoscopic adjustable gastric banding (LAGB).

Comparing LSG to the reference metabolic surgery procedure-RYGB, Vidal et al. (Vidal et al., 2012) found significant improvements in high-density lipoprotein cholesterol (HDL) and triglycerides (TRG) after LSG and the results, similar to those seen after gastric bypass but no change in low-density lipoprotein cholesterol (LDL) and total cholesterol (TC). Also Perathoner et al. (Perathoner et al., 2013) demonstrates the positive effect of LSG at 12 months follow-up with the resolution of diabetes in 85% of patients and dyslipidemia remission in 50%. Omana et al. (Omana et al., 2010) found a greater resolution or improvement of hyperlipidemia with LSG in comparison with laparoscopic adjustable gastric banding. Hyperlipidemia improved in 87% of patients after LSG and in 50% of patients after gastric banding after a 15-month follow-up period.

Long term follow-up (6-8 years) data published in 2012 by Eid et al. (Eid et al., 2012) showed a 77% improvement or remission of diabetes, lesser medications for diabetes, hypertension and hyperlipidemia in patients after sleeve gastrectomy. A systematic review of the literature, published by Khalifa in 2013 (Al Khalifa et al., 2013), concludes that LSG has a significant effect on hyperlipidemia, producing resolution or improvement in most of the cases. Therefore, LSG is an effective surgical option for weight loss and reduction in comorbidities such as hyperlipidemia.

In this way, our data are conclusive in terms of weight loss consecutive to LSG, comparable to other clinical trials that have reported similar 60-64 % for EWL% (Sieber et al., 2014) (Boza et al., 2014). This study confirms the existing data on the benefits of the laparoscopic sleeve gastrectrectomy (LSG) procedures on atherogenic dyslipidemia, but additional data are needed for the evaluation of the long-term effect on cardiovascular mortality, morbidity and risk factors in obese patients. The main drawback of the study was the small lot of patients. A larger study and a longer follow-up is necessary for establishing the metabolic impact for this bariatric surgery procedure.

I.4. The Financial Approach of Metabolic Surgery

I.4.1. Introduction

Administrative data is considered to be the information which is collected primarily for administrative purposes and not for research purposes. In most cases this type of data is collected by government departments or other types of organizations for the purposes of registration, transaction and record keeping, usually during the delivery of a service. The use of administrative data in the field of surgery data has grown popular for measuring the specific results of a procedure, the results of a surgery performed in a large population, and for estimating health economic costs. The use of this type of data is now allowing us to compare the results published by specific surgeons and those that occur in more large population samples. Furthermore, the use of administrative data analysis may help to find less commonly occurring outcomes that are found in larger populations, but are very difficult to quantify in smaller case series. Using administrative data may help defining the population risk level of adverse outcomes, such as complications after gastric bypass, but it shows clear limitation when we are trying to find the factors behind these outcomes (Flum and Dellinger, 2004).

The advantages and disadvantages of using administrative data in a study should be identified most easily comparing variant with the alternative of using survey data. The difference comes from how we formulate our hypothesis. The research hypotheses that are appropriately addressed for a use of administrative data are qualitatively different from those appropriately addressed by surveying a subset of the general population. Therefore, the particular question asked by the specific study should determine the type of data that one should use. For example, if an exhaustive study of a specific issue requires collected data from a population which is not included by the available administrative data or if important variables are missing; other data collection should be completed. Ideally, data from multiple sources should be used when we identify or rule out multiple potential causes of a particular phenomenon.

Few studies have examined the correlation between administrative data and survey data. A study compared information extracted from administrative databases collected for hospital billing purposes with specific clinical samples. No correlation of these data with that gathered from specifically designed clinical sources was found (Ferguson et al., 2000).

In other countries efforts are being made to raise the quality level of these administrative data. For example, in the United States, the STS National Cardiac Database has grown to include outcomes collected on more than 2 million patients from 60% of all cardiac surgery programs (Ferguson et al., 2000). This data base is a voluntary registry but is continuously audited on many levels for completeness and accuracy and is generally accepted in the specialty field of cardiac surgery as the benchmark for clinical outcomes analysis (Shahian et al., 2004). Studies have shown that this administrative database are correlated with the source data, the patient clinical hospital record. Results also showed that data are highly accurate for the reporting of major end points (Herbert et al., 2004). For example, the report of operative mortality was strongly correlated with source data, and there was an error rate of less than 1% for all major complications post-surgery (Herbert et al., 2004).

Personal contribution – published paper:

Timofte D, Pantea Stoian A, Hainarosie R, Diaconu C, Bulgaru Iliescu D, Bălan G, Ciuntu B, Neagoe RM. A review on the advantages and disadvantages of using administrative data in surgery outcome studies. J Surgery 2018, 14(3) :97-99.

The importance of finding advantages and disadvantages of using administrative data or clinical data in a study which measures an outcome of a medical operation, comes from the fact that using any statistical technique regardless of its sophistication, cannot compensate for flawed data, and it is the reason that motivates the present review. Administrative data is usually derived from discharge billing forms, and it is considered to be the most inexpensive and readily available source of information regarding hospitalizations outcome (Shahian et al., 2001) (Shahian et al., 2004). Although this type of data was not originally intended for this purpose, it is now used to assess healthcare provider performance (Daley, 1994) (Iezzoni, 2003).

I.4.2. Advantages

The primary advantage of administrative datasets comes from the fact that they are typically very large, covering samples of individuals and also time periods which are not achievable financially or logistically through any survey method. Beside cost saving advantage, the comprehensiveness of administrative data is often considered to be the one of the main advantage for research purposes. Other advantage that should be included here is that it may provide data on individuals who would not normally respond to surveys.

The use of administrative data, in the surgery field, is superior to other data sources for identifying program participation (such as bariatric programs). In other words, using administrative data allows us to answer the following questions: what benefits were provided to whom, when, and in what amount. Other advantage may be in the fact that administrative data is collected on an entire population of individuals or families participating in a given program. This is allowing us to study low-incidence phenomena that may be expensive to uncover in a survey of the general population. Furthermore, this also makes it possible to analyze the spread of events over a geographical area. For example, the prevalence of obesity in certain areas, gender differences or how different cultures may influence the bariatric surgery outcome. In addition, given that information about events is usually collected in the actual moment when the event happened, there is a less probability for errors because of poor recollection.

Another important advantage for the use of administrative data is uncovering information that a survey respondent is unlikely to provide in an interview. It is expected that many patients will underreport the substance abuse, for example. Although survey methods have progressed significantly in addressing this kind of sensitive issues, administrative data can prove to be an accurate source of indicators for phenomena that are not easily reported by individuals, if this sensitive or confidential data can be accessed.

Administrative data may be also superior because the data record for an individual can be corrected and updated constantly. The value of this is even greater when the old information is maintained in addition to the updates. In this case, an exact history of the patients can be observed; his trajectory over time can be established. For example, for

bariatric programs the weight fluctuations may help the specialists find the point in time in which a patient became obese. Further investigations can then be made to find the trigger behind the patient weight gain, possibly making assisting him in his weight loss program easier.

Therefore the main advantages of using administrative data in surgery outcome studies include: already collected data for operational purposes and therefore no additional costs of collection; the acquisition process is in no way intrusive to the target population; this type of databases are regularly and continuously updated; administrative databases can provide historical information and allow consistent time-series to be built up; they are collected in a consistent way because they are usually part of a national/local system; it covers near 100% of the target population; control groups can be created or selected post hoc; captures data of individuals who may not normally respond to surveys.

I.4.3. Disadvantages

As in the case of advantages, the disadvantages of administrative data are also listed as a contrast to the characteristics of survey data. The most important disadvantages are related to the reliability of administrative data for research purposes and the lack of adequate control variables. Also, the administrative data may be difficult to access because of confidentiality issues and because of bureaucratic issues in obtaining official approval. Furthermore, administrative data often lacks any documentation and information about the quality of the data. Time must be spent by the researcher to find out qualitative information about the condition of the data. In addition, time must be also spent for understanding how the administrative data was collected, processed, and stored.

In addition, one possible disadvantage of using administrative data in surgery data in surgery outcome studies refers to the incorrect number of cases for analysis. This problem results from errors in diagnosis or procedure coding and from the use of software algorithms that cannot reliably identify isolated complications cases (Mack et al., 2005).

Other disadvantage is linked to the restricted study populations. These results are strictly based on only one group (health care patients for example), especially in studies based on administrative databases, which are not necessarily representative of overall program quality and may bias the results (Hannan et al., 1997). Therefore, the results of these studies should only be extrapolated to the population from the respective administrative database. For example, it is important that in surgery studies that use administrative hospital data, the final results to be considered representative only for the hospitalized population.
Furthermore, various problems are linked to the non-standardized mortality end points that are found in administrative data bases. This problem emerges from the fact that most administrative data registries record only in-hospital mortality, whereas clinical databases often record 30-day mortality. The additional statistical and clinical implications of no standardized time of death intervals have been studied broadly in the literature (Osswald et al., 1999).

In addition, misalignment of data sources with their original intended use is also an issue. Many administrative databases are created for claims benefits coordination. Therefore, coding practices and algorithms are developed for such reimbursement issues, not for clinical outcomes profiling in surgery outcome studies (Romano et al., 2002).

Perhaps the most important disadvantage is linked with the absence of some critical clinical variables. This is problematic especially in surgical studies which measure various outcomes after various surgical operations. It has been shown that much of the predictive power of these surgical risk models is derived from a limited number of critical clinical variables that sometimes are missing in administrative databases (Iezzoni, 2003).

In the literature, it was found that complications and comorbidities are often confused in the administrative data. With administrative data, it is difficult to accurately code clinically relevant comorbidities or complications, to consistently capture all complications and comorbidities, and most important to distinguish comorbidities from complications (Jones et al., 1996).

Failure to distinguish complications from comorbidities leads to an exaggeration of risk models based on these administrative data. This bias comes from the inclusion of predictors in the risk model that are actually late-hospitalization, pre-terminal events and therefore present a high predictive power of mortality (Iezzoni, 1997) (Iezzoni et al., 1998).
Given all these disadvantages, several studies have failed to found a correlation between administrative databases and clinical ones. These studies showed differences in the outliers determined using administrative versus clinical data, even when they compared the clinical databases with relatively sophisticated administrative databases (Geraci et al., 2005) (Parker et al., 2006).

I.4.4. Discussion

Using the administrative databases in clinical studies on surgery outcomes can be used to reduce costs and to obtain a large sample of patients rapidly. To ensure that the results are not biased certain things can be done: the internal consistency of the data should be verified. It is important to understand how the data was collected and updated. If it is possible the data should be compared to any other available data sets through record linkage.

These ameliorations are required because hospital data records are still, for the most part, in their first generation of information systems. These systems are typically really old and do not take advantage of much of today's technology. In hospitals, front desk workers are typically not trained or do not have the time or resources to take on the data entry task. The modernization of hospitals correlated with the development of new graphical user interfaces is likely to have a positive effect on data entry and on the quality of administrative data bases.

I.5. The Surgeon in the Adipose Tissue Research

I.5.1. Introduction

Adipose tissue (AT) is no longer considered to be simply a passive lipid reservoir but rather an endocrine organ capable of secreting factors that profoundly influence processes such as feeding behavior, energy flux, and immune-inflammation (Mutch et al., 2009). Moreover, adipose tissue displays enormous plasticity and is capable of changing its size, phenotype and metabolic functions (Schoettl et al., 2018).

The classical abdominal adipose tissue (AT) compartmentalization into subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) has been widely studied in relation to obesity-related complications. The anatomical distinction of SCAT compartments into

superficial subcutaneous adipose tissue (sSCAT) and deep subcutaneous adipose tissue (dSCAT), divided by Scarpa's fascia, is well documented in literature. A few studies have shown that dSCAT is strongly related to insulin resistance (IR) in a manner nearly identical to that of VAT (Marinou et al., 2014).

The main white adipose tissues (WATs) are abdominal subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT). VAT surrounds the inner organs and can be divided in omental, mesenteric, retroperitoneal (surrounding the kidney), gonadal (attached to the uterus and ovaries in females and epididymis and testis in men), and pericardial. The gluteo-femoral adipose tissue (g) is the SCAT located to the lower body parts and is measured by hip, thigh, and leg circumference. WAT can also be found intramuscularly. The adipose tissue depots that have been linked to risk of developing obesity-related diseases are the omental and mesenteric (Bjorndal et al., 2011).

Commonly obesity is characterized by adipocyte hypertrophy, followed by increased angiogenesis. There is a chronic state of low-grade inflammation with progressive immune cell infiltration, extracellular matrix overproduction into obese adipose tissue. Production of proinflammatory adipocytokines is increased during the progression of chronic inflammation (Bremer and Jialal 2013).

The subcutaneous fat is readily accessible to study and has been shown to be metabolically correlated to indices of insulin resistance. Obtaining adipose tissue samples is paramount to the understanding of the pathophysiology of human obesity and its analysis may provide insightful overviews of mechanisms relating to metabolism and disease (Mutch et al., 2009).

Personal contribution – published paper:

Pricope-Veselin A E, Mocanu V, **Timofte D.** Open surgical and needle biopsy to study abdominal subcutaneous adipose tissue in obesity. J Surgery 2018, 14 (3):111-113.

I.5.2. Biopsy techniques: advantages and limits

Regarding the open (surgical) SCAT biopsies, the surgical subcutaneous fat biopsies can offer tissue samples that may provide a more comprehensive overview of the complexities of biological indices in white adipose tissue. Subcutaneous superficial AT samples are obtained by surgical biopsy from the periumbilical area, under local anesthesia (1% xylocaine) (Mutch et al., 2009) (Hansson et al., 2011) (Heinonen et al., 2015). First, the skin is cleaned and covered with special surgical drapes. An incision of <5 mm is made with a plain scalpel to access the subcutaneous AT. The surgeon held the tissue with atraumatic forceps and cut the tissue pieces with scissors. About 2-3 cm^3 (corresponding to about 3-5 g) AT could be removed. The skin incision is then closed with absorbable suture material (Bogov et al., 2008).

Side effects includes local discomfort, infections, bleeding or skin lesions in the tissue after diathermy.

In regards to ***punch biopsy***, the biopsy site is prepared with three betadine scrubs and covered with a fenestrated sterile drape (Guidelli et al., 2015). The dermis at the biopsy site (at the right anterior axillary line at the level of the umbilicus) is infiltrated with 0.5 ml of 1%

lidocaine followed by injection of 4 mL in the very superficial layers of adipose tissue immediately below the skin (no more than 1 cm deep). Punch biopsy is performed using a circular blade (3.0 mm in diameter) attached to a pencil-like handle. The instrument is rotated down through the epidermis and dermis and into the s.c. fat. Punch biopsy yields a cylindrical core of tissue that requires gentle handling (usually with a needle) to prevent a crush artefactat the pathological evaluation. The biopsy site is closed with a single absorbable suture. The procedure yield about 150 mg of intact SCAT. Precautions must be taken in patients who have a history of bleeding disorders and those that are on medications that aect hemostasis. The punch biopsy itself takes roughly 15 minutes and does not require any hospitalization. A patient may return to normal daily life immediately following a punch biopsy (**TABLE 5** and **TABLE 6**).

TABLE 5 *Techniques to study Subcutaneous Adipose Tissue (SCAT*)

Procedure	**Open SCAT biopsy**	**CNB**	**FNA**
Preparation	8 h fast	8 h fast	8 h fast
Setting	Bedside procedure	Bedside procedure	Can be performed anywhere
Anesthesia	Local anesthesia	Local anesthesia	No anesthesia
Location	Abdominal region: periumbilical (5 cm from the umbilicus		
Procedure	Incision: Open biopsy: 5 mm skin incision. The tissue is held with atraumatic forceps and cut the tissue pieces with scissors. The procedures last about 40 min. Punch biopsy: circular blade (3.0 mm in diameter. The procedure last about 15 min. The skin incision is then closed with absorbable suture material	Incision: 5-mm skin incision. The needle biopsy is performed using a 20 – 50 mL plastic syringe (filled with saline) attached to a 14-gauge aspiration needle (or modified Bergstrom 6 mm). The needle is passed through the sc fat several times while applying negative pressure. The procedure last about 20 min.	No incision. The needle biopsy is performed using a 20 – 50 mL plastic syringe (filled with saline) attached to a 16-gauge aspiration needle The aspiration procedure takes only 5 – 8 minutes
Safety	Local discomfort, infections, blood collection under the skin	Well tolerated, low incidence of painful hematomas	Cause little pain or after effects
Tests	Adipocyte size and number Immunohistochemistry Tissue culture RNA extraction FISH studies	Tissue culture RNA extraction Flow cytometer FISH studies	RNA extraction Flow cytometer FISH studies
Tissue yield	3 – 5 g (open biopsy)	1 – 2g	100 – 500 mg

Also, the ***Needle muscle biopsy*** sampling offers a less invasive, more rapid alternative to conventional open SCAT biopsies for for research purposes. The cellular material could be obtained through needle puncture, either relying on the forward motion and

the intrinsic capillary action of an FNA or by using spring-loaded physical cutting action of a tissue core by CNB (Vander Laan, 2016). The external diameter of the needle is described by the respective needle gauge, with a higher gauge corresponding to a smaller needle outer diameter. The needle gauge and thickness of the needle wall dictates the maximal diameter of any tissue fragments obtained by FNA or CNB, although appreciable microscopic tissue architecture can be obtained even with high gauge needles.

Fine needle aspiration (FNA): FNA biopsy is a reliable, cost-effective procedure that may be performed anywhere (bedside, outpatient clinic, remote setting). The risk of a significant complication is minimal, and although such incidents have been reported, the chances of such an occurrence may be equated to the risk incurred when undergoing simple venipuncture. As with venipuncture, local anesthetic is generally not required (McGrath et al., 2008).

The technique involves application of negative pressure during the procedure, typically with the use of a syringe (10-20 mL) placed in a syringe holder (aspiration gun/handle). When using an aspiration gun or handle, one must remember to pull the plunger back only after the needle has been placed into the lesion; the plunger should remain pulled while rapid, short strokes are made. More important, it is imperative that suction be released before the needle is removed, because continued negative pressure results in suction of the material back into the syringe preventing preparation of direct smears.

Superficial subcutaneous AT samples of about 1-2 cm^3 (corresponding to bout 1-2 g) are obtained from the periumbilical area (Mutch et al., 2009). A region 5 cm lateral from the umbilicus (either to the left or right side of the abdomen) is sterilized. A needle 16 G, 40 mm, regular bevel is adapted to a 20 mL syringe and the piston compressed. Approximately onethird of the length of the needle is inserted into the subcutaneous fat, and the needle piston is released maximally until it is locked by a stopper, thereby creating a vacuum. Tissue resistance is created by the physician gripping the abdominal wall with one hand while the other hand rotated the needle throughout the tissue in an up-down motion. Once the tissue is aspirated by the syringe, the needle is withdrawn, and the piston is removed. The aspiration procedure takes only 5-8 min, causes little pain or after effects, and does not require sutures or entail a return visit by the patient (Kettewich et al., 2012) (Campbell et al., 2009) (Westermark, 2012).

Core-Needle Biopsy (CBN): CNB is generally performed with a larger-gauge needle, ranging from 13-gauge to 14-gauge (an outer diameter of 2.4 to 2.1 mm) (Chandalia et al., 2007). The other two common needle biopsy instruments used are the Bergstrom needle (Alderete et al., 2015) and the modified Bergstrom needle also known as the UCH biopsy needle. The most important modification of the UCH is the addition of a Luer lock attachment to the inner cannula to allow the application of suction during the procedure (Tarnopolsky et al., 2011). Suction applied during the procedure results in consistently larger samples. Superficial subcutaneous AT samples obtained are of about 1.5 grams, which ranged from 510 times greater than those obtained using a punch biopsy method (Chandalia et al., 2007) (Alderete et al., 2015) (Aron-Wisnewky et al., 2009) (Walker et al., 2014) (Biopsy needle modified Bergström 6 mm).

The biopsy site is prepared with three betadine scrubs and covered with a fenestrated sterile drape. The dermis at the biopsy site (at the right anterior axillary line at the level of the umbilicus) is infiltrated with 0.5 cc of 1% lidocaine followed by injection of 4 cc in the very superficial layers of adipose tissue immediately below the skin (no more than ½- inch deep). A 6-7 mm incision is made in the skin and a 6-mm Bergström side-cutting needle is introduced approximately 1-1.5 inches through the incision into the deeper SCAT.

TABLE 6 *Advantages and disadvatanges of different biopsy techniques used to Subcutaneous Adipose Tissue (SCAT)*

	Advantages	**Disadvantages**
Open SCAT Biopsy	Succes rate close to 100% Yield: adequate sample for any analysis accessible for sampling quick (40 min), larger samples	Surgical procedure, most invasive than needle biopsy, Performed under local anesthesia in a hospital or outpatient clinic Scarring Additional risk of conscious sedation Expensive side effects such as infection or blood collection under the skin
CNB	Provides larger intact tissue fragments than FNA with preserved architecture Validated tissue for ancillary studies or immunohistochemistry Higher diagnostic yield for fibrotic tissue lesions For most lesions, has higher sensitivity, specificity, and diagnostic accuracy measures	Success rate 95% Requires the presence/expertise of an interventionalist. More expensive (equipment, requires image guidance) Higher complication rate (i.e. hemorrhage) Requires use of anesthesia or local anesthetic
FNA	Can be performed by a trained physician Rapid procedure (few minutes) Usually does not require anesthetic or anesthesia (not painful) Less traumatic (low risk of bleeding) Can be performed anywhere (bedside, outpatient clinic, remote setting) Lower complication rate Less expensive (needle and other processing equipment) Collection of fresh, intact, viable cells Entire nucleus present for FISH studies Excellent collection method for flow cytometry.	Success rate 95% Limited tissue architecture Lower yield for fibrotic lesions Cytologic specimen processing may pose validation challenges for downstream testing (immunohistochemistry, molecular testing) For most lesions, has lower sensitivity, specificity, and diagnostic accuracy measures.

After the needle is angled obliquely, an assistant applied brief suction from a 60-cc irrigation syringe attached to the Bergström needle with gastrointestinal irrigation tubing (Kendall; no. 1; 16 Fr/Ch × 48 inches). Four cuts are made with the cutting trocar as the needle was further advanced and rotated 90 degrees. The procedure is repeated with a second

pass to generate eight total cuts. Ultrasound (US) guidance could be used to ensure sampling from deep SCAT below the Scarpa's fascia, which is often 2-3 inches below the skin in obese participants. Some patients could experience minor discomfort when the needle was advanced through Scarpa's fascia. To rectify the discomfort, 1% lidocaine is administrated through a spinal needle guided by US into the superficial layer of Scarpa's fascia.

The time period from prepping the biopsy site to tissue processing averaged about 20 minutes.

After the biopsy procedure, manual compression is applied for 10 minutes to prevent bleeding. Following compression, a single nylon suture is used to close the wound. Bacitracin ointment is applied to the wound, which is then covered with dressing. The suture will be removed 5-7 days following the biopsy (**TABLE 5** and **TABLE 6**).

Little research has been conducted on association between subcutaneous adipose derived stem cells (SC-ASCs) differentiation and adipose tissues inflammation after bariatric surgery. We propose to assess the association between changes in SC-ASCs differentiation and inflammatory tissue and systemic biomarkers in obese patients at baseline and after 1- and 3-months follow-up.

I.5.3 Personal experience in this field

Personal contribution – published paper and research project:

1. Hristov I, Mocanu V, Zugun-Eloae F, Lăbușcă L, Crețu-Silvestru I, Oboroceanu T, Tiron C, Tiron A, Burlacu A, Pinzariu AC, Armașu I, Neagoe RM, Covic A, Scripcariu V, **Timofte DV**. Association of intracellular lipid accumulation in subcutaneous adipocyte precursors and plasma adipokines in bariatric surgery candidates. Lipids in Health and Disease, 2019, 18:141.
2. Adipose tissue biomarkers in short-term remission of metabolic syndrome after bariatric surgery. Contract no. 30340/2017, 2018-2019. Coordnator "Grigore T. Popa" Uiversity of Medicine and Pharmacy Iasi. Daniel Timofte- Project Director.

The main achievement in this area was the project "Adipose tissue biomarkers in short-term remission of Metabolic Syndrome after Bariatric Surgery", in which I was project director.

I.5.3.1. Introduction.

Obesity is characterized by low-grade, chronic and systemic inflammation resulting, in part, from altered adipose tissue (AT) immune responses (Labrecque et al., 2017). In the obese state, expression of several proinflammatory adipokines is increased, suggesting significant contribution of AT circulating levels of these mediators. In many cases, the quantitative contribution of AT to elevated plasma levels of these molecules in obesity remains to be fully established.

The current definition of the International Diabetes Federation (IDF) for the metabolic syndrome (Alberti et al., 2009) includes the assets of the cardiovascular risk factors that are considered the best predictors for cardiovascular mortality for obese and non-obese patients and includes parameters that can be accessible for screening (Mongraw-Chaffin et al., 2018). Insulin resistance evaluation by HOMA-IR is considered as a good cardiovascular risk predictor (Meigs et al., 2006), being also demonstrated as a valuable

criteria for recognition of the obese individuals with a higher mortality risk (Hinnouho et al., 2013).

However, around 35% of the obese individuals do not develop insulin-resistance or associated metabolic disturbances (Lin et al., 2017), and the particularities of adiposity expansion in the obese patients in not understood.

Insulin resistance is closely associated with disturbances of fat metabolism [6]. Thus, exceeding the storage capacity of the subcutaneous adipose tissues results in lipotoxicity, a condition characterized by fatty acid infiltration of insulin target tissues i.e., skeletal muscle and liver, that eventually leads to insulin resistance (Unger 2003).

High leptin levels and leptin:adiponectin ratio are predictors of obesity related complications as type 2 diabetes mellitus (T2DM) and hypertension independent of BMI or the metabolic syndrome (MetS) criteria (kim et al., 2006; Rueda-Clausenet al., 2010; Oda et al., 2008).

The subcutaneous fat mass expansion is initiated by adipocyte hyperplasia, a physiological process that generates new mature adipose cells through activation and differentiation of multipotent stem progenitors. However, increasing expansion requirements eventually exceeds the individual adipogenic differentiation capacity of preadipocytes. When this adipogenic potential is reached, excessive lipid accumulation results in a dysfunctional adipose tissue (Patel and Abate 2013; Rosen and Spiegelman 2000), due to adipocyte hypertrophy, decreased adipogenesis and angiogenesis (Anderssonet al., 2014; Lonn et al., 2010).

Subcutaneous adipose tissue consists predominantly of adipocytes, but also contains other cell populations generally referred to as the stromal vascular fraction (SVF). Zuk identified in the SVF, a multipotent, undifferentiated, self-renewing progenitor cell population that is morphologically and phenotypically like mesenchymal stem cells (MSCs) (Zuk et al., 2001). These isolated adipose tissue-derived stem cells (ASCs) display a capacity of differentiation like MSCs and show the expression of the specific stem cell markers in vivo (Woo et al., 2016). ASCs however have a series of advantages as a multipotent differentiation source as they are more accessible by simple subcutaneous adipose tissue biopsy, a repeatable minimally invasive method, the isolation procedure is simple and the stem cell quality and proliferation capacity that does not decline with the age of the patient (Beane et al., 2014). After isolation and proliferation of these ASCs, they can be used for experimental study of the molecular processes in regulating adipocyte differentiation (Russo et al., 2013).

Under a positive energy imbalance, AT expansion occurs through hypertrophy (increase in size of existing adipocytes) and/or hyperplasia (increase in adipocyte number through adipogenic adipocyte differentiation). The inability of preadipocytes to differentiate and store lipids may result in excessive adipocyte hypertrophy. This, in turn, is associated with altered endocrine and immune responses, such as adverse adipokine secretion. The AT of obese individuals is also characterized by macrophage infiltration, which is viewed as both a cause and a consequence of this AT immune response and leads to chronic inflammation. Current literature suggests that bariatric surgery may improve inflammatory status in morbidly obese individuals (Appachi et al., 2013) (Cancello et al., 2005).

Our research project will assess the following aspects:

- Assessment of adipose tissue biomarkers related to adipogenic capacity of subcutaneous adipose derived stem cells (SC-ASCs) precursors in obese patients before and after 1 and 3 months after bariatric surgery;
- Assessment of adipose tissue and systemic biomarkers (TNF alfa, IL-6, chemerin and adiponectin) related to inflammation of SC-ASCs in obese patients before and after 1 and 3 months after bariatric surgery;
- Relationship between subcutaneous adipose derived stem cells (ASCs) differentiation and inflammatory biomarkers in obese patients before and after 1 and 3 months after bariatric surgery;
- Evaluation of the changes in adipocyte differentiation capacity after bariatric surgery and the correlation with improvement of the metabolic status.

I.5.3.2. Materials and methods

In our research protocol we harvesting tissue samples during the bariatric surgery from two areas:

- from the specimen – the gastric fragment we usually take about 4 cmc from the greater curvature;
- from subcutaneous adipose tissue we take 2 cmc from the site of the left hypochondrium trocar.

We collected adipose tissue from both subcutaneous and visceral areas, in order to evaluate the changes in adipocyte differentiation capacity before and after the bariatric surgery.

Sample collection

Ethical aspects: The use of clinical data, the blood samples and subcutaneous adipose tissue collection were performed at the baseline in bariatric patients (N=4). Subcutaneous adipose tissue was harvest during the surgical procedure with minimal patient discomfort, after a signed informed consent approved by the Ethical Committee of the University of Iasi, Romania.

Characteristics of the study group: The study included 20 obese female patients (OB group), referred for Laparoscopic Sleeve Gastrectomy (LSG) and seven normal weight females (NW group, control) with other abdominal surgery indications. The mean body mass index (BMI) was 45.02±6.31 kg/m^2 in OB group and 24.46±2.50 kg/m^2 in NW group. The age ranges of the two groups matched, with a mean of 42.05±9.91 years for OB group and 42.00±10.67 years for the NW group.

Biochemical measurements: The blood samples were collected after 12 hours of fasting, previous to bariatric surgery procedure.

- The lipid profile was evaluated including: Total Cholesterol (TC), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL) and Triglycerides levels (TGL), and the Total cholesterol/HDL ratio.
- Insulin resistance have been assessed using the homeostasis model assessment (HOMA) index will be used to estimate insulin resistance (HOMA = fasting insulin (μU/mL) x fasting glucose (mg/dL)/405).

- Evaluation of the nutritional status: total proteins, albumin, 25 OH Vitamin D, folic acid, Vitamin B12, plasma Iron levels.

These tests are included in the bariatric protocol and are performed to all patients during the hospitalization.

Anthropometric measurements were performed at baseline.

Evaluation of eating habits and eating disorders. We applied questionnaires to assess the eating habits at baseline.

Evaluation of serum inflammatory biomarkers. Serum has been stored at -80C for further measurement of C reactive protein (CRP), fibrinogen, TNF alfa, IL-6, chemerin and adiponectin (ELISA).

Subcutaneous (SC)-adipose tissue collection and processing. SC adipose tissue has been obtained during the surgery procedures and approximately 1 g of adipose subcutaneous tissue has been extracted.

Microscopic analysis and morphometry of the subcutaneous adipose tissue. Part of the subcutaneous adipose tissue obtained was analyzed by optical microscopy and we performed a quantification of the amount and size of adipocytes. The adipose tissue slides stained with H&E were evaluated using TISSUEGNOSTICS rig consisting in Zeiss AxioObserver Z1 microscope and acquisition and analysis software. The rig can realize a consecutive scan of 8 slides in one working session using TISSUEFAXS software v.4.2. After a preview of the entire tissue section at low magnification 5x, a predefined number of, 3.8mm² circular randomly located regions in every slide are acquired at 20x magnification.

We used an Image analysis software - ADIPOSOFT 1.13 (based on ImageJ 1.49j10) to quantify the adipocytes area in hematoxylin and eosin (H&E) sections. Adiposoft is an automated, OS software (Author CIMA, University of Navarra, maintainer Mikel Ariz) for the analysis of white adipose tissue on histological, hematoxylin and eosin (H&E) stained sections, build on the frame of ImageJ software

Immunofluorescence staining of adipose tissue biomarkers. For tissue sections, fat pads have been performed in 5 µm thick paraffin-preserved subcutaneous adipose tissue following standard protocols. Inflammation was assessed by TNF-α staining and CD68.

Adipocytokines, inflammatory status of the adipose tissue

We stored (-80 ˚C) part of the adipose subcutaneous tissue that should be maintained available for adipocytokines dosage, using ELISA techniques. We evaluated later: TNF alpha, IL6, chemerin and adiponectin, and we will correlate the results with the biochemical profile.

Adipose derived stem cell isolation (ASCs), proliferation and differentiation.

ASCs have been prepared from adipose tissue samples by collagenase digestion and cultured according to an established protocol. Briefly, the protocol include: Digestion using collagenase 0,1 ml (100 ml/mg) in 9,9 ml HEPES Solution; Cell Separation by centrifuge at 500 g for 15 min; Cells were plated in a 24-wells plate by uniform distribution, at a density of 1.5×10^5 cells/well; Mesenchymal cells culture: incubation in DMEM=Ham's F12 (1:1) medium containing 10% FBS (fetal bovine serum) and antibiotics, at a density of cells were plated in a 24-wells plate by uniform distribution, at a density of 1.5×10^5 cells/well; Adipocyte differentiation protocol: using a specific differentiation cocktail: Insulin, Dexamethasone, IBMX.

Complete adipogenic differentiation is expected after 21 days. We are following the progressive lipid accumulation under an inverted microscopy, equipped with phase contrast (CEMEX).

Red Nile staining: To determine the lipid accumulation, differentiated mature adipocytes were fixed with 10% formalin for 20 min and then stained with 0.1% Oil Red O (ORO) for 60 min.

Identification of adipocyte specific markers

Differentiated adipocytes were fixed with 10% formalin and permeabilized in 0.1% Triton X in PBS. Immunofluorescence staining for perilipin (PLIN) was performed on differentiated ASCs. Images was taken using automated inverted fluorescent microscope and image processing will be performed with Image J Analysis Software.

Evaluation of healthy eating behavior pre-bariatric surgery

In order to investigate whether preoperative problematic eating behaviors predict weight loss outcomes following bariatric surgery we applied Food frequency Questionnaire, Eating Inventory and Anxiety and Depression Scale before the bariatric surgery.

Statistical analysis

Anthropometrical, biochemical and hormones concentrations were expressed as mean ±standard deviation. Comparisons were performed with the Mann–Whitney test. Significance was defined as $p < 0.05$. All statistical analysis was performed using IBM SPSS Statistics 21 Software.

I.5.3.3. Results

Characteristics of the patients

Characteristics of the obese patients and normal weight controls are showed in **Table 7**. Plasma levels of total cholesterol, LDL-cholesterol, total cholesterol/HDL-cholesterol, insulin, and HOMA-IR index were significantly increased in OB group as compared with NW group. Significantly high leptin: adiponectin ratio values were found in in OB group as compared with NW group. No significant differences were obtained for HDLcholesterol, triglycerides, fasting glucose, morning cortisol and adiponectin (**TABLE 7**). Adipocyte area was found increased in obese patient as compared to control (**TABLE 7**, **Figs. 12-15**)

The percentage od large adipocytes (area larger than 2500) was higher (52% of cells) in obese patient as compared to normal weight patient (19%) (**TABLE 7**).

Identification of inflammatory status in adipose tissue

In our study, we evaluated the immunofluorescence staining of TNFa and CD68 to identify the inflammatory status in adipose tissue (**Fig. 16**).

Adipose derived stem cell (ASCs) isolation, proliferation and differentiation.

The stromal fractions were obtained from subcutaneous abdominal adipose tissue derived from OB and NW group and grown in culture at a starting density of 1.5×10^5 cells/ml.

TABLE 7 *Characteristics of the obese patients and normal weight controls. The data are presented as the means±S.D. or percentage.*

Parameter	Obese patients (OB) N=20	Normal weight controls (NW) N=7
Age (years)	42.1 (9.9)/45.5 (23–63)	42.0 (10.7)/42.0 (25–58))
BMI (kg/m^2)	45.0 (6.3)/43.7 (36.4–58.9)	24.5 (2.5)/24.7 (21.2–29.0)
Total cholesterol (mg/dL)	210.6 (32.1)/212.5 (138–265)	166.7 (18.3)/159.0 (151–202)
LDL-cholesterol (mg/dL)	141.0 (29.0)/139.5 (80–184)	90.0 (2.8)/90.0 (86–93)
HDL-cholesterol (mg/dL)	49.7 (12.2)/49.0 (23–75)	54.2 (3.7)/54.0 (48–59)
TC/HDL (<4)	4.4 (1.0)/(3.1–6.2)	3.1 (0.3)/3.1 (2.7–3.4)
Triglycerides (mg/dL)	165.3 (103.5)/140.5 (69–448)	96.3 (14.4)/93.0 (73–115)
Fasting plasma glucose (mg/dL)	126.8 (74.1)/102.0 (77–362)	86.9 (8.9)/87.0 (73–99)
Insulin (μU/mL)	16.8 (9.1)/15.8 (6.0–43.4)	7.4 (1.6)/6.89 (6.3–10.7)
HOMA-IR	6.1 (7.0)/3.8 (1.4–28.7)	1.6 (0.4)/1.5 (1.3–2.5)
Adipocyte area	2721 ± 951	797 ± 13.71
Adipocyte area ≥ 2500 (%)	52	19

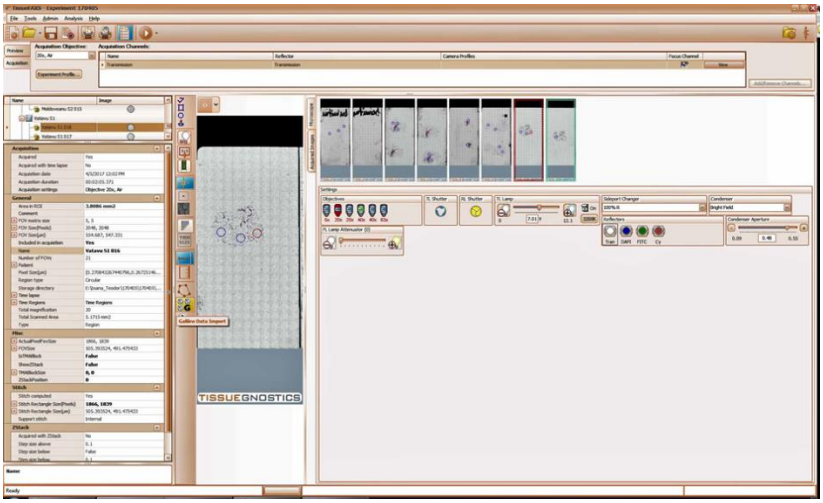

Fig. 12. *Acquisition of images by scanning smears from subcutaneous tissue*

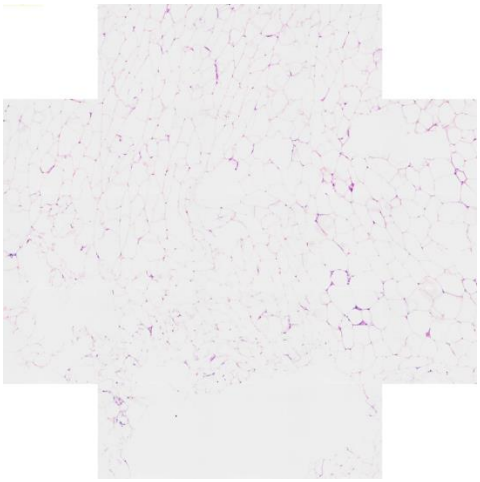

Fig. 13. *Acquisition of images by selecting images with subcutaneous fatty areas*

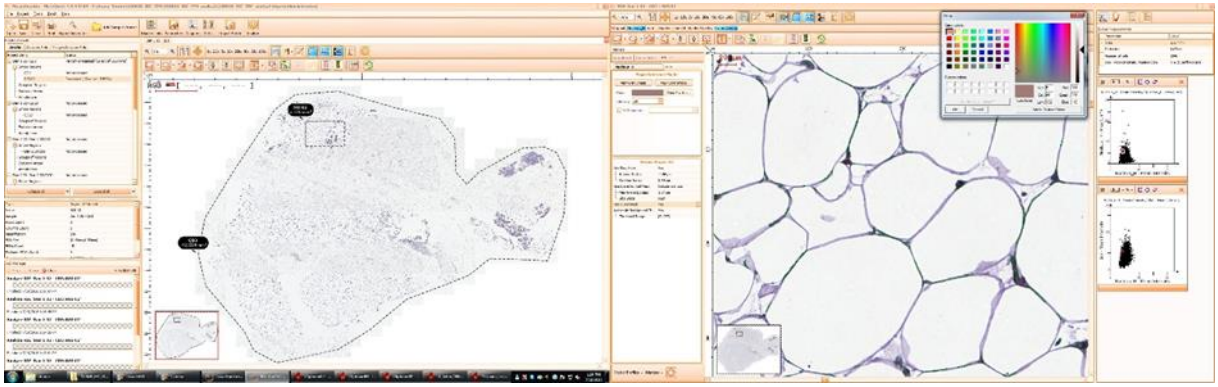

Fig. 14 .*Image processing with Adiposoft*

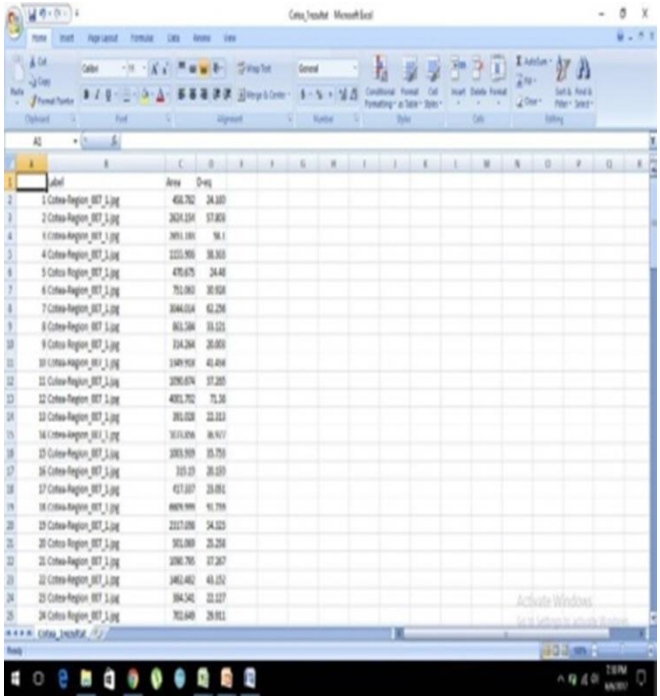

Fig. 15. *Measurement of adipocyte area with Adiposoft program*

The cell culture viability was evaluated by MTT–formazan (3, 4, 5-dimethylthiazol2–5-diphenyltetrazolium bromide). The metabolic activity of mesenchymal stem cells evaluated using MTT assay was 93±3% for the OB group with a similar result: 94±2% for the control NW group.

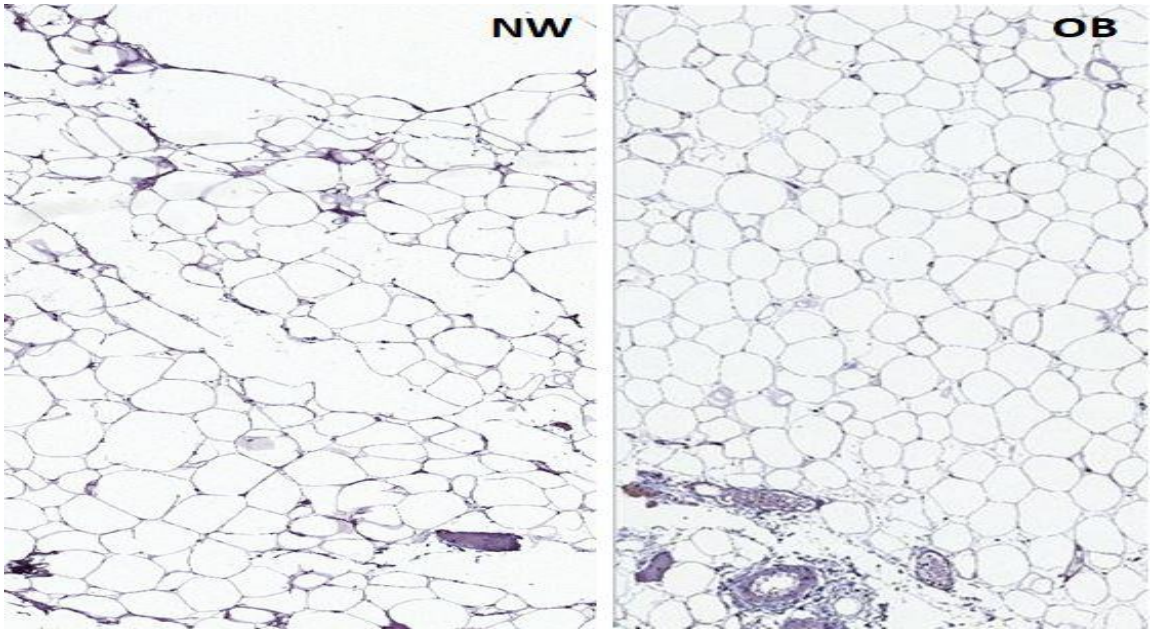

Fig. 16 .*Immunohistochemical analysis of the inflammatory response from adipose tissue: CD68 for the detection of macrophages in the subcutaneous adipose tissue in the studied groups*

Adipogenic differentiation protocol was monitored in cell cultures using optic microscopy and lipid accumulation was observed from day 10±3 in NW samples and from day 12±4 in OB. Also, the lipid droplets accumulated were fewer and smaller in the OB samples **Fig. 17**.

Intracellular lipid accumulation (Red Nile staining)

Specific lipid stain was evaluated using Red Nile staining for lipid accumulation obtained for adipocytes differentiated from OB and NW (**Fig. 18**).

I.5.3.4. Discussion

Bariatric surgery is widely acknowledged as the most effective treatment for obesity (Frikke-Schmidt et al., 2016). The most obvious effect of bariatric surgery is loss of up to half of total adipose tissue mass within the first year after surgery along with improvements in systemic metabolism.

The altered lipid accumulation capacity of subcutaneous preadipocytes in obese patients is currently being evaluated as a precocious marker for insulin resistance as it translates the incapacity of the mesenchymal cell line progenitors to differentiate mature, functional adipocytes with optimal lipid storage capacity. Several studies found that lipid accumulation evaluates the expansion capacity of the pre-adipogenic mesenchymal cell line

and is associated with a poor metabolic profile for obese patients (Isakson et al., 2009; Tsatsoulis et al., 2013; McLaughlin et al., 2010; Vidal-Puig 2013)

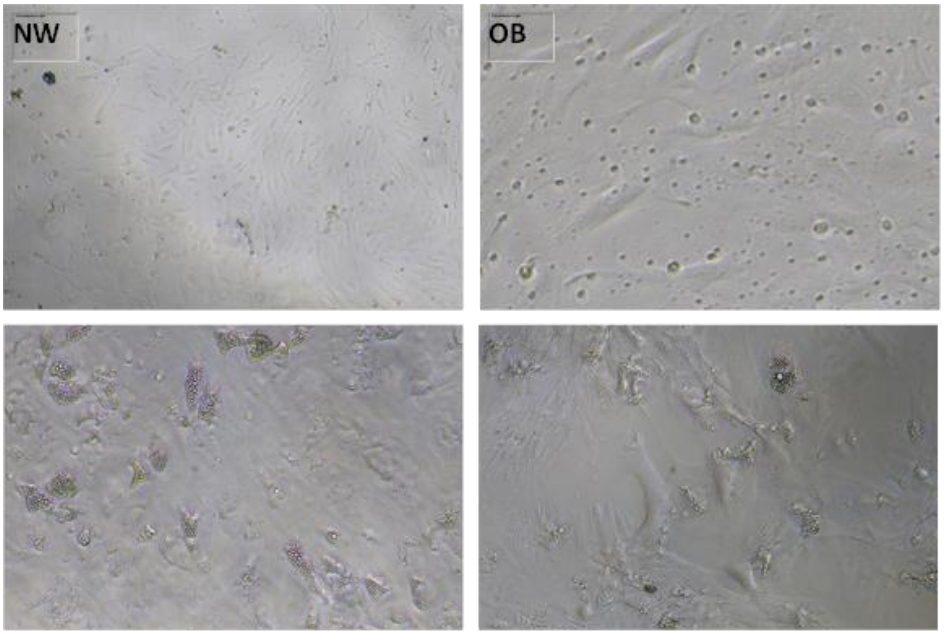

Fig. 17.*Adipocytes differentiated of ASCs derived from NW and OB patients.*

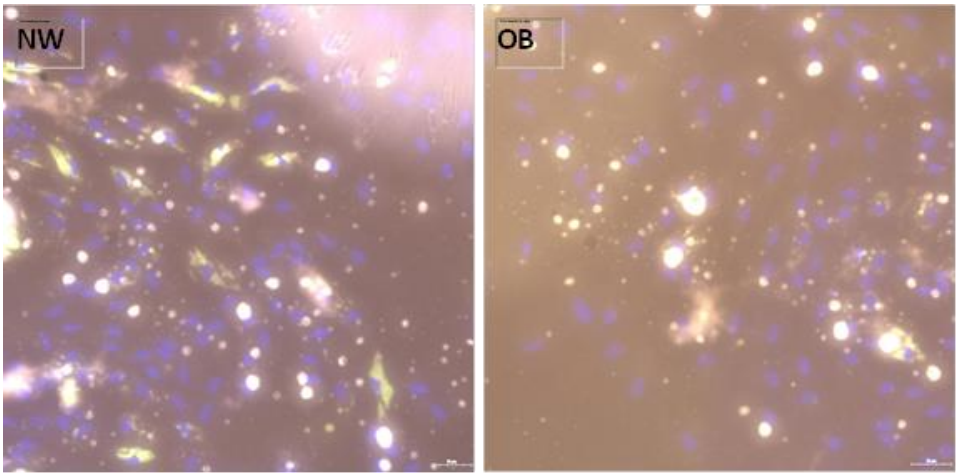

Fig. 18. *Immunoflourescence analysis of the lipid accumulation in differentiated ASCs derived from NW and OB patients. Merge image cytosolic triglycerides (orange), nuclear DAPI (blue), perilipin (green)*

These metabolic improvements associated with bariatric surgery do not correlate directly with reduction of adipose mass per se, but also relate to the extent different adipose tissue anatomic depots are affected.

The subcutaneous adipose tissue represents 90% of total fat mass, it has potential to greatly affect systemic insulin resistance via adipokine secretion, that plays an important role in glucose uptake impairment, as chemerin was reported to be associated positively with BMI and the markers of inflammation and metabolic syndrome in humans (Jeffery et al., 2015). Adiponectin expression has been demonstrated to accelerate 3T3-L1 proliferation and also

lipid accumulation evaluated by Oil red O staining was found to be 4-fold greater in adipogenic differentiated pre-adipocytes that overexpressed adiponectin (Fu et al., 2005).

We report a decreased lipid accumulation capacity for adipogenic differentiated ASCs of obese female patients versus those from normal weight controls, and significant correlations between the adipocyte fat accumulation potential and insulin, adiponectin, leptin: adiponectin ratio (LAR).

In our study we did not find different levels of morning cortisol for obese patients and normoponderal controls. Also, there was no statistically significant correlation with lipid accumulation, showing that morning cortisol is not a valuable predictor for adipogenesis dysfunctions in obese patients. Literature data are inconsistent in this matter, some data suggest that glucocorticoids (GC) increase the lipid turnover in adipose tissue (Lee and Fried 2014), but despite the major impact of GC on adipogenesis, normal or low circulating cortisol values are found in obese patients studies (Ljung et al., 1996).

The fact that obesity alters the adipogenic differentiation capability of ASCs from subcutaneous adipose depots is supported by the data from other research groups (Weyer et al., 2000; Perez et al., 2013). De Girolamo et al. evaluated the ASCs adipogenic potential in obese versus non-obese controls and they also found a reduced proliferative rate for obese ASCs (Vidal-Puig 2013). Alteration of the pre-adipocytes lineage in obese bariatric patients was also demonstrated by Perez that showed an altered lipid accumulation in obese-ASCs derived adipocytes as compared to adipocytes lineage coming from normal weight humans or mice (Perez et al., 2013). In another study, Perez et al. reported a significantly enhanced apoptosis and a reduced proliferative capacity of ASCs isolated from obese subjects; the impaired adipogenesis was correlated with the *in vivo* environmental obesity-related altered mitochondrial biogenesis, increased reactive oxygen species production and increased extracellular acidification (Perez et al., 2015).

We demonstrated that plasma insulin is correlated with decreased adipocyte ability to accumulate lipids. Previous research data published by Weyer, also show that the pre-adipocytes differentiation potential is correlated with insulin resistance in humans, in both obese and normal weight individuals (Weyer et al., 2000). Reported data from *in vitro* studies confirmed the decrease in the adipogenic differentiation ability of the ASCs from obese subjects and have described the mechanisms of obesity induced adipose-tissue remodeling, that include a disproportionate synthesis of extracellular matrix components (Henegar et al., 2008) but also a decreasing number of adipocytes. Thus, the variations in adipose cellularity that occur during the development of insulin resistance seem to determine a decrease in the clonogenic and proliferative potential of ASCs. Their role in unhealthy adipose tissue expansion associated with metabolic syndrome is an important predictor for obesity associated co-morbidities.

Chronic low-grade inflammation in adipose tissue contributes to levels of inflammatory markers in the circulation and for this reason bariatric surgery follow-up studies frequently apply measurements of common biomarkers such as C-Reactive Protein (CRP), TNF-alpha and/or IL-6. IL-6 is mostly consistently reported to decrease after surgery. There is less consensus with TNF-alpha levels as they have been reported to decrease in patients after surgery as compared with levels before surgery and between groups of obese versus operated patients. To the best of our knowledge, very few studies have successfully

measured local inflammation within adipose tissues after surgery in animal studies. However, these limited findings do indicate that inflammation decreases within the distinct adipose depots as assessed by TNF-alpha and IL-6 mRNA expression as well as number of macrophages and T-cells residing within the mesenteric depot in particular. With inflammation comes fibrotic remodeling and potential excessive synthesis of extracellular matrix components and accordingly studies within animals' models have shown that adipose fibrosis is reduced when macrophages are depleted.

In our study, the inflammation has been evaluated within the adipose depots by TNFalpha and CD68 immunofluorescence staining in OB and NW groups. We reported an increase inflammatory status in adipose tissue of OB patients as compared to NW patients.

The novel finding in our study was the negative correlation between adipogenic potential (assessed by lipid accumulation in mature adipocytes) with triglycerides and LAR as key markers of impaired metabolic profile. These results point to abnormal adipogenesis as a link between obesity and its clinical complications.

Overall strengths of our study include direct measurement of the lipid accumulation in differentiated ASCs and the assessment of the relation between impaired adipogenesis with plasma metabolic and adipokine parameters in females with increased adiposity. A limitation of our study is the small size of the study and control groups and the relatively young age of the patients.

I.5.3.5. Conclusion

These observations support the contention that bariatric surgery reduces inflammation associated with obesity. Our study demonstrates that in severely obese female patients, the ASCs from subcutaneous adipose tissue have a decreased potential for adipogenesis as compared with normal weight controls. The abnormal lipid accumulation in the mature adipocyte derived from obese ASCs could possible predict the further metabolic changes and influence the selection of patients for bariatric surgery.

I.6. The relationship between the adipose tissue and associated comorbidities

I.6.1. Introduction

As already discussed, obesity is nowadays one of the most important public health issues around the world, due to its increasing prevalence and its associated comorbidities, which involve important mortality and significant costs. It is already stated that obesity associates a low grade chronic inflammation although the pathogenic mechanisms underlying this phenomenon are not fully elucidated. The importance of the inflammatory status in obese patients is primarily related to the risk of carcinogenesis, enhanced by the secretion of adipokines and promotion of inflammatory pathways (Nunez et al., 2009). The central pathogenic phenomenon underlying the link between obesity and inflammation is insulin resistance (IR), as the increased secretion of active adipokines by excessive adipose tissue interfere with the anti-inflammatory effect of insulin, which will promote inflammation (Dandona et al., 2004).

Personal contribution – published paper:

Livadariu RM, **Timofte D**, Dănilă R, Sângeap AM, Constantinescu D, Trifan A. Obesity is linked with inflammation-evaluation of subclinical inflammatory status in obese patients. J Surgery, 2017, 13(4): 127-131.

The aim of the study was to evaluate the status of serum inflammatory markers in a group of obese patients with medical indication of bariatric surgery. **We also aimed to define** to what extend the pathological changes in serum markers of inflammation are influenced by anthropometric indices and by the presence of associated comorbidities to obesity, such as metabolic syndrome (MS) and obstructive sleep apnea (OSA).

I.6.2. Material and methods

We performed a retrospective study on 64 obese patients successively hospitalized for bariatric surgery in our Surgical Unit between November 2014 and November 2016. We only included patients over 18 years with medical indication of bariatric surgery. Patients diagnosed with acute or chronic infections were excluded; we also excluded the patients known to have chronic medical conditions that could affect the inflammatory status. All patients signed the Informed Consent approved by the Ethics Committees of the University of Medicine and Pharmacy "Grigore T. Popa" Iași before joining the study. All patients received a full evaluation including medical and personal history, complete clinical examination, anthropometric measurements (BMI calculated by formula G (kg)/T^2 (cm) and waist circumference measured in centimeters-half point between the last coast and iliac crest) as well as general and special biological tests. Blood samples were collected in the morning of surgery in order to measure routine and special biological tests. Insulin resistance was assessed by the homeostasis model assessment of insulin resistance index (HOMA-IR=fasting blood glucose (mg/dl) x fasting insulinemia (mU/L)/405). The values of C Reactive Protein (CRP) and serum fibrinogen, NLR and PLR scores (obtained by dividing the number of neutrophils and platelet counts by the number of lymphocytes) were noted. Leptin dosing was performed by the enzyme-linked immunosorbent assay (ELISA) using an SANOFI Pasteur ELISA, with HRP-labelled antibody sandwich ELISA, with 7.36 ± 3.73 ng/ml and for men 3.84 ± 1.79 ng/ml.

Among the comorbidities, we noted the presence of type II diabetes (DM II), dyslipidemia and hypertension as constitutive parts of the metabolic syndrome, but also the presence of obstructive sleep apnea syndrome-according to the pneumological reports.

The database was completed using Microsoft Excel 2013 version and the statistically analysis was performed in SPSS V.19.0. Continuous variables were expressed using mean, median and standard deviation (SD) values. Significance level (p-value) was considered to be 0.05 (5%) with 95% probability (confidence interval). The regression analysis was used for estimating the relationships among different variables; the statistical significance of every model was verified using the t-Student, the ANOVA test or chi-square test.

I.6.3. Results

Considering age as a categorical variable with 45 years threshold, in our study group there was a higher percentage (62.5%) of patients aged ≤45 years. The number of male

patients was significantly lower (23.4%) than the number of females (76.6%). All patients enrolled in the study had BMI >35, with a mean value of 45.06 ± 6.67 SD, ranging between 35 and 67 kg/m^2. The mean value of waist circumference was 133.39 cm ± 17.47 SD, ranging between 89 and 159 cm. The inflammatory status of each patient was evaluated by serum values of fibrinogen, CRP, Leptin and NLR and PLR scores. As showed in **TABLE 8**, the mean values of serum inflammatory markers were abnormally elevated. Leptin: 54.017 ng/ml ± 37.32 SD, Fibrinogen: 403.64 mg/dl ± 48.53 SD, CRP: 0.89 mg/dl ± 0.86 SD, suggesting the existence of a subclinical inflammatory state in our group of patients.

We used the regression analysis to evaluate the possible influence of anthropometric indexes on serum values of inflammatory markers (**Fig. 19**) and the results showed that there is a directly proportional relationship between variation of waist circumference and serum fibrinogen (p=0.04) and between waist circumference and CRP values variation (p=0.003).

TABLE 8 *Inflammatory markers values in obese patients.*

	Mean	95% Cl		Median	SD	Min	Max
		Min.	Max.				
Leptin	54.017	44.69	63.34	44.40	37.32	6.70	187.50
PLR	115.87	108.06	123.68	113.20	31.26	52.66	236.00
NLR	2.10	1.91	2.28	2.03	0.74	1.01	5.10
Fibrinogen	403.64	391.52	415.77	406.50	48.53	288.00	500.00
CRP	0.89	0.67	1.10	0.51	0.86	0.20	4.60

As showed in **Fig. 20**, there is also a direct proportional relationship between the value of the serum leptin and the waist circumference, statistically significant when tested using ANOVA test (p=0.01). Concerning the BMI value, the regression analysis showed that there is a directly proportional relation between serum fibrinogen and PCR values variation and BMI, with no statistical significance in the studied group. The only statistically significance (p=0.05) was noted in the directly correlation between BMI and leptin serum values (**Fig. 21**).

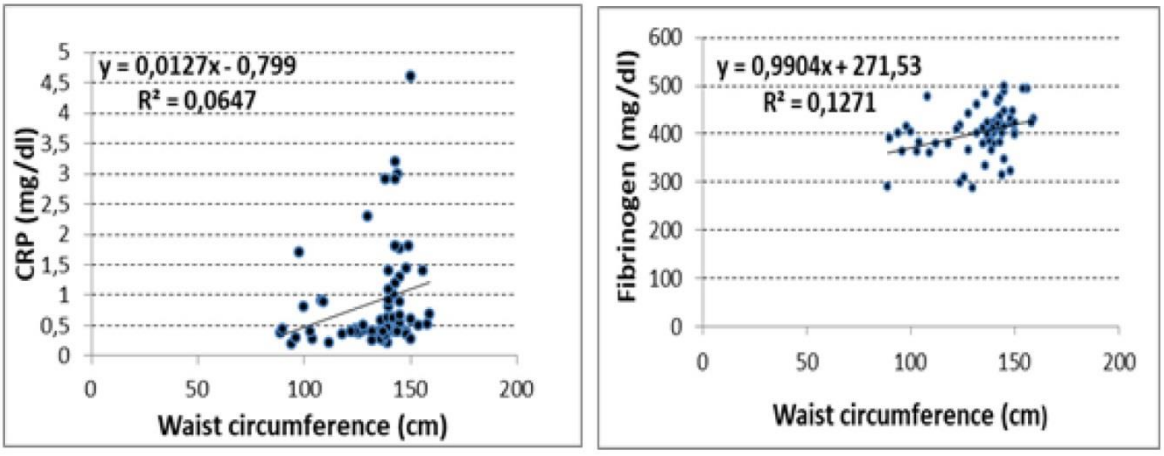

Fig. 19. *Regression analysis: fibrinogen and CRP values vs. waist circumference*

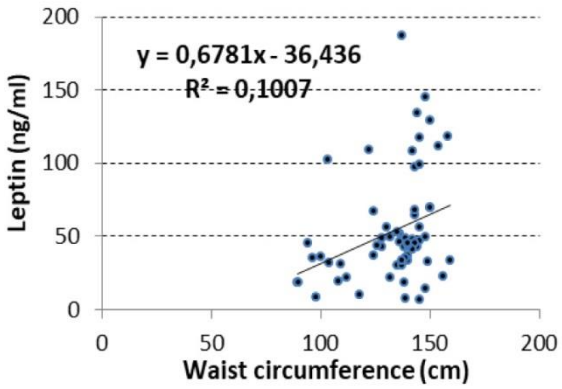

Fig. 20. *Regresion analysis: leptin serum values vs. waist circumference.*

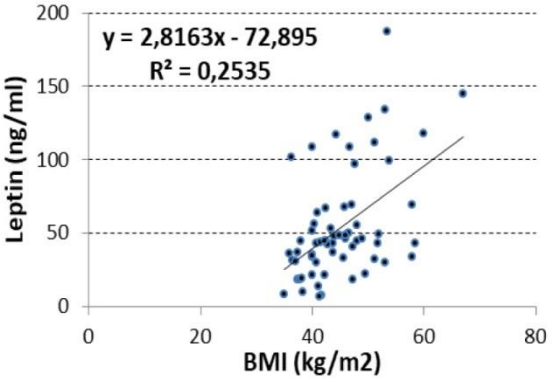

Fig. 21. *Regresion analysis: leptin values vs. BMI values.*

The results also showed that the increased values of anthropometric indexes have no influence on PLR and NLR variation. When assessing the correlations between serum markers of inflammation and MS and its components, we noticed that elevated serum fibrinogen values correlated (p=0.04) with MS (***TABLE 9***).

TABLE 9 *Differences between inflammatory serum markers in relation to MS*

	MS	**Mean**	**95% Cl**		**Std. Dev.**	**Min**	**Max**	**Sig**
			Min.	**Max.**				
Fibrinogen	Abs.	392.29	374.30	410.29	51.57	288.00	492.00	0.04
	Pres.	416.50	400.79	432.21	42.08	290.00	500.00	
PLR	Abs.	114.82	105.75	123.90	26.01	63.00	188.70	0.77
	Pres.	117.06	103.34	130.78	36.75	52.66	236.00	
NLR	Abs.	2.03	1.82	2.24	0.60	1.03	3.72	0.42
	Pres.	2.18	1.85	2.51	0.89	1.01	5.10	
CRP	Abs.	0.90	0.59	1.21	0.89	0.20	3.20	0.92
	Pres.	0.88	0.56	1.20	0.86	0.22	4.60	
Leptin	Abs.	50.32	38.98	61.67	32.51	7.91	145.20	0.40
	Pres.	58.21	42.41	74.00	42.31	6.72	187.50	

We also noted that in patients with DM II, the mean serum levels of fibrinogen, leptin and CRP are within pathological limits and higher than in non-diabetic patients. Continuing with the evaluation of inflammatory markers in relation to pathological changes of glucose metabolism, the regression analysis showed that between the variation of the serum values of

fibrinogen and CRP on the one hand and the variation of HOMA IR value on the other hand there is a directly proportional relationship. Testing the results using the ANOVA test did not reveal statistical significance. In contrast, the directly proportional relationship between increasing in serum leptin and HOMA IR values (**Fig. 22**) was statistically significant in the studied group (p=0.04).

TABLE 10 *Differences between serum inflammatory markers in relation to DM II*

Inflammatory marker	DMII	Mean	95%Cl		Std. dev.	Min	Max	T-test p-Sig
			Min	Max				
Fibrinogen	Abs.	398.17	384.29	412.05	49.85	288	500	0.06
	Pres.	427.33	405.17	449.49	34.88	363	493	
PLR	Abs.	116.15	107.46	124.84	31.20	62.23	236.00	0.88
	Pres.	114.64	93.77	135.52	32.85	52.66	179.40	
NLR	Abs.	2.12	1.9173	2.3396	0.758	1.01	5.10	0.53
	Pres.	1.97	1.5226	2.4341	0.717	1.10	3.50	
PCR	Abs.	0.806	0.5931	1.0207	0.768	0.20	3.20	0.10
	Pres.	1.25	0.4973	2.0127	1.19	0.22	4.60	
Leptin	Abs.	53.60	43.610	63.592	35.86	10.2	187.5	0.85
	Pres.	55.81	27.370	84.264	44.77	6.7	118.0	

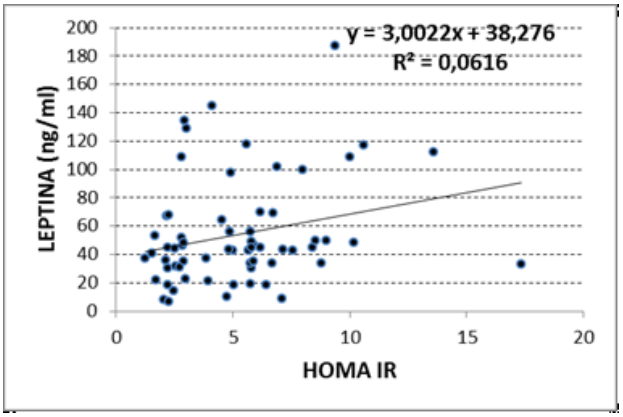

Fig. 22. *Regression analysis of serum leptin value vs. HOMA-IR value*

TABLE 11 *Mean values of serum fibrinogen, CRP and leptin are higher in the patients diagnosed with OSA*

OSA		Mean	95%Cl		Dev. Std.	Min	Max	Sig.
			Min.	Max.				
Fibrinogen	Abs.	380.23	359.95	400.51	45.74	288.00	475.00	0.004
	Pres.	415.90	401.62	430.19	45.83	290.00	500.00	
PLR	Abs.	122.73	110.97	134.48	25.52	77.08	188.70	0.20
	Pres.	112.28	101.93	122.63	33.21	52.66	236.00	
NLR	Abs.	2.06	1.75	2.36	0.69	1.10	4.20	0.74
	Pres.	0.007	1.88	2.37	0.78	1.01	5.10	
PCR	Abs.	0.82	0.43	1.21	0.87	0.20	3.20	0.64
	Pres.	0.93	0.65	1.20	0.88	0.22	4.60	
Leptin	Abs.	44.28	34.71	53.85	21.59	18.20	102.00	0.007
	Pres.	59.12	45.81	72.43	42.72	6.72	187.50	

In our study, we observed that the mean values of serum Fibrinogen, CRP and leptin are higher in the patients diagnosed with OSA, mean values exceeding the upper limit of normal in this group comparing with the non-OSA group. Both elevated plasma fibrinogen (p=0.004) and elevated serum leptin values (p=0.007) were positively and significantly correlated with the presence of OSA (**TABLE 11**).

I.6.4. Discussions

Chronic inflammation specific to patients with obesity and SM has some special features, as there is no involvement of autoimmune diseases or infectious pathologies that could induce significant damage to an organism. The results of our study showed that the increased serum inflammatory markers values (Fibrinogen, CRP and Leptin) directly correlated with obesity, especially abdominal obesity quantified by waist circumference. The variation of the CRP and cytokines serum values (IL 6) proportional to the anthropometric index variation and the distribution of adipose tissue has been demonstrated by other authors (Yudkin et al., 1999). The pathogenic mechanisms explaining this link are related to the capability of the abdominal excessive adipose tissue to induce chronic inflammation through abnormal production of cytokines, chemokines, acute phase proteins and other mediators of inflammation, and through activating alternative inflammatory pathways (Hotamisligil, 2006). Regarding NLR and PLR scores, literature data supports correlations between these-as markers of inflammation and obesity (Koca, 2017).

Our study results do not confirm the importance of these scores as markers of inflammation in obese patients. Analyzing the correlations between the presence of MS and the changes in serum markers of inflammation, we found that the mean serum values of fibrinogen and leptin are pathologically elevated in patients with diagnosed MS, and the correlation between elevated fibrinogen and MS was statistically significant (p=0.04). Other authors who studied these relationships concluded that the connection between obesity, inflammation and MS is related to the insulin resistant, hypertrophied and dysfunctional adipocytes of the obese patients; the excessive fatty tissue is not metabolically inert, as it is characterized by an enhanced release of proinflammatory factors that will further support chronic inflammation through lipo-toxicity and oxidative stress (Sun et al., 2016). Elevated serum fibrinogen in connection with MS and obesity is even more important since hyperfibrinogenemia increases the cardiometabolic risk, with authors claiming that this is even a component of SM (Imperatore et al., 1998). Since the glucose metabolic disorders, either in the form of IR or in the form of constituted DM II are important components of MS and involve complex pathogenic mechanisms that interfere with the rest of the SM components, we studied the possible correlations between the imbalance of carbohydrate metabolism and changes in serum inflammatory markers.

In our study group, the mean values of fibrinogen (p=0.06) and leptin were higher in patients with diagnosed DM II. The regression analysis showed a direct relationship between the increase of fibrinogen and PCR serum values and HOMA IR variation, but no statistical significance was noted. The only statistically significant correlation was recorded for the proportional regression of leptin serum values in relation to those of HOMA-IR (p=0.04). Current literature unambiguously supports the link between hyperfibrinogenemia and DM II and this correlation is considered one of the main pathogenic factors involved in micro- and

macroangiopathic complications of DM II development (Bembde, 2012) (Iyer and Desai, 2010).

OSA is another comorbidity intensively associated to obesity. In this study we tried to find out to what extent the serum values of the studied inflammatory markers are correlated with OSA. Many authors have already demonstrated the link between OSA, its severity and inflammation, using common inflammatory biomarkers such as fibrinogen, PCR, TNF α, IL 6 and even showing reduction in inflammation biological status with OSA treatment (Sahlman et al., 2010) (Shamsuzzaman et al., 2014); also, the evaluation of inflammation by modern biomarkers involved in endothelial dysfunction (Pentraxin 3, Nesfatin 1) revealed the same direct relationship between their increase and the existence of OSA (Unnikrishnan et al., 2015). The results obtained in our study group support the link between the increased inflammatory markers and OSA. The mechanism involved in inflammation induced by OSA is primarily a direct one, related to the episodes of intermittent hypoxia and hypercapnia leading to activation of hypoxia induced factor 1 (HIF1) and nuclear factor kB (NF-kB), a transcription factor that participates in the immune response to infection. However, these mechanisms are intricate with other pathways activated by the presence of OSA, such as sympathetic stimulation that will lead to IR installation and increased FFA release, which contributes to the activation of inflammatory pathways to endothelial dysfunction. Thus, a vicious circle is installed in which each of the components is maintained and stimulates by each other (Unnikrishnan et al., 2015). Thus, it seems that Patients with obesity are characterized by a subclinical inflammatory status. Of all inflammatory markers assessed in the study, elevated fibrinogen appears to be most sensitively related to the presence of MS, OSA and DM II.

I.7. The relationship between the adipose tissue and the calcium metabolism

I.7.1. Introduction

As previously discussed, obesity is a multifactorial disease and is characterized by a positive energy balance that results from excess energy intake, insufficient energy expenditure and it is manifested by an excess of adipose tissue (Del Pozo et al., 2011). In the last decades, it has become a major public health problem that increases health care costs, reduces life years and the quality of life (Aronne, 2001). The World Health Organization estimates that, by 2020, 2.3 billion adults will be overweight and more than 700 million will become obese (McArdle et al., 2013). Furthermore, obesity has a strong correlation with the development of many comorbid conditions such as insulin resistance, type 2 diabetes mellitus, hypertension, dyslipidemia, metabolic syndrome, as well as other associated complications, such as those related to a variety of biological and biochemical modifications (e.g. increased oxidative stress status), some cancers, pain and neuropsychiatric-connected manifestations etc. (Wilson et al., 2005) (Antioch et al., 2015). Such medical conditions were found to be specific also to pediatric patients' populations, making childhood obesity an even more important healthcare issue.

In the literature there is a hypothesis that an inverse relationship exists between calcium intake and obesity markers such as body weight, weight gain or body fat percentage. However, numerous studies question the idea whether adequate calcium nutrition can prevent or even reduce obesity. The conflicting results have led to a lack of general agreement. Many reviews and meta-analyses have tried to address the apparently contradicting information in an effort to reach a consensus. However, the disagreement may have more to do with the interpretation of the data than the data itself, with different hypothesis which could be valid in different specific populations.

In regard to observational studies, it is almost invariably shown that an inverse association between the intake of dietary calcium and body fat levels exists, which may suggest that calcium influences energy balance in a positive manner, especially in obese participants (Shalileh et al., 2010). Most calcium intake derives from the consumption of dairy products, and thus it may be speculated that the observed association between calcium and body weight may be confounded by other nutrients found in dairy products, such as protein or bioactive peptides. On the other hand, this association could be due to the fact that individuals who consume a high dairy diet may live a healthier lifestyle or on the contrary, individuals with a low-quality diet are more likely to also consume low dairy intakes. To determine whether this association is causal and it is not only a correlation, it is necessary to find the exact mechanisms underlying how calcium influences the energy balance.

One possible explanation on how calcium intake influences the energy balance in human body was proposed by Zemel et al (Zemel et al., 2000). These authors hypothesized that dietary calcium, via its influence on plasma 1,25- dihydroxy vitamin D3 concentrations, regulates the concentrations of intracellular adipocyte calcium and in this way it regulates fat metabolism in the adipocytes. In addition, a low dietary calcium diet leads to an inhibited lipolysis, and to a decreased fat oxidation. Consequently, a diet low in calcium may lead to weight gain, and a high dietary calcium intake may have the opposite effect. Other authors (Bortolotti et al., 2008) who wanted to test this hypothesis conducted an experiment on two groups (placebo vs. experimental).

The participants from the experimental group received 800mg dairy Ca per day for a 5-week period, while the control group received the placebo treatment. The results presented by the authors failed to find any effect of calcium supplementation on fat tissue metabolism. These findings together with other results from different studies (Boon et al., 2007) seem to provide sufficient evidence to question the hypothesis according to which dietary calcium may play a vital role in human energy balance through a calcium-controlled pathway in the fat tissues. Therefore, another theory emerged suggesting that the effect of calcium on adipose tissue may appear mainly during calorie restriction periods and only in individuals who consume a low calcium diet on daily basis. In addition, a calcium-deficient diet, which can easily be experienced during a weight loss diet, has been found to lead to hunger, noncompliance, and poor weight-loss outcome (Major et al. 2008). Therefore, a low calcium diet is not only detrimental for all attempts to control body fat, but also it may be most relevant to study its effects during body fat loss phase of an individual, when a difference in appetite and hunger sensations is a vital determinant of compliance and also of the outcome in terms of total body fat lost (Tordoff, 2001).

Although the data on this subject is still conflicting, there is still good evidence that dietary calcium intake plays an important role in human body-weight regulation. Perhaps future studies should concentrate more on describing the exact mechanism or how calcium-deficient diets can amplify hunger and impair compliance.

In regard to low calcium levels presented by patients after bariatric surgeries, different studies showed that malabsorptive procedures may be considered as a risk factor for developing bone disease (Mellstrom et al., 1993) (Eddy, 1971) (Zittel et al., 1997) as a result of altered calcium metabolism and compromised calcium absorption (Sellin et al., 1984) (Nunan et al., 1986) (Charles et al., 1984) (Dano and Christiansen, 1978). For example, some studies have investigated calcium absorption after jejunal-ileal bypass surgery. The results showed that calcium absorption decreased by roughly 50% after this type of surgery (Hylander et al., 1980). Considering that inadequate calcium intake is common after gastric bypass (Brolin and Leung, 1999) (Alvarez-Leite, 2004) (El-Kadre et al., 2004), this may also contribute to an altered calcium metabolism and bone loss.

Personal contribution – published paper:

Timofte D, Ochiuz L, Ursaru M, Ciuntu B, Ionescu L, Calu V, Mocanu V, Puia I. Biochemical modifications related to Calcium deficiencies in obesity and after laparoscopic sleeve gastrectomy. Rev Chim (Bucharest), 2017, 68(10): 2341 - 2345

Thus, the aim of this study is to lead to a better understanding of the extent to which calcium absorption and intakes are decreased after laparoscopic sleeve gastrectomy.

I.7.2. Material and Methods

This study was conducted on 170 patients, all Romanians, hospitalized for bariatric surgery in the Surgery Service, Sf. Spiridon Clinical Emergency Hospital in Iasi (Romania). Eighty-five patients (41 males and 44 females) were recruited to be part of the experimental group. These patients were investigated before and after 1 year following the laparoscopic sleeve gastrectomy. The data obtained from this laparoscopic sleeve gastrectomy group was compared to the data from a control group, which was recruited from the waiting list for laparoscopic sleeve gastrectomy, consisting of 85 patients, 31 males and 54 females. The control group was recruited to match weight, abdominal sagittal diameter (ASD) and serum calcium in relation to the corresponding baseline values in the group who underwent laparoscopic sleeve gastrectomy. The exact characteristics of the population sample used in this study can be seen in **TABLE 12**.

All patients from the experimental group were prescribed the following supplements as mandatory postoperative care: 1 multivitamin daily; iron (325 mg with B_{12} and folate) daily; calcium citrate (1800 mg) daily; and fat-soluble vitamin supplements (10,000 IU of vitamin A, 1200 IU of vitamin D, 60 IU of vitamin E, and 300 µg of vitamin K) daily. Serum calcium levels were measured by atomic absorption spectrophotometry, and the coefficient of variation is <2% for this method.

TABLE 12 *Clinical characteristics at baseline in patients before laparoscopic sleeve gastrectomy surgery and in patients from control group. data given are arithmetic mean values (±SD). ASD= abdominal sagittal diameter.*

	Laparoscopic sleeve gastrectomy group	Control group	Group comparisons (p value)
Gender (male/female)	41/44	31/54	
Age (years)	40.16	42.00	0.098
Weight (kg)	122.00	120.00	0.215
Height (cm)	171.00	169.00	0.276
ASD (cm)	29.30	29.50	0.860
Calcium (mg/dL)	9.10	9.00	0.328

Abdominal sagittal diameter, a variable reflecting visceral adipose tissue, was recorded at the umbilical level as the height (cm) of the abdomen measured from the examination couch.

All patients signed a specific study inclusion agreement annexed to the informed consent form, and all experimental biochemical studies were performed in the light of the uniformly accepted ethical principles stated by the Helsinki Declaration.

I.7.3. Results

Thus, our results showed that at baseline, before patients underwent laparoscopic sleeve gastrectomy surgery, there were no statistically significant differences between the group of patients directed for surgical treatment and the control group, regarding age, height, weight, ASD or serum calcium concentrations (**TABLE 12**). However, a statistically significant positive correlation between serum calcium concentrations and ASD was found ($r = 0.227$, $p = 0.003$). None of the patients in this study had any complications during the surgical performance or during the 1-year follow-up period.

Regarding the results after 12-months follow-up, serum calcium concentration decreased significantly in the experimental group, from 9.1 to 8.82 mg/dL ($p=0.004$), while the exact same trend was observed during the corresponding period in the control group. However, the decrease in the control group was non-significant, from 9.0 to 8.93 mg/dL ($p = 0.388$). The intergroup difference in serum calcium concentrations after 12 months post operation ($p = 0.156$) was non-significant (**Fig. 23**).

Regarding the abdominal sagittal diameter, in the experimental group, the mean ASD decreased from 29.3 cm at baseline to 22.4 cm, ($p < 0.001$). In the control group, a very small change but non-significant in mean ASD was observed between baseline and 12 months follow-up, 29.5 cm to 29.3 cm, respectively ($p = 0.830$). ASD was significantly different in the two groups at the 12 months follow-up ($p < 0.001$) (**Fig. 24**).

To find out if any of the post operation variance is explained by the preoperation values we used the analysis of covariance. When we controlled the preoperation values, using ANCOVA, the statistical significance difference between control and experimental groups maintained for ASD after 12 months ($p < 0.001$) and there was still no significant difference for serum calcium levels 12 months post operation ($p = 0.218$).

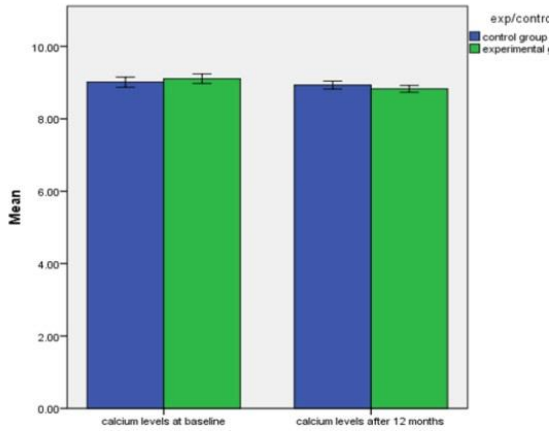

Fig. 23. *Changes in serum calcium (mg/dL) from baseline to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

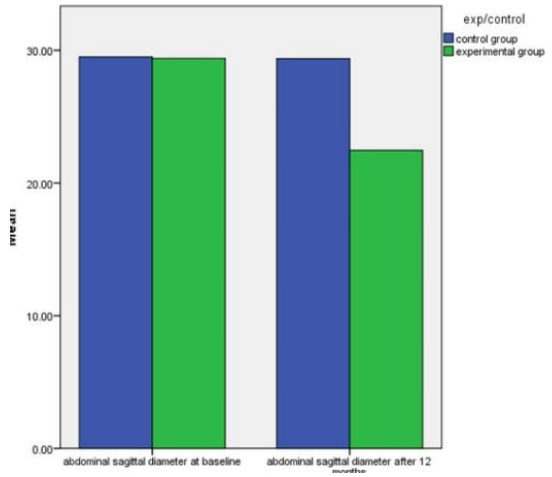

Fig. 24. *Changes in ASD (cm) baseline to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

I.7.4. Discussion

Our major findings are in line with previous research that demonstrates that lower levels of serum calcium are common after bariatric surgeries (El-Kadre et al., 2004) (Diniz et al., 2004) (Goode et al., 2004) (Hamoui et al., 2003) (Johnson et al., 2006). Although there are studies that presented no significant change in calcium metabolism after bariatric surgery (Sanchez-Hernandez et al., 2005) (Ybarra et al., 2005). Furthermore, there is some evidence suggesting that obesity can be protective against osteoporosis, and when even a moderate weight loss occurs, a decrease in bone mineral density appears (Shapes and Dawson-Hughes, 2001). In consequence, with more dramatic weight loss, which is expected to take place after bariatric surgeries, there is an increased risk of low bone mass and metabolic bone disease (Bano et al., 1999) (Coates et al., 2004).

Two different studies have showed that a low level of dietary calcium increases the risk of hypertension and insulin resistance syndrome (Griffith et al., 1999) (McCarron and Reusser, 1999). Another study found that dairy products consumption was inversely proportional to obesity. In this study, each daily serving of a dairy product was associated with 21% lower odds of developing obesity (Pereira et al., 2002).

As mentioned, although the exact mechanism by which calcium intake regulates visceral fat is unclear, two potential mediators include estrogen and cortisol. Estrogen is associated with less central fat deposition including a lower sagittal abdominal diameter compared to subcutaneous fat deposition (Elbers et al., 2003) (Puder et al., 2006). In addition, studies have shown that dietary calcium is associated with the metabolism of estrogens to relatively more active forms (Napoli et al., 2005) (Napoli et al., 2007). Furthermore, cortisol is known to promote intraabdominal adipose tissue accumulation (Bujalska et al., 1997). It has been suggested that high dietary calcium intake may result in lower cortisol production by inhibiting 1,25- dihydroxy vitamin D3-mediated expression of adipocyte 11-β-hydroxysteroid dehydrogenase type 1, the enzyme that converts cortisone to cortisol (Morris and Zemel, 2005). However, the level of calcium intake needed to influence

estrogen and cortisol effects on intra-abdominal adipose tissue accumulation and whether this differs by gender or age is not known.

Hypocalcemia is a well-recognized complication of malabsorptive operations, such as laparoscopic sleeve gastrectomy. The results of this study have similarly shown a significant decrease of calcium levels after 12 months. It is very important to note that even if there was a significant decrease in serum calcium levels in the experimental group (from 9.1 to 8.82 mg/dL, $p = 0.004$), the values remained in the normal ranges (8.5-10.2 mg/dL). However, in parallel with the development of hypocalcemia, the incidence of secondary hyperparathyroidism increases with time. That is one of the main reasons why postoperative care and long-term monitoring is vital. Furthermore, patient education is a critical component of both preoperative preparation and continual postoperative care. This is already incorporated into the bariatric programs by the surgeons, but it should be handled with the knowledge that nutritional deficiencies are a rare but distinct complication after these operations.

Hormones regulating calcium metabolism are often disturbed in severe obesity, but also after bariatric surgeries. For example, serum parathyroid hormone is found to be higher in severely obese subjects when compared with non-obese subjects (Hey et al., 1982). As before mentioned this hormone has been also shown to decline in patients who suffer dramatic weight loss (Andersen et al., 1988). However, surprisingly, the data shows that patients who underwent bariatric surgery present persistently elevated serum parathyroid hormone (Ott et al., 1992) (Shaker et al., 1991) (Sorensen et al., 1992). In addition, other studies on patients after bariatric surgeries present results that confirm a decrease in 25hydroxy-vitamin D levels (Mosekilde et al., 1980) (Rickers et al., 1984) (Slatter et al., 2004). Furthermore, the literature shows that serum estrogen levels are typically elevated in severe obese populations (Kirschner et al., 1990) and that these levels decrease when the individuals are starting to lose body fat (Kopelman et al., 1981) (Ricci et al., 2001). This hormonal profile may stand behind the disturbed calcium metabolism that occurs after bariatric surgeries (Cifuentes et al., 2004) (Cifuentes et al., 2004). On the other hand, bone regulating hormones in bariatric patients and their relationship to calcium absorption and bone loss has not been broadly studied previously in bariatric patients, despite the prove effectiveness of these procedures (von Mach et al., 2004) (Wei et al., 2006).

Waist circumference it is known to be a strong marker for health risk, since the abdominal fat distribution is associated with many risk factors such as coronary heart diseases and type 2 diabetes (Han et al., 1995) (Ohlson et al., 1985). Furthermore, it has been proven that individuals with a large waist circumference suffer impairments not only in general health but also in the quality of life (Lean et al., 1998). However, the main reason why we chose to use sagittal abdominal diameter as the measure of visceral obesity was that, among both men and women, sagittal abdominal diameter was found to have stronger correlations with the risk factors in the metabolic syndrome when compared to other measured anthropometric variables, such as waist circumference, waist-to-hip ratio and body mass index. Furthermore, in the regression analysis of one study, the most important risk factor for coronary heart disease and for the metabolic syndrome was found to be the sagittal abdominal diameter (Ohrvall et al., 2000).

In addition, Pouliot et al have shown that waist circumference and sagittal abdominal diameter are simple, yet most powerful anthropometric indices of abdominal and visceral adipose tissue accumulation and of cardiovascular risk in men and women (Pouliot et al., 1994). These authors present the mechanism of the proposed link between increased visceral adipose tissue and risk factors for coronary heart disease. This mechanism may be based on the occurrence of elevated concentrations of free fatty acids in blood from the enlarged abdominal fat depots. With a multiscan CT technique the sagittal abdominal diameter at the umbilical level has been shown to predict the amount of visceral adipose tissue (Pouliot et al., 1994). In recent studies (Despreas et al., 1991) (Richelsen and Pedersen, 1995), sagittal abdominal diameter is measured on CT scans, but in our study, it was measured with the patient in a supine position using a simple ruler. We considered that the measurement of sagittal abdominal diameter by this easy method is reproducible and accurate.

The literature presents unambiguous results: despite counseling, the noncompliance showed by patients after bariatric surgery is typically up to 40% (Brolin and Leung 1999). Patient compliance is a major concern after bariatric surgeries especially because surgeons have no control over it. This study reflects the true incidence of calcium deficiency after laparoscopic sleeve gastrectomy surgery performed within a comprehensive bariatric surgery program that involves comprehensive nutritional counseling, monitoring, and supplementation. Thus, in conclusion, undeniably, malabsorptive bariatric surgeries result in an altered calcium metabolism in some individuals. It is our opinion that these patients need long-term follow-up with frequent evaluations and immediate replacement of these essential nutrients with the help of a better diet or with dietary supplements. The specialized clinics performing these operations as a treatment for morbid obesity need to prepare to follow these patients long-term and to address potential nutrient deficiencies. The programs developed by these clinics should include physical education and nutrition information classes and contribute to a better understanding on how to live a longer healthier lifestyle. Further studies should be conducted to explore the exact incidence of long-term metabolic consequences after bariatric surgeries and what is to be done in order to prevent them.

I.8. The relationship between the adipose tissue and the oligoelements dynamics

I.8.1. Introduction

As mentioned above, the growing incidence of obesity is widely recognized as one of the most challenging contemporary problems regarding the public health (Danaei et al., 2011). Obesity has been proven to lead to numerous macrovascular and microvascular complications, including myocardial infarction, diabetic cardiomyopathy, stroke, neuropathy and renal failure in many patients and is currently difficult to control by the available medical treatment, including diet, drug therapy and behavioral or biochemical modifications (Shamseddeen et al., 2011). Therefore, bariatric surgery should not be disregarded as a possible solution. It has been demonstrated that bariatric surgery can be one of the most effective treatment of morbid obesity and, depending on the type of operation, is also been shown that it can be very effective in the resolution of diabetes (Rubino et al., 2010). Studies

demonstrated that this effect occurs even before the start of the weight loss, and it may be explained by changes in the gut hormones and the diet of the patient (Cummings et al., 2007).

Regarding the specificity of Romania's population, the prevalence of obesity in the adult population in our country has previously been found to range between 7.9 % (OECD, Health at a Glance: Europe 2014 completed with Eurostat 2014) and 21.7 % (World Health Organization, 2014). It is important to be mentioned that these obesity rates are self-reported through estimates of anthropometric data. Other two epidemiological studies we found, performed back in 2005 and 2006, presented an estimated obesity prevalence of 24 % and, respectively, 26.3 % (Cinteza et al.,2007) (Dorobantu et al., 2008).

The importance of studying the effects of serum oligoelements levels on general health comes from data that shows that a low serum magnesium concentration increases the risk of all-cause mortality when added to the conventional cardiovascular disease risk factors (Haglin et al., 2007). In addition, the same analysis shows that low serum magnesium has a significant correlation with all-cause mortality in type 2 diabetes patients (Jorgensen et al., 1997). However, some studies presented results that a weight-reducing surgical method, the jejunoileal bypass, has been shown to correlate with an increased risk of magnesium depletion (Bloomberg et al., 2005).

Regarding laparoscopic sleeve gastrectomy in morbidly obese patients, there is relatively little information about possible changes in circulating magnesium concentrations (Food and Agriculture Organization, 2004).

Regarding the impact of Laparoscopic Sleeve Gastrectomy on Serum Zinc or Copper and Body Composition, bariatric surgery is widely accepted as the most effective strategy leading to long-term weight loss. The results of this operation are impressive: studies have shown a decrease in mortality, in cardiovascular events and cancer incidence (Buchwald et al., 2004) (Adams et al., 2007). Furthermore, it has been observed a major improvement in metabolic obesity-related comorbidities (Sjostrom et al., 2004) (Buchwald and Williams, 2004) (Maggard et al., 2005), as our group and others also previously described on various levels, related areas of research and experimental designs.

However, how much fat mass (FM) or muscle mass (MM) is lost, is still uncertain. In the ideal case, there would be an expectation of significant loss of body fat and a desired limited loss of muscle tissue. In the event that an excessive loss of lean body mass (LBM) occurs, certain metabolic consequences are expected, due to LBM being a key determinant of weight loss and also to glycemic regulation. Furthermore, a significant loss of muscle mass (MM) in particular, may lead to a poorer quality of life, with an occurrence of functional impairment due to an accelerated onset of fatigue in daily activities. In addition, muscle tissue is a major determinant of insulin sensitivity, and also involved in post-prandial glucose disposal. Other studies have shown that the percentage of lean body mass lost after bariatric surgery is expected to be approximately 31% of the total mass lost (Chaston et al., 2007) (Carey et al., 2006) (Das et al., 2003) (Ciangura et al., 2010) (Moize et al., 2013) (Levitt et al., 2010).

It is well documented that obese individuals experience chronic inflammation resembling that found in various infectious disease (Wisse et al., 2007) (Fogarty et al., 2008). It seems likely that zinc and copper metabolism are altered similarly by cytokines and

signaling pathways (Wisse et al., 2007) (Fogarty et al., 2008) (Pepys, 1996) in infection and obesity. However, it is not certain that the quality and the quantity of inflammation are identical in both of these conditions.

Although there is no available literature studying a possible hypomagnesaemia in Romanian population, magnesium deficiency commonly occurs throughout the world. For example, in the United States, the estimated average requirement for magnesium is set at 255–265 mg/ day for females and 330–350 mg/day for males. Yet, the same presented data indicates that about 60% of all adults do not meet the previous presented requirements (Food and Nutrition Board, 1997). In addition, the same data estimates that about 10% of adults older than 19 years have magnesium intakes that are about half of the US recommended dietary allowance (Moshfegh et al., 2009) (Ma et al., 1995). Despite this, widespread pathological conditions attributed to dietary magnesium deficiency are rarely reported. On the other hand, epidemiological and correlation studies clearly show that a low magnesium status is associated with various pathological conditions, such as atherosclerosis (Abbott et al., 2003) (Touyz, 2003), hypertension (Abbott et al., 2003) (Rude et al., 2009), osteoporosis (Barbagallo et al., 2003), diabetes mellitus, neurological or psychiatric manifestations (Dai, Shrubsole and Ness n.d.) and some form of cancers (Ridker, 2007). From this data one may conclude that magnesium deficiency may be a greater nutritional problem than currently recognized.

Personal contribution – published paper:

Timofte D, Ochiuz L, Ursaru M, Ciuntu B, Hristov I, Puia I, Calu V, Mocanu V. The biochemical effect of laparoscopic sleeve gastrectomy on serum magnesium levels. Rev Chim (Bucharest) 2017, 68(9): 1997 - 2001.
Timofte D, Ochiuz L, Ursaru M, Ciuntu B, Ionescu L, Calu V, Mocanu V, Puia IC. The impact of laparoscopic sleeve gastrectomy on serum zinc or copper and body composition. Rev Chim (Bucharest) 2017, 68(11): 2628- 2634.

The aim of this study was to investigate the impact this procedure has on body composition, serum zinc and serum copper levels in obese patients over a period of 12 months following laparoscopic sleeve gastrectomy, as well as to study how laparoscopic sleeve gastrectomy affects the levels of serum magnesium in obese populations.

I.8.2. Materials and Methods

I.8.2.1. *Bariatric surgery and serum Magnesium levels*

The study was conducted on eighty patients (38 males and 42 females), all Romanians, hospitalized for bariatric surgery in the Surgery Service, Sf. Spiridon Clinical Emergency Hospital in Iasi (Romania). These patients were investigated before and after 6 months and again after 1 year following the laparoscopic sleeve gastrectomy. These data were compared to that of a control group, recruited from the waiting list for laparoscopic sleeve gastrectomy, consisting of 80 patients, 29 males and 51 females. The control group was recruited to match weight, body mass index (BMI) and serum magnesium in relation to the corresponding baseline values in the group who underwent laparoscopic sleeve gastrectomy. All the included patients signed an informed consent and the experimental

procedures were carried out in accordance with the mandatory principles of the ethics. Baseline characteristics of the subjects are shown in the **TABLE 13**.

TABLE 13 *clinical characteristics at baseline in patients before laparoscopic sleeve gastrectomy surgery and in patients from control group. data given are arithmetic mean values (±SD). BMI=body mass*

	Experimental data	**Control group**	**Group comparisons (p value)**
Sex (male/female)	38/42	29/51	
Age (years)	40.14 (7.2)	42.03 (7.8)	0.116
Weight (kg)	122.3 (19.2)	120.8 (21)	0.629
Height (cm)	171.1 (9.7)	169.6 (7.2)	0.280
BMI (kg/m^2)	43.2 (6.1)	42.4 (4.6)	0.331
Magnesium (mmol/L)	0.778 (0.11)	0.780 (0.118)	0.885

All patients treated by surgical intervention were given the same kind of dietary advice and were recommended to take a daily oral supplement containing vitamins and minerals but not magnesium. BMI (kg/m^2) was calculated as weight (kg) divided by height (m) squared. The serum magnesium was measured by spectrophotometric determination in serum with xylidyl blue. The coefficient of variation is <2% for this method.

I.8.2.2. *Bariatric surgery and serum Zinc and Copper*

This study was conducted on 90 patients (44 men and 46 women), all Romanians, who were hospitalized for laparoscopic sleeve gastrectomy surgery in the Surgery Service, Sf. Spiridon Clinical Emergency Hospital in Iasi (Romania). These patients were investigated before and 12 months after the laparoscopic sleeve gastrectomy. Data from the experimental group was compared to that of a control group, recruited from the waiting list for laparoscopic sleeve gastrectomy, consisting of 89 patients, 33 males and 56 females. The control group was recruited to match weight, lean body mass, fat mass, muscle mass, serum zinc and serum copper in relation to the corresponding baseline values in the group who underwent laparoscopic sleeve gastrectomy, as it can be seen in **TABLE 14**. All patients signed a specific study inclusion agreement annexed to the informed consent form, and all experimental biochemical studies were performed in the light of the uniformly accepted ethical principles stated by the Helsinki Declaration. Weight was measured with a digital scale to the nearest 0.1 kg, and height was determined by a wall-mounted stadiometer to the nearest 0.5 cm. Body composition was measured by DEXA, using a whole-body scanner. For all patients, right-side half-body scans were carried out from which whole-body composition was extrapolated. MM corresponded to the appendicular LBM (the MM of the arms and legs), and was determined as the difference between total LBM and truncal LBM after removing the contribution of bone.

Copper and zinc concentrations in serum were measured by fame atomic absorption spectrometry following a one in four dilution with water. Typical between-batch precision for these assays was 3.9 and 2.27%, respectively.

TABLE 14 *Characteristics of study participants before and 12 months after laparoscopic sleeve gastrectomy. Data are presented as mean (SD).*

	Before surgery		P	After 12 months		P
	Experimental group	Control group		Experimental group	Control group	
Age (years)	40.23 (6.8)	42 (7.6)	0.088	-	-	
Gender (M/F)	44/46	33/56	-			
Body composition analysis						
Weight (kg)	122.1 (18.3)	120.6 (20)	0.605	-	-	
Height (cm)	171 (9.2)	169.6 (7.1)	0.275	-	-	
Lean body mass (kg)	57.2 (10.9)	57.1 (10.9)	0.965	47.2 (10.8)	57.2 (10.8)	<0.001
Fat mass (kg)	56.8 (10.9)	56.7 (10.9)	0.961	30.6 (10.4)	56.8 (10.8)	<0.001
Muscle mass (kg)	25.5 (10.6)	25.4 (10.6)	0.963	20.6 (9.7)	25.4 (10.5)	0.002
Biochemical variables						
Serum zinc (ug/dL)	77.3 (8.7)	77.1 (12.6)	0.897	63 (11.1)	76.6 (12.6)	<0.001
Serum copper (ug/dl)	147 (11)	147.1 (16.8)	0.966	90.2 (14.7)	147.6 (16.9)	<0.001

I.8.2.3. *Laparoscopic sleeve gastrectomy*

Laparoscopic sleeve gastrectomy involved a longitudinal resection of the stomach on the greater curvature from the antrum starting opposite of the nerve of Latarjet up to the angle of His. The first step of the procedure was the division of the vascular supply of the greater curvature of the stomach, which was achieved with the section of the gastro-colic and gastrosplenic ligaments close to the stomach. The greater curvature was completely freed up to the left crus of the diaphragm to completely resect the gastric fundus that harbors the ghrelin secreting cells of the stomach. The second step of the procedure was the longitudinal gastrectomy that "sleeved" the stomach to reduce it to a narrow tube. A naso-gastric tube was used to obtain a precise calibration and to avoid stenosis of the gastric plasty.

I.8.2.4. *Statistics*

All analyses were defined a priori. The results were given as arithmetic mean with SD. ANOVA was used for group comparisons. Adjusted analyses were made using ANCOVA. Baseline associations between continuous variables were analyzed using Pearson correlation coefficients. Tests were two-tailed and a p value <0.05 was considered significant. The statistical analysis was performed using Windows 19.0 version of SPSS software (SPSS Inc., Chicago, IL, USA).

I.8.3. Results

I.8.3.1. *Study of the serum Magnesium levels after bariatric surgery*

At baseline, before patients underwent laparoscopic sleeve gastrectomy surgery, there were no statistically significant differences between the group of patients directed for surgical treatment and the control group, regarding age, height, weight, BMI or serum magnesium concentrations. In addition, the correlations between serum magnesium concentrations on the one hand, and BMI were not statistically significant ($r = 0.060$, $p = 0.447$). None of the patients in this study had any complications during the surgical performance or during the 1-year follow-up period.

At 6-months follow-up serum magnesium concentration increased in the experimental group, from 0.77 to 0.82 mmol/L (p = 0.005), while an opposite trend was observed during the corresponding period in the control group, from 0.78 to 0.76 mmol/L (p = 0.238). The intergroup difference in serum magnesium concentrations at the 6months follow-up (p< 0.001) was significant (Fig. 26). In the experimental group, the mean BMI decreased from 43.2 kg/m^2 at baseline to 33.2 kg/m^2, (p < 0.001). In the control group, a small change but non-significant in mean BMI was observed between baseline and 6 months follow-up, BMI 42.4 kg/m^2 and 42.1 kg/m^2, respectively p = 0.076. BMI was significantly different in the two groups at the 6 months follow-up (p < 0.001) (**Fig. 25**).

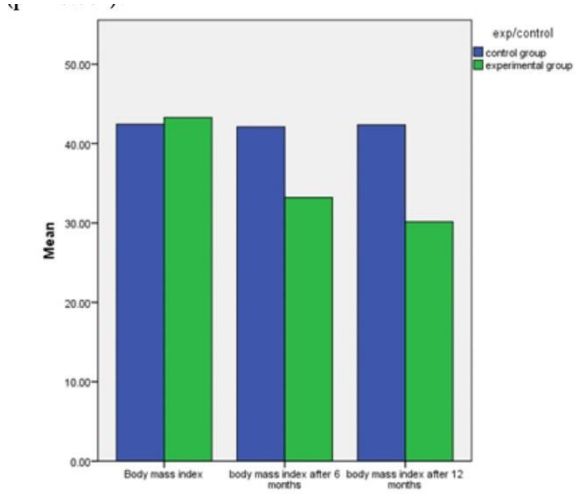

Fig. 25. *Changes in BMI (kg/m2) baseline to 6 month to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

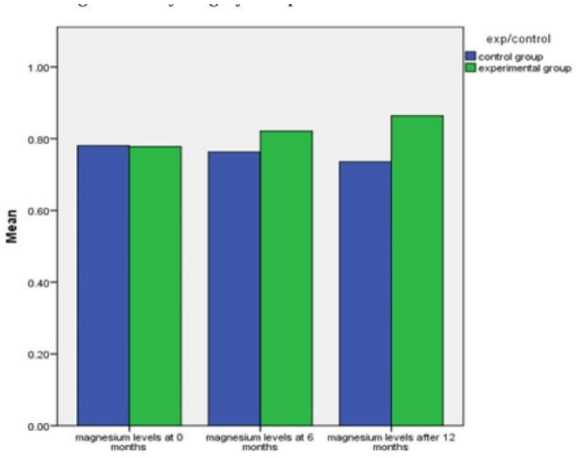

Fig. 26. *Changes in serum magnesium (mmol/L) baseline to 6 month to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

At 1 year follow-up, the serum magnesium concentrations increased from 0.77 mmol/L before laparoscopic sleeve gastrectomy surgery to 0.86 mmol/L (p < 0.001) and decreased 0.78 to 0.73 mmol/L in the control group (p = 0.002). The intergroup difference regarding serum magnesium at the 1-year follow-up was statistically significant (p < 0.001) (**Fig. 26**). In the experimental group, the mean BMI decreased from 43.2 kg/m^2 at baseline to 30.1 kg/m^2, after 1 year (p< 0.001). In the control group, a small change but again non-significant in mean BMI was observed between baseline and 1 year follow-up, BMI 42.4 kg/m^2 and 42.3 kg/m^2, respectively p = 0.794. BMI was significantly different in the two groups at the 1 year follow-up (p < 0.001) (**Fig. 25**). When we analyzed the difference between the data 6 months and after 1 year, serum magnesium concentration increased in the experimental group, from 0.82 to 0.86 mmol/L (p < 0.001), while an opposite trend was noted during the 6 months period in the control group, from 0.76 to 0.73 mmol/L (p = 0.009). The intergroup difference in serum magnesium concentrations at the 6-months follow-up (p < 0.001) was significant. In the experimental group, the mean BMI decreased from 33.2 kg/m^2 at 6 months after the operation to 30.1 kg/m^2, at 1 year after the operation (p < 0.001). In the control group, a small change but nonsignificant in mean BMI was observed between 6 months after operation and 1 year followup, BMI 42.1 kg/m^2 and 42.3 kg/m^2, respectively (p

= 0.472). BMI was significantly different in the two groups at the 1 year follow-up ($p < 0.001$).

When we controlled the preoperation values, using ANCOVA, the **statistical significance** difference between control and experimental groups maintained on serum magnesium levels after 6 months ($p < 0.001$), after 12 months ($p < 0.001$) and also for BMI 6 months post operation ($p < 0.001$) and BMI 12 months post operation ($p < 0.001$).

I.8.3.2. *Study of the serum Zinc and Copper levels after bariatric surgery*

At baseline, before patients underwent laparoscopic sleeve gastrectomy surgery, there were no statistically significant differences between the group of patients directed for surgical treatment and the control group, regarding age, height, weight, LBM, FM, MM, serum copper or serum zinc concentrations (**TABLE 14**). None of the patients in this study had any complications during the surgical performance or during the 1-year follow-up period.

At 12-months follow-up Serum zinc concentration decreased in the experimental group, from 77.3 to 63 μg/dL ($p < 0.001$). The same trend was observed during the corresponding period in the control group, from 77.1 to 76.6 μg/dL ($p = 0.008$). The intergroup difference in serum zinc concentrations at the 12-months follow-up ($p < 0.001$) was significant (**Fig. 27**).

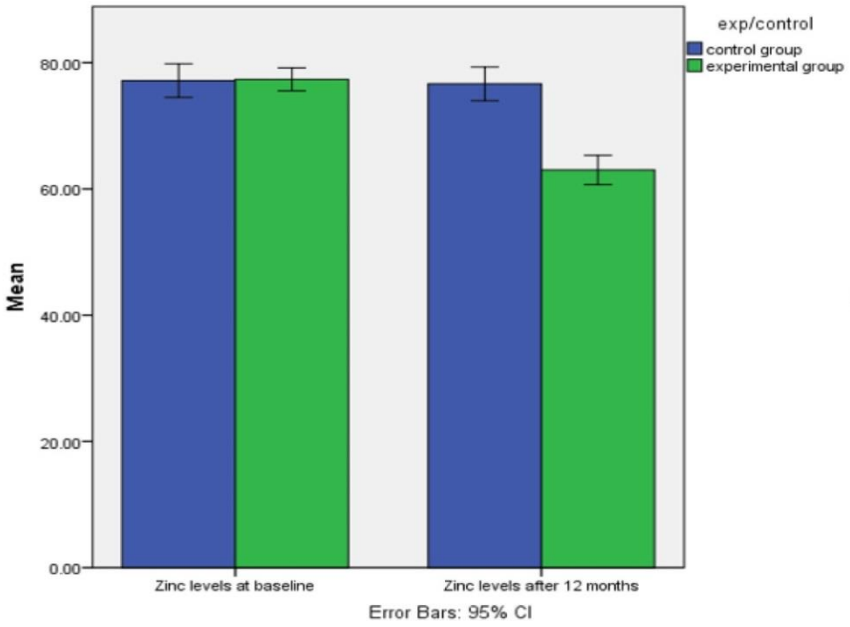

Fig. 27. *Changes in serum zinc (μg/dL) from baseline to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

Serum copper concentrations decreased from 147 μg/dL before laparoscopic sleeve gastrectomy surgery to 90.2 μg/dL at the 1-year follow-up ($p < 0.001$) and increased from 147.1 to 147.6 μg/dL in the control group ($p = 0.021$). The intergroup difference regarding serum magnesium at the 12-months follow-up was statistically significant ($p < 0.001$) (**Fig. 28**).

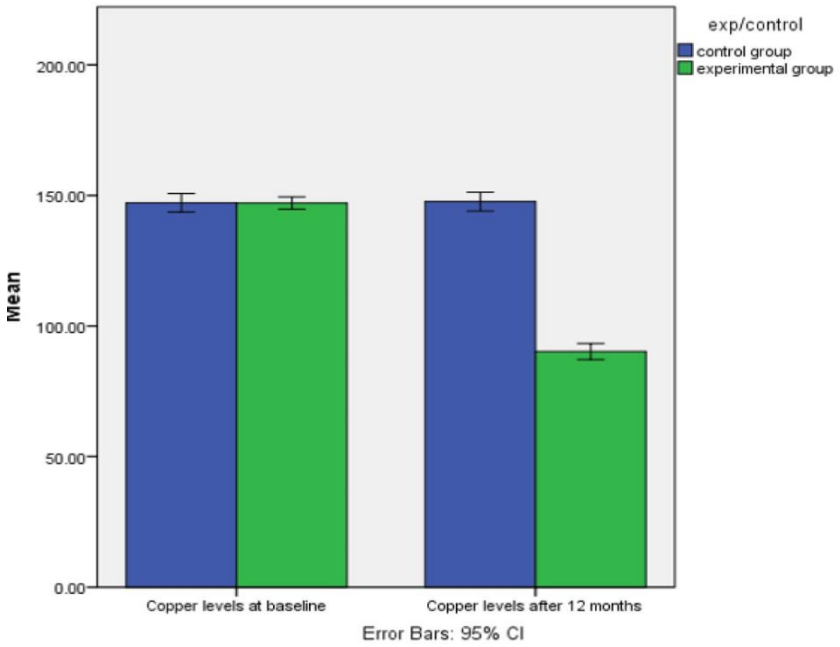

Fig. 28. *Changes in serum copper(µg/dL) from baseline to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

In the experimental group, the mean LBM decreased control group, a small change but non-significant in mean LBM was observed between baseline and 12 months follow-up, LBM 57.1 kg and 57.2 kg, respectively, p = 0.095. LBM was significantly different in the two groups at the 12 months follow-up (p < 0.001) (**Fig. 29**).

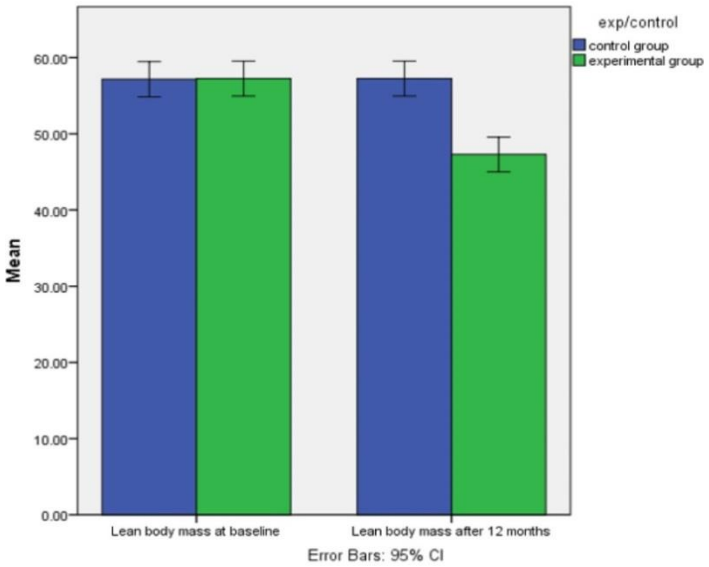

Fig. 29. *Changes in lean body mass (kg) from baseline to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls.*

Regarding the fat mass, in the experimental group, the mean FM decreased from 56.8 kg at baseline to 30.6 kg after 1 year (p < 0.001). In the control group, a small change but again non-significant in mean FM was observed between baseline and 1-year follow-up, FM

56.7 kg and 56.8 kg, respectively p = 0.067. FM was significantly different in the two groups at the 1-year follow-up (p < 0.001) (**Fig. 30**).

When we analyzed the difference between the baseline and after 1-year data regarding the muscle mass, a decrease was observed in the experimental group, from 25.5 to 20.6 kg (p < 0.001), while the MM in the control group remained approximately the same during the 12months period, 25.43 and 25.46 kg (p = 0.278). The intergroup difference regarding the MM at the 12-months follow-up (p = 0.002) was significant (**Fig. 31**).

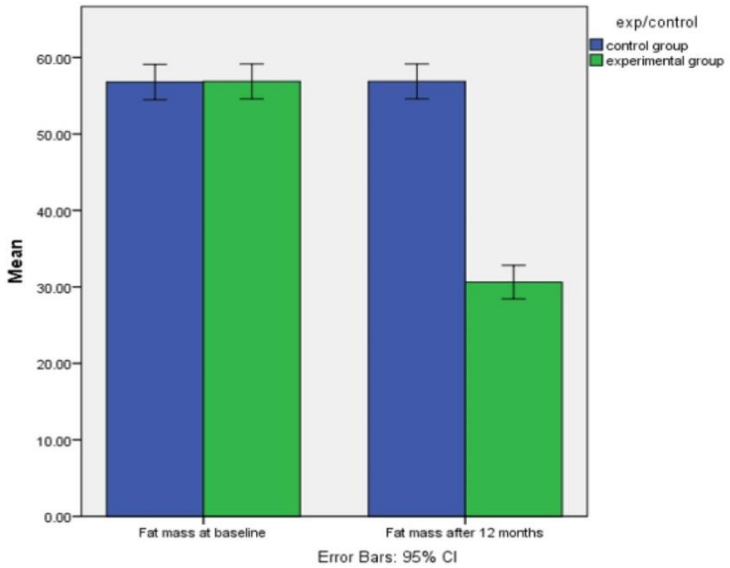

Fig. 30. *Changes in fat mass (kg) from baseline to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

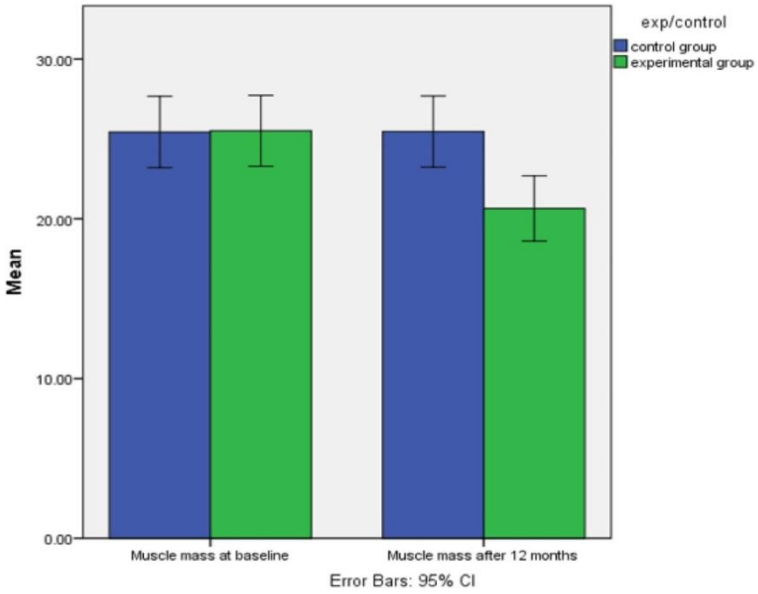

Fig. 31. *Changes in muscle mass (kg) from baseline to 1-year followup in obese patients treated with laparoscopic sleeve gastrectomy surgery compared to untreated controls*

When we controlled the preoperation values, using ANCOVA, the **statistical significance** difference between control and experimental groups 12 months post operation maintained for all the measured variables (serum zinc, serum copper, LBM, FM and MM) ($p < 0.001$). Average LBM taken at baseline was 57.2±10.9 kg, with average ponderal loss of 10.0 kg 12 months after bariatric surgery.

I.8.4. Discussion

I.8.4.1. Study of the serum magnesium levels after bariatric surgery

In our study, we observed that the serum magnesium concentrations increased significantly from 0.77 to 0.82 mmol/L after the first six months and to 0.86 mmol/L during the first year after the laparoscopic sleeve gastrectomy surgery. The increase in serum magnesium was accompanied by an expected major decrease in BMI, from 43.2 kg/m^2 before the operation to 33.2 kg/m^2 after 6 months and to 30.1 kg/m^2 after another 6 months at the 1 year mark after the operation. In the control group no statistical significant difference was observed regarding either the BMI or the serum magnesium level, after 6 months or after 1 year. Although, a non-significant decrease in serum magnesium levels was observed in the control group from 0.78 mmol/L to 0.76 mmol/L after 6 months and to 0.73 mmol/L after 1 year. It is important to be mentioned that although a significant increase for the experimental group and an non-significant decrease for the control were observed, the values were always in the normal range (between 0.7 and 1 mmol/L).

In the literature, the data about potential changes in magnesium levels after bariatric operations is conflicting. For example, we found a study (Diniz et al., 2004) which reported a similar but non-significant increase in serum magnesium concentrations in a reduced sample of only eight obese patients who underwent a bariatric surgery. In another research (Goode et al., 2004), which studied the potential impact of bariatric surgery on bone metabolism on a larger sample of 110 patients observed that none of the patients showed hypomagnesemia. In a study with an extended period of follow up (5 years), Goode et al. (Goode et al., 2004) reported normal serum magnesium levels in the experimental group which underwent bariatric surgery, as well as in the control group, which was BMI matched. The authors concluded that the magnesium levels might be associated to the change in BMI per se rather than the method of treatment. A possible explanation of these contradictory results may be that different methods of bariatric surgery might have different effects on the serum magnesium levels because hypomagnesemia has been reported more frequent in patients who underwent jejunoileal bypass for example (Singh et al., 2009). These differences are notable although the weight reduction is similar as in the laparoscopic sleeve gastrectomy treated subjects in the present study. Different confound variables such as altered magnesium absorption or induced side effects like diarrhea might help explaining the observed discrepancies between different bariatric surgical methods.

The recommended diet regime after laparoscopic sleeve gastrectomy is characterized by a lower calorie intake but with a high content of nutrients. However, the diet is not supplemented with magnesium. Further studies with dietary registration consisting of a food diary should be carried out to determine the exact influence of the post operation diet on magnesium levels.

I.8.4.2. Study of the serum zinc or copper levels after bariatric surgery

These results are in concordance with those found in the literature. For example, in a study by Aquino et al. (de Aquino et al., 2012), where 114 patients undergoing bariatric surgery were evaluated and found to have a LBM average of 64.43 kg preoperation to 51.39 kg 6 months post-surgery. The same study found an average drop in FM from 58.41 at baseline to 36.40 after 6 months. Another study on 123 patients, by Hartwig el al. (Hartwig et al., 2013), found a drop in LBM from 65.4 kg to 61.0 kg only one month after bariatric surgery. The same study showed a drop in mean FM from 60.1 kg to 50.4 kg. A bariatric surgery is considered to be successful when patients show a ponderal loss over 30% of their preoperative weight. A satisfactory outcome of a bariatric surgery is considered when 25% of their preoperative weight is lost; and unsatisfactory when they lose fewer than 25% of their total weight (Maclean et al., 1981). Therefore, the patients we studied lost 28.5% of their FM after 12 months after laparoscopic sleeve gastrectomy. Surgery could thus be classified as successful. However, the patients also lost 19.2% of their muscle mass, which may be a reason to raise concerns about future health.

As before mentioned, bariatric surgery's main benefit lies on reducing comorbidities associated with obesity. It is demonstrated that even a slight drop in weight is enough to have a positive effect on type 2 diabetes, hypertension, and dyslipidemia (Busetto et al., 2000) (Inge et al., 2007). Furthermore, a 10% drop in weight leads to better long-term control of these same conditions (Kuhlmann et al., 2000). The significant decrease in MM (from 25.5 kg to 20.6 kg) observed in the experimental group may be explained by the large caloric deficit that is created after the drastic total body weight lost. Large drops in weight are usually followed by a significant drop in basal metabolic rate (BMR), to the tune of hundreds of kilocalories (Hill et al., 1987). If we add the hypocaloric diets often prescribed post-surgery that boost proteolysis to meet metabolic demands, we might explain the drop in MM. Furthermore, when this caloric deficit is not followed by sustained physical exercise, which has many benefits including boosting resting metabolic rate and stimulating protein synthesis in the muscles (Stiegler and Cunliffe, 2006) (Ciolac and Guimares, 2004), basal metabolic rate was found to drop by 15% to 30% in some studies, making it impossible to maintain a healthy body composition in the long term (Ravussin et al., 1988).

However, there is a consensus that obese individuals, regardless of age, have a greater absolute maximum muscle mass and strength compared to non-obese persons, suggesting that increased adiposity acts as a chronic overload stimulus on the antigravity muscles (quadriceps, calf and other muscles), thus increasing muscle size and strength (Tomlinson et al., 2016). Therefore, when the overload stimulus is decreased (the body-weight) a drop in muscle mass should be expected. In addition, protein intake was found to predict the extent of muscle lost after bariatric surgeries. At the end of the first postoperative year, greater protein intake was conducive to the weight lost being primarily in fat. In a study where the authors used stable isotope techniques, on 82 individuals following bariatric surgery, the results showed an 18% drop in lean mass and 82% drop in fat after 4 months (Palombo et al., 1981). Another study found that 12-months after bariatric surgery, a 20–30% loss in lean mass was found. The amount of fat lost was found to range between 70% and 80% of total weight loss by the patients (van Gemert et al., 2000).

The importance of physical activity after bariatric surgery is undeniable, physical exercise can speed up weight loss and preserve LBM. It has been proven that patients who performed physical exercises after bariatric surgery gained 15% LBM, while those who remained sedentary lost 11% LBM by the end of the first year of observation (Metcalf et al., 2005). Therefore, engaging in physical activities is a determining factor in the drop in FM and gains in LBM and MM. Another, study by Herring et al (Herring et al., 2017), 24 patients who underwent bariatric surgery, recorded a 5.6 kg difference between groups in body mass change from baseline to 24 weeks favoring the exercise group. In addition, it has been found that body fat percentage and FM are more direct indicators of the gravity of obesity than BMI (Nagaya et al., 1999). Furthermore, it has also been shown that premature death is inversely proportional to gains in FM and reductions in LBM (Jackson et al., 2002) (Residori et al., 2003). As before mentioned, a significant drop in LBM results in a drop in basal metabolic rate (Karhunen et al., 1997), which is of a higher importance during postoperative care, given how an adequate protein intake and engaging in rigorous physical exercises may moderate, or even outweigh the negative effects of a carbohydrate restrictive, caloric-deficit diet. That is the reason why regularly physical activities play such an important role in the maintenance or in the improvement of lean muscle mass (Stiegler and Cunliffe, 2006) (Liou et al., 2010). However, despite physical activity being an important method for optimizing surgical outcomes after laparoscopic sleeve gastrectomy, it should be taken into consideration that it can sometimes lead to a compensatory response of increased caloric intake (American Society of Metabolic and Bariatric Surgery, 2008). The American Society for Metabolic and Bariatric Surgery has reported that exercise changes body composition, with increased lean body mass resulting in slower loss of overall body mass (American Society of Metabolic and Bariatric Surgery, 2008). The frequency and intensity of exercise may also affect metabolic rate contributing to weight loss plateaus, further studies should asses how different types of exercises, with different frequencies performed at different levels of intensity, influence the total weight lost and the body composition after bariatric surgeries.

In connection to physical activities, there is well known that trace elements like zinc and copper are directly involved as enzymatic co-factors in maintaining and regulating many physiological processes, especially those related to physical exercise (Anderson et al., 1984) (Ohno et al., 1985) (Olha et al., 1982). In fact, zinc is a structural component of several enzymes, among which are carbonic anhydrase and copper is in the structure of SOD, LDH and cytochrome oxidase. Therefore, it is important to determine whether bariatric surgery alters plasma levels of these metals, as this could be a possible cause of deficit, necessitating a supplementation to maintain enzyme efficiency especially if the patients are regularly engaging in physical activities.

In addition, patients undergoing laparoscopic sleeve gastrectomy are at risk for impaired copper status due to hypo acidity in the remnant stomach pouch and because of bypass of the duodenum. In our study we find a clear trend for change in serum copper levels after bariatric procedures, in the sense of a statistically significant difference between the experimental group and the controls, 90.2 µg/dL compared to 147.6 µg/dL, respectively ($p < 0.001$). In concordance with our results, previous studies have shown that the concentration of blood copper decreases following bariatric surgery (Rojas et al., 2011) (Gletsu-Miller and

Wright, 2013) (Gletsu-Miller et al., 2012). Furthermore, there are numerous reports of severe cases of copper deficiency after these surgeries (Gletsu-Miller and Wright, 2013) (Gletsu-Miller et al., 2012) (O'Donnell and Simmons, 2011). The prevalence of copper deficiency ranges from 10 to 15% in crosssectional studies and 4 to 18% in longitudinal studies (Griffith, et al. 2009) (Rojas, et al. 2011) (Gletsu-Miller and Wright, 2013) (Ernst, et al., 2009). Other than bariatric surgeries, other risk factors for copper deficiency include high zinc supplementation, and insufficient copper in micro nutrient supplementation (Rojas et al., 2011).

Regarding the serum zinc level, in our sample we found a statistically significant difference between the laparoscopic sleeve gastrectomy group and the control group after 12 months, 76.6 µg/dL compared to 63.0, respectively ($p < 0.001$). Before the operation, we did not observe significant deficiencies in any of the trace elements that we measured and it is important to mention that our preoperative sample (experimental group plus control group) was significant ($n = 179$). The mean serum zinc was 77.2 µg/dL and for serum copper 147.0µg/dL. These results at baseline are in contrast to other studies which have demonstrated that obesity is associated with lower circulating concentrations of many micronutrients and greater prevalence of biochemical deficiencies. The mechanisms underlying these observations are likely diverse but, include effects of inflammation on nutrient transporter proteins as well as greater volumes of distribution given expanded adipose and body water compartments (Saltzman and Karl, 2013).

Recent findings seem to provide some possible reasons for these conflicting conclusions about the real importance of magnesium. One of these findings is based on the connection between serum magnesium levels and inflammation in humans. Human studies clearly indicate that a low magnesium status is associated with increased inflammatory and oxidative stress. More so, C-reactive protein is a well-documented indicator of chronic inflammation (King et al., 2007). In addition, several studies showed that magnesium intake was inversely related to elevated serum or plasma C-reactive protein. However, perhaps the most eloquent proof was an analysis of no less than 5,007 children (with ages ranging between 6–17 years old). This analysis found that the children who consumed less than 75% of the recommended daily allowance were 1.94 times more likely to have elevated serum C-reactive protein when compared to children who were consuming more than the recommended daily allowance for magnesium (King et al., 2005). A similar analysis on an adult sample this time, showed a 1.48–1.75 times increased likelihood of elevated serum C reactive protein for those consuming less than the recommended daily allowance for magnesium when compared with those who consumed more than the recommended dose (Rodriguez and Guerrero, 2004).

The second recent finding is about a potential magnesium deficiency in obese populations. Recent studies show that an obese person who has a low magnesium level is much likely to present chronic inflammation indicators when compared to an obese with normal levels of magnesium. For example, in a study on 192 subjects (Corica et al., 1997), the results showed a correlation between low serum magnesium and elevated TNF-a concentrations in obese these subjects. In another interesting study about magnesium levels and obesity, Corica et al. (Corica et al., 1997) found that hypertensive obese subjects had significantly lower plasma magnesium concentrations than the non-obese healthy controls.

But more interesting, the same study found that the obese subjects with normal blood pressure presented normal values of serum magnesium. This finding is consistent with the idea from the literature that magnesium supplementation lowers blood pressure in hypertensive, but not in normotensive, overweight populations (Huerta et al., 2005).

There is still a lot to learn about the connection between chronic low-grade inflammation and obesity. Not all obese people present increased indicators of inflammatory stress. That is the reason, why many authors suggest that other factors may be involved in the development of inflammation in this population. As it was before presented, a low magnesium status occurs more often in obese populations individuals (Corica et al., 1997) (Lee et al., 2009) (Corica et al., 1999), thus it is plausible to assume that one of the variables causing the activation of inflammatory responses may be a magnesium deficiency.

The response to acute and chronic inflammation is described as a number of plasma proteins, such as ceruloplasmin, being synthesized in liver under the influence of cytokines and secreted into the circulation. Furthermore, a few other proteins, such as albumin, present a simultaneous decrease. Some of the albumin decline presented in the literature may have resulted from inflammation. It is well known that albumin is the principal zinc-binding protein (National Academy of Sciences, 2001) in serum. Therefore, with lower albumin, there are fewer binding sites for zinc. Consequently, the zinc deficiencies observed in obese populations may be due to cytokine effects on albumin. However, low values for serum copper are relatively rare in the literature (Fogarty et al., 2008). But some author suggests that even if a copper deficiency was to be present, diagnosis may have been masked by cytokine effects that increased serum copper values because ceruloplasmin is the predominant carrier of circulating copper (Linder and Goode, 1991). Perhaps copper deficiency would have been found if patients had been evaluated with some of the newer, potentially more sensitive, indices of copper status such as erythrocyte and extracellular superoxide dismutases, leukocyte copper, platelet cytochrome c oxidase or serum lysyl oxidase (Kinsman et al., 1990) (Milne, 1994) (Bergomi et al., 1997) (Mielcarz et al., 1997) (Johnson et al., 2005). Although the Western diet, for example, is often low in copper (Klevay, 1998) (Pang et al., 2001), copper deficiency has not been described in patients before bariatric surgery. However, a copper deficiency is often reported after bariatric surgeries (Kumar et al., 2004) (Kelkar et al., 2008) (Griffith et al., 2009). Studies in the literature show that patients who undergo laparoscopic sleeve gastrectomy show a 65% to 80% loss in body weight over a 12- to 18-month postoperative follow up (Shan et al., 2006) (Sugarman, 2001), but there are relatively few studies that present how much of this is muscle mass or fat mass. These changes are the result of a calorie deficit, low protein intake, and quick weight loss, with patients who undergo bariatric surgery likely to see changes in body composition over the course of treatment.

In addition, obesity and weight gain have been associated with poor food choices, suggesting that despite high caloric intake, micronutrient intake in obese patients is deficient (Saltzman and Karl, 2013) (Fung et al., 2001) (Leidkwe et al., 2004). In addition to the inadequate nutrient intake in obesity, it has been showed that nutrient metabolism is also altered in the obese population. Some authors suggest that chronic inflammation associated with obesity may dysregulate nutrient homeostasis, alter synthesis of binding proteins, or even increase oxidative stress, therefore, resulting in an increase usage of antioxidant

nutrients (Aasheim and Bohmer, 2008) (Calder et al., 2011). Additionally, although our mean values for both zinc serum and copper serum were significantly lower when compared to the matched control group, the values remained in the normal ranges for both minerals (70 150 µg/dL for copper and 60-144 for zinc). However, it still remains a concern and frequent and thorough evaluations are necessary.

I.8.5. Conclusions

The patients who underwent laparoscopic sleeve gastrectomy surgery were characterized by an expected BMI decreased and by an increased circulating magnesium level. These findings, may suggest an inverse association between a lower body fat level and an improved magnesium status. However, further detailed investigations are needed to identify the exact underlying mechanisms. Furthermore, the role of oligoelements in obesity and comorbid conditions should be established, to answer the question whether or not dietary mineral deficiency is a significant nutritional concern.

Thus, bariatric surgery has been proven to be an effective tool in achieving absolute weight loss and reducing fat tissue, in morbidly obese population. However, a diet rich in micronutrients and with an adequate protein intake should be prescribed to minimize the loss of lean body mass, and to counteract the post operation mineral deficiencies. In addition, physical exercises, aerobic or strength training should be performed regularly to reduce the negative impact that this operation has on muscle mass and muscle strength.

I.9. EXPRESSION OF THE GHRELIN RECEPTOR (GHSR-1A) IN SUBCUTANEOUS ADIPOSE TISSUE AND THE EFFECT ON PROLIFERATION AND DIFFERENTIATION OF PREADIPOCYTES

I.9.1. Introduction

Ghrelin acting through GHS-R, theoretically causes weight gain based on the increase in height of individuals and/or lean tissue, similar to GH administration. Basically, the available data clearly demonstrate that central or peripheral administration of ghrelin increases the body fat mass, adipogenesis and lipogenesis (by increasing PPARγ level), with concomitant reduction of lipolysis and use of lipids as energy substrates (Mihalache, 2016). Peripheral daily administration of acyl-ghrelin for two weeks caused a significant increase in fat mass as measured by dual energy X-ray absorptiometry (Li, 2016). On the other hand, the blockade of the ghrelin receptor abolished the effect of acyl-ghrelin on adiposity (Davies et al., 2009). *In vitro* experiments demonstrated that ghrelin increases white adipose tissue volume by either stimulating adipogenesis or inhibiting lipolysis and lipid efflux from adipocytes. Choi reported that ghrelin stimulates adipogenesis via activation of ghrelin receptor subtype 1a *in vitro* culture of rat preadipocytes (Choi et al. 2003). In contrast, Ott demonstrated no direct effect of ghrelin on adipogenesis by using a well-characterized brown adipocyte model, even though ghrelin directly suppressed expression of adiponectin, an adipokine involved in the pathogenesis of insulin resistance and obesity (Ott et al. 2002). Using a stable cell line overexpressing ghrelin, Zhang et al (2004) demonstrated that ghrelin inhibits adipogenesis in 3T3-L1 preadipocytes. Ghrelin exposure stimulates proliferation in 3T3-L1 cells and prevents the progression of adipocyte differentiation. Ghrelin may inhibit

adipogenesis by a mechanism involving down-regulation of PPAR-g activity (Bhattacharya et al., 2014).

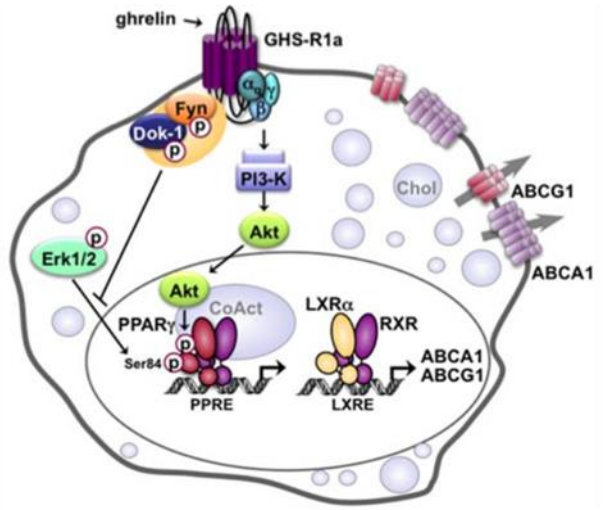

Fig. 32. *GHS-R1a-induced signaling to PPARgamma in macrophages. Activation of GHS-R1a by ghrelin promotes the recruitment and activation of a Fyn/Dok-1 complex with the subsequent decrease in Erk1/2-mediated phosphorylation of PPARc Ser-84, restraining its inhibitory potential. Ghrelin also activates the PI3-K/Akt pathway through a Gaq–dependent mechanism, which then promotes PPARc AF-1 phosphorylation independently of Ser-84, resulting in receptor transcriptional activation and increase in the PPARc-LXRa-ABCA1/G1 metabolic cascade in macrophages (Demers et al., 2009).*

Little research has been conducted on association between the effect of ghrelin and its receptor on subcutaneous preadipocyte proliferation and differentiation. This effect could be studied using agonist and antagonist substances of ghrelin receptor (GHSR 1a).

Personal contribution – Research project:

Expression of the ghrelin receptor (GHSR-1a) in subcutaneous adipose tissue and the effect on proliferation and differentiation of preadipocytes. Contract no. 29032/2016, 2017-2018. Coordonator ”Grigore T. Popa” Uiversity of Medicine and Pharmacy. Veronica Mocanu – project director; Daniel Timofte – member in the research team

We proposed to assess the role of agonist and antagonist ghrelin receptor on adipogenesis in normal weight and morbid obese patients.

I.9.2. Materials and Methods

Characteristics of the study group

The study included 20 obese female patients (OB group), referred for Laparoscopic Sleeve Gastrectomy (LSG) and 4 normal weight females (NW group, control) with other abdominal surgery indications. The mean body mass index (BMI) was 45.02±6.31 kg/m2 in OB group and 23.14±1.8 kg/m2 in NW group. The age ranges of the two groups matched, with a mean of 42.05±9.91 years for OB group and 38.0±9.4 years for the NW group.

Biochemical measurements

Blood samples were collected after 12 hours of fast, before the bariatric surgery procedure. The lipid profile was established based on the following serum determinations: Fasting plasma glucose (FPG), Total Cholesterol (TC), Low Density Lipoproteins (LDL) Cholesterol, High Density Lipoproteins (HDL) Cholesterol, Triglycerides (TG) and TC/HDL ratio. Plasma concentrations of fasting insulin were measured using a radioimmunoassay

(RIA) method and HOMA-IR was calculated using the formula: $\frac{FPI\ x\ FPG}{405}$, where FPI = fasting plasma insulin (μUI/ml) and FPG = fasting plasma glucose (mg/dl).

Plasma concentrations of total morning cortisol (8:00 a.m), leptin and adiponectin was measured in plasma using a ELISA method.

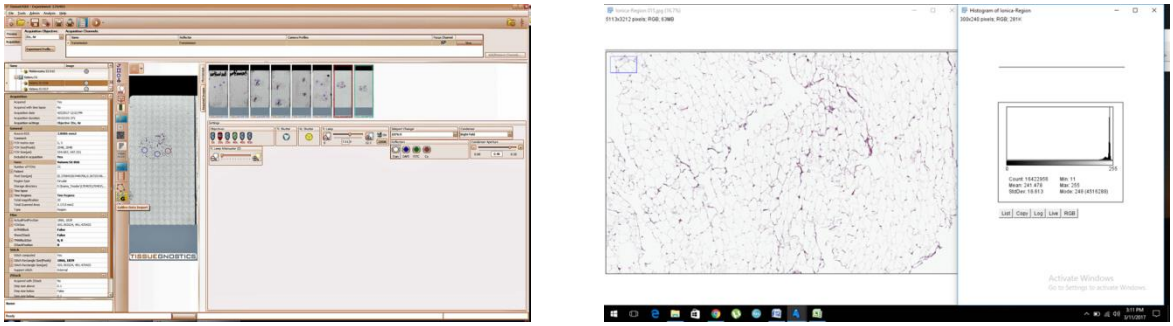

Fig. 33. *Selection of adipose tissue region for morphometry using Adiposoft*

Microscopic analysis and morphometry of the subcutaneous adipose tissue

Part of the subcutaneous adipose tissue obtained will be analysed by optical microscopy and we will perform a quantification of the amount and size of adipocytes. The microscopic analysis of adipocyte cellularity in hematoxylin and eosin (H&E) stained histological sections can be realized manually on histological images that are captured using a wide field optical microscope, printed and count the number and measure the diameter of every cell. From the diameter, the average adipocyte volume and lipid content can be mathematically derived

Qualitative and quantitative evaluation GHSR 1s and PPARγ

ARN extractions and RT-PCR were used to assess *GHSR 1s* and PPARγ expression in adipose tissue.

Immunohistochemistry analysis of GHSR 1s

For tissue sections, fat pads will be be performed in 5 μm thick paraffin-preserved subcutaneous adipose tissue following standard protocols. Immunohistochemistry staining was used to explore ghrelin receptor, GHSR1a, and also to assess PPARg, AMPK-alfa-1 + AMPK-alfa-2, CD 68 (macrophages) in adipose tissue.

ASCs isolation and proliferation

ASC culture was derived using a protocol described by Lyons (Silva, et al. 2015), starting from a small amount of subcutaneous abdominal adipose tissue (less than 1 g) remained in the trocar during the laparoscopic procedure, so that to not create supplemental patient discomfort to the bariatric surgery procedure. The sample was washed in 0.9% saline solution and digested in 10 mg/ml collagenase type I (Sigma Aldrich lot # SLBM2283V) for 15 minutes. Dulbecco's modified Eagle's medium (DMEM)/F12. The washed digested tissue was subjected to red blood cells lysis and released cells were passed through a 70μm-cell strainer before being re-suspended at a density of 1.5×10^5 cells/ml culture medium (DMEM/F12 +10% foetal bovine serum(FBS),100 U/ml penicillin and 100μg/ml

streptomycine). Cells were cultured at 37ºC under 5% CO2 atmosphere and the medium was refreshed every 2-3 days. Progression towards ASCs confluence was followed for a medium duration of 16±3 days using an inverted optical microscope (Olympus CKXC3).

ASCs characterization. Cells present in the ADSCs fractions will be fixed with 4% paraformaldehyde/PBS, washed with PBS, treated with 0.2% Triton-X-100, and then blocked with a 4% BSA solution. The samples will be incubated with the primary antibodies overnight at 4oC, washed with PBS and incubated for one hour with secondary antibodies labeled with Alexa Fluor 568 and 488. Primary antibodies will include: 1) CD68, a macrophage, monocyte, and dendritic cell marker 2) CD34, an adult hematopoietic stem cell marker 3) CD105, CD 29 (Integrina-beta-1) and CD90, the mesenchymal stem cell markers. The monoclonal anti-vimentin antibody (mesenchymal marker) and DAPI (nucleous) were also evaluated under a fluorescent microscope ZeissAxio Observer Z1 microscope.

Images will be taken using automated inverted fluorescent microscope and image processing will be performed with Image Analysis Software.

Effects of ghrelin receptor GHSR 1 a on adipogenic differentiation. ASCs will be exposed to control complete medium DMEM +10%FBS (control) or adipogenic differentiation (induction) medium (DMEM, 10% FBS, 1% ITS, Dexamethasone, IBMX, Indomethacin and human ghrelin analog (hexarelina) at the final concentrations of 1 nmol/L in the presence or absence of 1 μmol/L, PF-04628935, a ghrelin receptor inverse agonist of GHS-R1α (Bhattacharya et al., 2014) alternating with maintenance medium (DMEM, 10% FBS, 1% ITS) until the preadipocyte are fully differentiated (day 10).

Oil Red O staining

To determine the lipid accumulation, cells were fixed in 10% formalin for 20 min and stained with 0.1% Oil Red O (ORO) for 60 min as described by Kraus A et al. (2016).
Lipids were spectrophotometrically quantified using a microplate reader Tecan Sunrise by reading the absorbance values at 492 nm wave length, after elution of ORO-stained cells in 90% isopropanol.

Statistical analysis

Data was analysed using IBM SPSS Statistics 21 Software and expressed as mean ±standard deviation. Comparisons between parameters in obese patients and normoponderal controls were performed using the Mann–Whitney test. Pearson's correlation analysis was used to evaluate correlations between lipid accumulation evaluation and biochemical and hormonal parameters. Significance was defined as $p < 0.05$.

I.9.3. Results

Characteristics of patients

Plasma levels of total cholesterol, LDL-cholesterol, total cholesterol/HDL-cholesterol, insulin, C-peptide, and HOMA-IR index were significantly increased in OB group as compared with NW group. Significantly high leptin and leptin:adiponectin ratio values were found in in OB group as compared with NW group (

TABLE *15*).

Microscopic analysis and morphometry of the subcutaneous adipose tissue

The results are showed in

TABLE *15* and **Fig. 32**.

Qualitative and quantitative evaluation GHSR 1s and PPARγ

ARN extractions and RT-PCR were used to assess *GHSR 1s* and PPARγ expression in adipose tissue. We found significantly higher PPAR γ expression in the subcutaneous adipose tissue and a positive correlation between PPAR γ expression and body mass index (BMI). The ghrelin receptor mARN expression was lower in obese patients as compared to control.

Immunohistochemistry analysis of GHSR 1s

For tissue sections, fat pads will be be performed in 5 µm thick paraffin-preserved subcutaneous adipose tissue following standard protocols. Immunohistochemistry staining was used to explore ghrelin receptor, GHSR1a, and also to assess PPARg, CD 68 (macrophages) in adipose tissue (**Fig. 34**). In obese patients, the adipocyte the expressed GHSR1 immunohistochemistry staining was diminished as compare to normal weight patients. The surface decreased expression of GHSR1 was associated with increased adipocyte size and macrophage infiltration of adipose tissue.

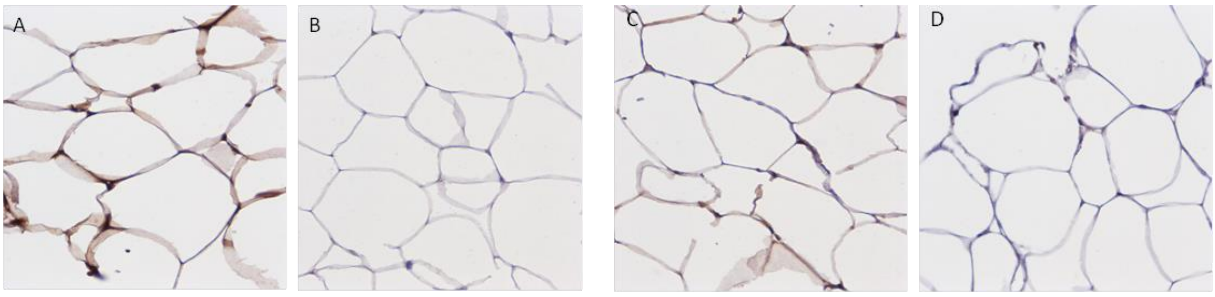

Fig. 34. *Immunohistochemistry of ghrelin receptor)GHSR 1 a) in NW (A) and OB (C) patients and CD68 (macrophages) in in NW (C) and OB (D) patients.*

Adipose derived stem cell (ASCs) isolation, proliferation and characterization.

The stromal fractions were obtained from subcutaneous abdominal adipose tissue derived from OB and NW group and grown in culture at a starting density of 1.5×10^5 cells/ml.

The ASCs proliferation to confluence was obtained after 18±4 days for OB patients isolated ASCs and after 16±3 days for ASCs in NW patients, with no statistical significant difference between OB and NW groups (**Fig. 35**).

The cell culture viability was evaluated by MTT–formazan (3, 4, 5- dimethylthiazol-2–5-diphenyltetrazolium bromide) according to the protocol of Mossman et al. (Mossman et al., 1983). The metabolic activity of mesenchymal stem cells evaluated using MTT assay was 93±3% for the OB group with a similar result: 94±2% for the control NW group.

Identification of mesenchimal specific markers

In our study, in order to characterize the cell culture population as mesenchymal stem cells, we evaluated the expression of vimentin and CD 90 (**Fig. 36**, **Fig. 37**).

TABLE 15 *Characteristics of the obese patients and normal weight controls. The data are presented as the means±S.D.*

Parameter	Normal weight controls (N=4)	Obese patients (N=20)	p- value
Age (years)	38.00 ± 9.42	42.05 ± 9.91	0.431
BMI (kg/m2)	23.14 ± 1.79	45.02 ± 6.31	<0.001
Lipid profile			
Total cholesterol (mg/dL)	172.00 ± 3.76	217.80 ± 20.72	0.045
LDL-cholesterol (mg/dL)	88.75 ± 3.09	141.30 ± 22.43	0.005
HDL-cholesterol (mg/dL)	55.75 ± 3.30	49.7 ± 12.78	0.241
TC/HDL (Normal <4)	3.08 ± 0.35	4.56 ± 0.85	0.005
Triglycerides (mg/dL)	105.29 ± 9.81	143.90 ± 60.08	0.210
Glucose homeostasis			
Fasting plasma glucose (mg/dL)	86.75 ± 11.26	126.80 ± 74.06	0.056
Insulin (μIU/mL)	6.68 ± 0.47	16.29 ± 9.25	0.005
C-peptide (ng/mL)	1.64 ± 0.40	3.20 ± 0.99	0.001
HOMA-IR	1.42 ± 0.14	5.96 ± 7.08	0.001
Plasma hormones			
Morning cortisol (μg/dL)	9.78 ± 4.16	12.88 ± 4.13	0.115
Leptin (ng/dL)	4.30 ± 0.48	24.38 ± 8.49	<0.001
Adiponectin (μg/dL)	30.66 ± 1.20	30.22 ± 1.76	0.970
Leptin: Adiponectin Ratio	0.14 ± 0.19	0.82 ± 0.32	<0.001
Adipocyte differentiation dysfunction			
Lipid accumulation (OD)	0.485 ± 0.06	0.791 ± 0.14	<0.001

P-values were assessed by Mann–Whitney test to compare non-normal distributed variables. P-value ≤ 0.05 was considered significant.

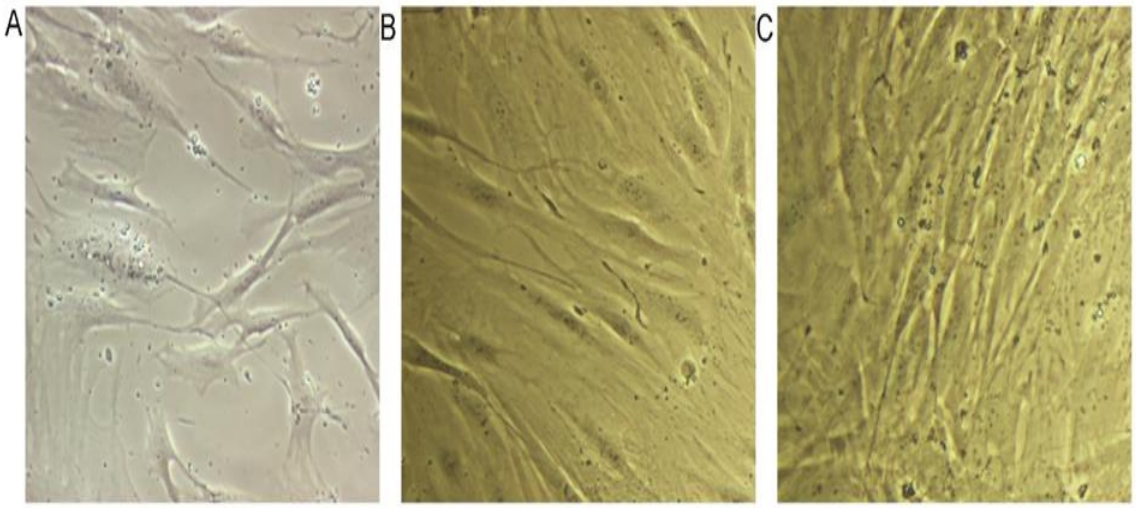

Fig. 35 .*Adipose derived stem cells isolation and proliferation (2-3 weeks). A. Stem cells isolation (10x brightfield); B. Stem cells proliferation (10x phase contrast); C. Stem cells culture confluence 90%-day 18 (10x phase contrast)*

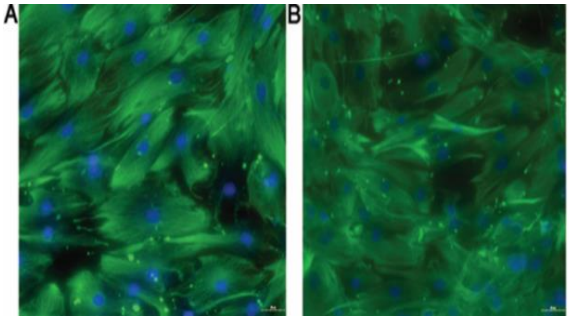

Fig. 36. *ASCs characterization as mesechymal cells using Vimentin IF stain.-Merge image cytosolic Vimentin (green) and nuclear DAPI (blue) A. Normoponderal ASCs B . Obese ASCs*

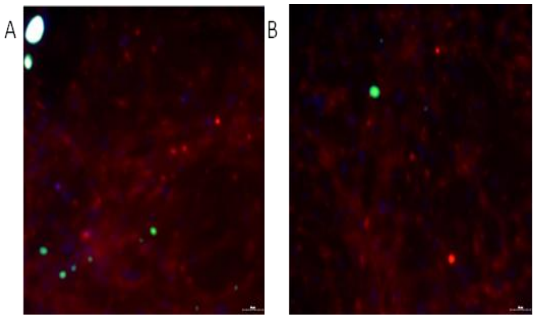

Fig. 37. *ASCs characterization as mesechymal cells using immunofluoresce staining for CD 90, CD 80 and CD 34. Merge image CD 90 (green), Red Nile (red) and nuclear DAPI (blue). Presence of CD 90 (mesenchimal cells) and absence of CD 80 (nacrophages) and CD 34 (hematopoetic cells). A. Normoponderal ASCs B. Obese ASCs*

Effects of ghrelin receptor GHSR 1 a on adipogenic differentiation

Adipogenic differentiation protocol was monitored in cell cultures using optic microscopy and lipid accumulation. The lipid droplets accumulated were fewer and smaller in the OB samples as compared to NW. The addition of hexarelin (Hex) to differentiation medium resulted in decreased adipogenic differentiation. Moreover, the addition of PF-04628935 (PF) resulted in inhibition of adipogenic differentiation.

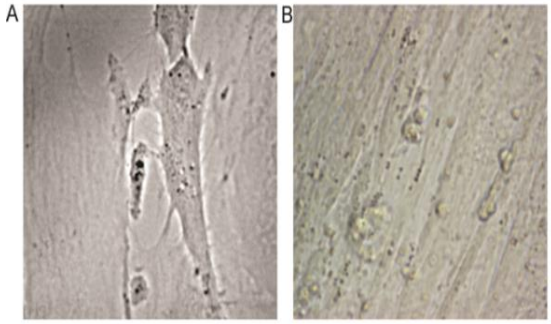

Fig. 38. *Adipogenic differentiation stages: progressive lipid accumulation A. Adipogenic differentiation protocol day 5- inverted microscopy image: Phase contrast PH2, 20x; B. Adipogenic differentiation protocol day 10- inverted microscopy image: Phase contrast PH2, 5x*

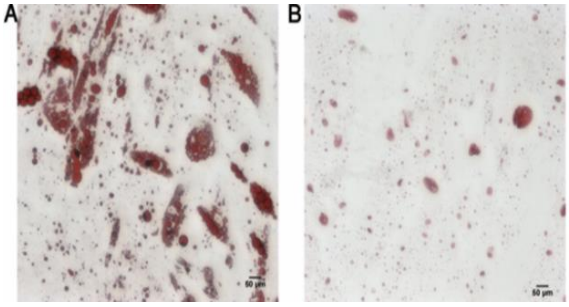

Fig. 39. *Specific lipid stain with ORO brightfield optic microscopy. A. Adipocytes differentiated from NW ASCs. B. Adipocytes differentiated from OB ASCs.*

Intracellular lipid acumulation (Oil red O staining)

Specific lipid stain was evaluated using ORO and the lipid accumulation obtained for adipocytes differentiated from NW (**Fig. 38**) ORO absorbance at 492 nm by spectrophotometry showed a significant increase ($p < 0.001$) in lipid deposition in ASCs derived from obese patients as compared to normal weight patients) (**TABLE 16** and **TABLE 17**).

Identification of adipocyte specific markers

Differentiated adipocytes were characterised be immunofluorescence staining for PPARg (green), triglicerides (orange) and nucleus (blue). Images will be taken using automated inverted fluorescent microscope and image processing will be performed with Image J Analysis Software (**Fig. 39**).

TABLE 16 *Lipid accumulation (Oil Red O –Absorption, OD) in differentiated ASCs*

	Normal weight	**Obesity**	**p value**
CONTROL	0.236±0.08	0.224±0.10	0.02
INDUCTION	0.791±0.14	0.485±0.06	0.01
HEXARELIN	0.667±0.06	0.445±0.06	0.03
GHSR 1a ANTAG	0.133±0.02	0.102±0.03	NS

Correlation of lipid accumulation with metabolic parameters

A negative correlation was found between the lipid accumulation (OD) and plasma insulin ($r = -0.44$, $p<0.05$), C-peptide ($r = -0.63$, $p<0.01$), leptin ($r = -0.45$, $p<0.05$), and leptin:adiponectin ratio ($r = -0.46$, $p<0.05$) (**TABLE 16**, **Fig. 39**).

Fig. 40. *Differentiation of ASCs isolated from subcutaneous adipose tissue of obese patients using complete medium (A), induction medium with hexarelin (ghrelin agonist)(B) or hexarelin and PF-4628935 (GHSR-1a antagonist)(C).*

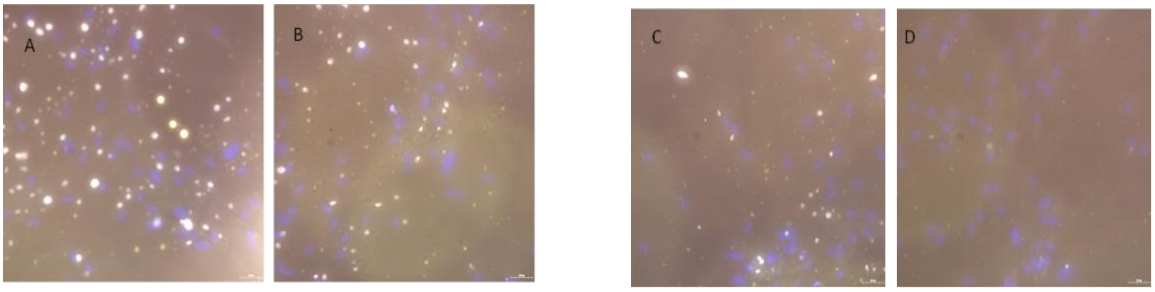

Fig. 41. *Differentiation of ASCs isolated from subcutaneous adipose tissue. Immunohistochemistry of PPAR g in adipogenic differentiated ASC from NW patients and obese using complete medium (A and B), and induction medium with hexarelin (ghrelin agonist) (C) or hexarelin and PF-4628935 (GHSR-1a antagonist)(D) in obese patients*

No correlations were noticed between lipid accumulation and other plasma metabolic parameters (fasting glucose, total cholesterol, LDL-cholesterol or HDL-cholesterol), neither

between lipid accumulation and HOMA-IR index or plasma concentrations of morning cortisol and adiponectin

TABLE 17 *Pearson's correlation of adipocyte lipid accumulation with anthropometric, metabolic and hormonal parameters.*

Co-variables	Pearson's correlations	
	r	p-value
Age	0.32	0.89
BMI	-0.19	0.41
Total cholesterol (TC)	-0.08	0.75
LDL-cholesterol (LDL-C)	-0.04	0.87
HDL-cholesterol (HDL-C)	0.37	0.29
TC/HDL	-0.60	0.80
Triglycerides (TG)*	-0.45	0.05
Fasting plasma glucose (FPG)	-0.32	0.17
Insulin*	-0.44	0.05
C-peptide*	-0.63	<0.01
HOMA-IR	-0.37	0.10
Morning cortisol	0.27	0.26
Leptin*	-0.45	0.05
Adiponectin	0.26	0.26
Leptin:Adiponectin Ratio*	-0.46	0.04

r, Pearson's correlation coefficient. *Significant correlation ($p<0.05$)

I.9.4. Discussion

The altered lipid accumulation capacity of subcutaneous preadipocytes in obese patients is currently being evaluated as a precocious marker for insulin resistance as it translates the incapacity of the mesenchymal cell line progenitors to differentiate mature, functional adipocytes with optimal lipid storage capacity. Several studies found that lipid accumulation evaluates the expansion capacity of the pre-adipogenic mesechymal cell line and is associated with a poor metabolic profile for obese patients (Isakson et al., 2009) (Tsatsoulis et al., 2013) (Vidal-Puig, 2013).

Peroxisome proliferator-activated receptor gamma (PPAR γ) represents a master regulator of adipogenesis. It is expressed since the early stages of the process, being induced during preadipocyte differentiation into mature adipocyte, subsequently increasing the number of new formed small and insulin sensitive adipocytes. Moreover, PPAR γ is expressed throughout all the adipose tissue, playing a role in cell proliferation, energy metabolism and inflammation. Due to these functions, PPAR γ expression regulation may have a major impact in obesity, which is considered a state of chronic low-grade inflammation (McLaughlin et al., 2010).

Ghrelin, a stomach-derived hormone implicated in numerous behaviors including feeding, reward, stress, and addictive behaviors, acts by binding to the growth hormone secretagogue receptor (GHSR). Ghrelin induced increase adiposity, hepatic steatosis, increasing lipid droplet number and triacylglycerol content by a GHS-R(1a)-dependent mechanism. We evaluated PPAR γ and ghrelin mRNA expression in adipose tissue

samples from 6 obese patients and 2 nW patients before undergoing bariatric surgery. PPAR γ and ghrelin mRNA was assessed using real-time PCR.

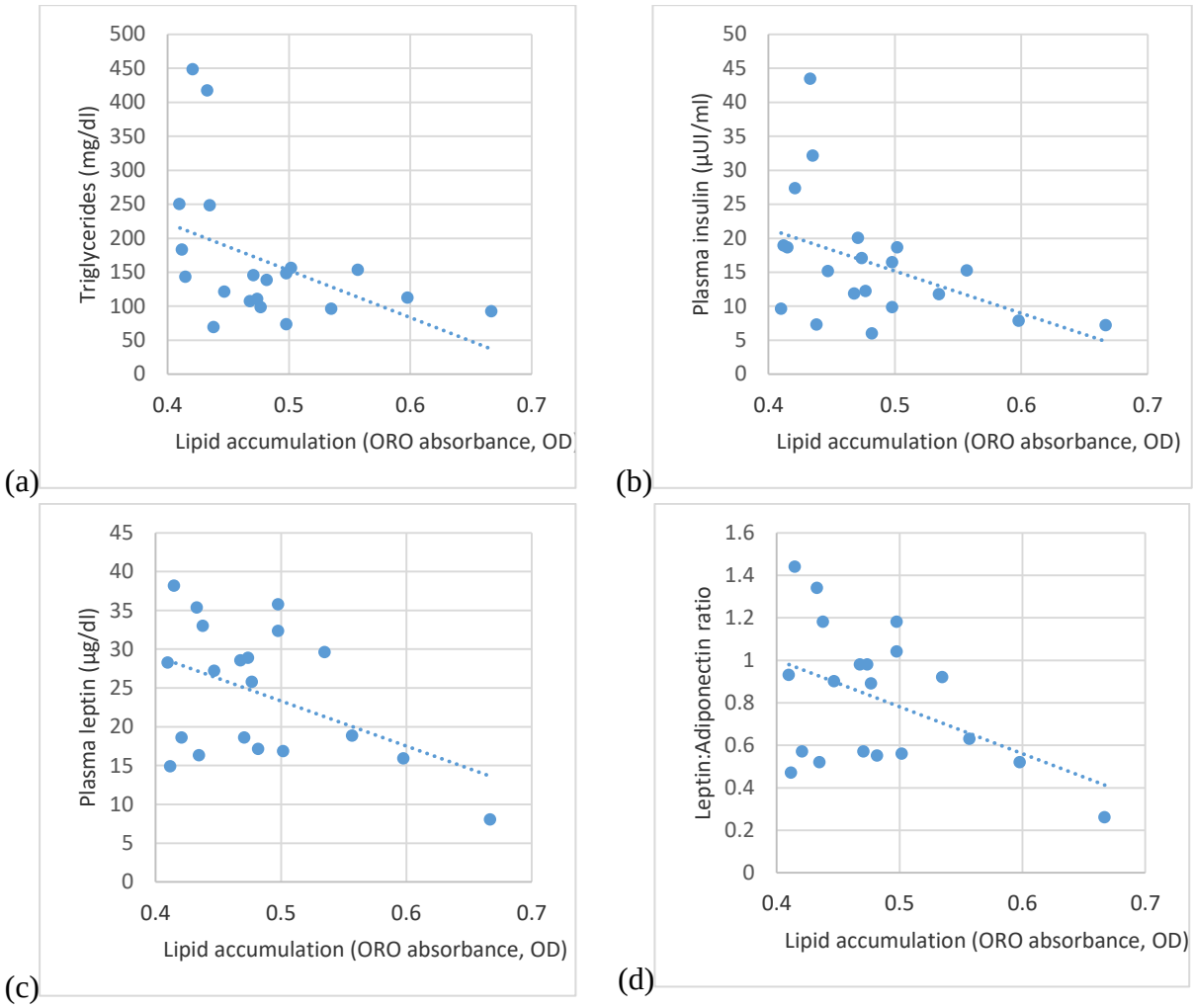

Fig. 42. *Graphic illustration for the correlation between Oil Red O absorbance and metabolic and hormonal parameters: A. Correlation with plasma triglycerides level; B. Correlation with serum insulin levels; C. Correlation with plasma leptin; D. Correlation with plasma leptin: adiponectin ratio.*

In obese patients, the adipocyte the expressed GHSR1 immunohistochemistry staining was diminished as compare to normal weight patients. The surface decreased expression of GHSR1 was associated with increased adipocyte size and macrophage infiltration of adipose tissue.

We found significantly higher PPAR γ expression in the subcutaneous adipose tissue and a positive correlation between PPAR γ expression and body mass index (BMI). The ghrelin receptor mARN expression was lower in obese patients as compared to control.

Adipocyte expression of PPAR γ and GHSR-1a were connected with the amount of adipose tissue found in an individual. Our data imply that exposure to ghrelin may limit adipose tissue expression of GHS-R(1a) in relationship with lipid retention in adipocyte.

The subcutaneous adipose tissue represents 90% of total fat mass it has potential to greatly affect systemic insulin resistance via adipokine secretion, that plays an important role in glucose uptake impairment, as chemerin was reported to be associated positively with BMI

and the markers of inflammation and metabolic syndrome in humans (Jeffery et al., 2015). Adiponectin expression has been demonstrated to accelerate 3T3-L1 proliferation and also lipid accumulation evaluated by Oil red O staining was found to be 4-fold greater in adipogenic differentiated pre-adipocytes that overexpressed adiponectin (Fu et al., 2005). Also, in animal studies on mice the same results were found (Combs et al., 2004). These results support the role of adiponectin as a key autocrine/paracrine factor that could play an essential role in the regulation of adipocyte metabolism and adipose tissue mass.

We report a decreased lipid accumulation capacity for adipogenic differentiated ASCs of obese female patients versus those from normal weight controls, and significant correlations between the adipocyte fat accumulation potential and insulin, leptin, leptin: adiponectin ratio (LAR).

The fact that obesity alters the adipogenic differentiation capability of ASCs from subcutaneous adipose depots is supported by the data from other research groups (Louwen et al., 2018) (Weyer et al., 2000) (Perez et al., 2013). De Girolamo et al. evaluated the ASCs adipogenic potential in obese versus non-obese controls and they also found a reduced proliferative rate for obese ASCs (Vidal-Puig, 2013). Alteration of the preadipocytes lineage in obese bariatric patients was also demonstrated by Perez et al. that showed an altered lipid accumulation in obese-ASCs derived adipocytes as compared to adipocytes lineage coming from normal weight humans or mice (Perez et al., 2013). In another study, Perez et al. reported a significantly enhanced apoptosis and a reduced proliferative capacity of ASCs isolated from obese subjects; the impaired adipogenesis was correlated with the in vivo environmental obesity-related altered mitochondrial biogenesis, increased reactive oxygen species production and increased extracellular acidification (Perez et al., 2015)

We demonstrated that plasma insulin and C- peptide are correlated with decreased adipocyte ability to accumulate lipids. Previous research data published by Weyer et al, also show that the pre-adipocytes differentiation potential is correlated with insulin resistance in humans, in both obese and normal weight individuals (Weyer et al., 2000).

The novel finding in our study was the negative correlation between adipogenic potential (assessed by lipid accumulation in mature adipocytes) with triglycerides and LAR as key markers of impaired metabolic profile. These results point to abnormal adipogenesis as a precocious link between obesity and its clinical complications.

Overall strengths of our study include direct measurement of the lipid accumulation in differentiated ASCs and the assessment of the relation between impaired adipogenesis with plasma metabolic and adipokine parameters in females with increased adiposity. A limitation of our study is the small size of the study and control groups and the relatively young age of the patients.

I.9.5. Conclusions

Our results suggested that GHSR 1a expression is diminished in obese patients as compared to normal weight patients. The abnormal GHSR 1 is associated with multiple changes in adipocyte size, adipose tissue inflammation and altered adipogenesis the evaluation of GHSR 1a on adipocyte could bring a better understanding of metabolic changes associated with adiposopathy in obese patients.

CHAPTER II. OBESITY – A MULTISISTEMIC DISEASE

II.1. State of the Art

Obesity is a real and serious health problem by increasing global cardiovascular risk, lowering life expectancy and improving quality of life. The existence and the degree of obesity are closely related to the global cardiovascular risk that mainly associates three comorbidities: type II diabetes, hypertension and cardiovascular disease (Angrisani, 2017). Dyslipidemia, consisting of hypertriglyceridemia and decreased plasma HDL levels, is commonly found in obese patients and further contributes to increased cardiovascular risk. Other cardiovascular disorders encountered in obese patients are thrombophlebitis, pulmonary thromboembolism and chronic peripheral venous insufficiency with varicose ulcers (Buchwald, et al., 2004).

The essential elements of metabolic syndrome (X syndrome) are the association of insulin resistance and hyperinsulinemia with dyslipidemia and essential hypertension, whose central feature is central-type obesity and is at increased risk for cardiovascular accidents (Kim and Richards, 2010).

Hypoventilation syndrome and sleep apnea are another severe complication of obesity. Apnea is followed by hypoxemia and hypercapnia, as well as a response to stress characterized by increased levels of catecholamine and endothelin. Sleep apnea is associated with an increased risk of high blood pressure and increases the mortality of these patients by sudden death (Agrawal, 2016).

Osteoarticular disorders of the type of osteoarthritis (knee, hip) and discopathy that arise as a consequence of overuse of the joints by excess body mass, as well as gout, a consequence of hyperuricemia, invalidate these patients by limiting their possibilities for movement (Blackstone, 2016).

Malignancies such as breast and endometrial cancer in obese women as well as prostate and colon cancer in obese men are more frequent than in the general population (Blackstone 2016).

Gastroesophageal reflux disease and hepato-biliary diseases such as non-alcoholic hepatic steatosis, biliary lithiasis, liver enzymatic abnormalities are commonly associated in obese patients and require adequate therapeutic behavior (Blackstone, 2016).

Obesity can induce endocrine disorders. Obese women often develop polycystic ovary syndrome that associates hirsutism and menstrual cycle disorder that can go up to amenorrhea with anovulatory cycles and sterility (Blackstone, 2016).

Central obesity (faciotroncular) is associated with overproduction of androgens, which in man is the cause of hypothalamic hypogonadism. Postmenopausal hypoestrogenism in obese women contributes to the increase in malignancy (breast and endometrial cancer) (Blackstone, 2016).

The incidence of psychiatric disorders in obese patients was found to be higher than in the general population. Obese patients experience depressive episodes of high frequency, usually of medium intensity, dysthymia, anxiety and phobias (social phobia, obsessive compulsive disorder) (Kopelman, 2010).

However, the recent increase in the prevalence of obesity is mainly due to negative environmental factors that may outweigh the potential of regulatory systems. These include a large-scale availability of high-energy foods along with an unprecedented drop in physical activity (Agrawal, 2016).

The main preoccupations that I had in this direction of research are reflected in the next articles and projects:

1. Bilha S, Nistor I, Nedelcu A, Kanbay M, Scripcariu V, **Timofte D**, Siriopol D, Covic A. The effects of bariatric surgery on renal outcomes: a systematic review and meta-analysis. Obes Surg 2018, 28(12): 3815 - 3833.
2. Livadariu R, **Timofte D**, Trifan A, Dănilă R, Ionescu L, Sângeap AM, Ciobanu D. Vitamin D deficiency, a noninvasive marker of steatohepatitis in patitents with obesity and biopsy proven nonalcholic fatty liver disease. Acta Endocrinologica (Buc), 2018, 14(1): 76 – 84.
3. Livadariu R, Dănilă R, Ionescu L, Ciobanu D, **Timofte D.** Study of biochemical and clinical markers in steatohepatitis related to obesity. Rev Chim, 2018, 69(6):1501 – 1506.
4. **Timofte D**, Ciuntu B, Bulgaru Iliescu D, Hainarosie R, Pantea Stoian A, Mocanu V. Laparoscopic sleeve gastrectomy is associated with reduced depressive symptoms: a one-year follow-up study. Revista de Cercetare și Intervenție Socială, 2018, 61: 147 – 154.
5. **Timofte D**, Ciuntu B, Iliescu DB, Hainarosie R, Neagoe R, Hristov I, Stoian AP, Mocanu V. The impact of bariatric surgery on anxiety symptoms. Revista de Cercetare și Intervenție Socială, 2018, 62: 185 – 195.

II.2. The metabolic surgery and the renal function

II.2.1. Introduction

Obesity and chronic kidney disease (CKD) are two of the greatest epidemics of the twenty-first century. In 2014, 10.8% of men and 14.9% of women worldwide had a body mass index (BMI) $\geq$ 30 kg m^2 (NCD Risk Factor Collaboration 2016); also, 13.4% of the global population has CKD (Hill et al., 2016). High body fat increases the risk of developing CKD indirectly—not only via diabetes mellitus and hypertension (NCD Risk Factor Collaboration 2016) (Hill et al., 2016) but also through direct renal functional and structural modifications. This is due to an increased renal sodium tubular reabsorption, secondary to kidney compression (Hall et al., 2014) that triggers the vasodilation of the afferent arteriole (via tubuloglomerular feedback) leading to hyperperfusion, hyperfiltration, and increased glomerular capillary pressure with subsequent albuminuria/proteinuria (Hall et al., 2014) (Garland, 2014). As obesity-associated kidney damage progresses, hyperfiltration (increased glomerular filtration rate (GFR)) is replaced by a declining GFR, with progression toward end-stage renal disease (ESRD) (Hall et al., 2014) (Garland, 2014). If obesity is responsible for kidney damage, is it possible to reverse the damage through weight loss therapies? Many studies have addressed this question and most of them reported beneficial effects of different weight loss interventions for improving obesity-induced kidney damage (Navaneethan et al.,

2009) (Afshinnia et al., 2010) (Bolignano and Zoccali, 2013). For each 1 kg reduction in weight, there is a 4% decrease in proteinuria and albuminuria, independently of blood pressure decline (Afshinnia et al., 2010).

Bariatric surgery (BS) is the most efficient intervention for obtaining and maintaining substantial weight loss and the only curative method that significantly ameliorates obesity-related comorbidities (Piche et al., 2015). BS also seems to have a positive impact on renal function (Afshinnia et al., 2010); however, it is associated with hyperoxaluria, nephrolithiasis, and oxalate nephropathy (Duffey et al., 2010). Most importantly, data regarding the impact and safety of BS in patients with kidney impairment are insufficient. This systematic review particularly addresses the effects of BS on kidney function outcomes in non-CKD and CKD patients. Thus, in one of our previous published work (Bilha, et al. 2018) we wanted to evaluate the benefits and harms of BS for weight loss on kidney function. We searched MEDLINE (inception to August 2017), the Cochrane Library (Issue 10–12, October 2017), and the website clinicaltrials.gov (August 2017) without language restriction. Hand search for relevant articles was done on reference lists from textbooks, articles, and scientific proceedings.

Personal contribution – published paper:

Bilha S, Nistor I, Nedelcu A, Kanbay M, Scripcariu V, **Timofte D**, Siriopol D, Covic A. The effects of bariatric surgery on renal outcomes: a systematic review and meta-analysis. Obes Surg 2018, 28(12): 3815 - 3833.

II.2.2. Material and Methods

We conducted a systematic review and meta-analysis on observational cohort studies in adults with obesity that were treated with BS for weight loss and have reported data about the impact of BS (any techniques) on kidney function endpoints (serum creatinine, creatinine clearance, GFR, proteinuria, nephrolithiasis, and need for renal replacement therapy (RRT)). Patients (non-CKD, CKD or transplanted) were included in this analysis if their biochemical renal function values were reported before and after BS. The surgery itself could be sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB), adjustable gastric banding (AGB), and biliopancreatic diversion (BPD) done either by open or laparoscopic surgery. Patients were acting as their own control group, since renal endpoints were compared before (perioperative) and after the surgery. Patients undergoing reoperative intervention for obesity were excluded.

Data extraction was done independently by two authors (AN and SB) using standard data extraction forms. When more than one publication of a study was found, reports were grouped together and only the publication with the most complete data was included. Data extracted included identifying information, study outcomes, details of the study protocol, and demographic data. We extracted characteristics of each study including baseline renal function values, baseline clinical characteristics of the study population, CKD status, known comorbidities, type of study design, types of surgery, and total duration of follow-up. Any unclear or missing information was requested from the authors by written correspondence, and any relevant information obtained was included in the review. Disagreements were resolved by consultation between all authors.

We used a random-effects model for meta-analysis and expressed treatment effects as a risk ratio (RR) with 95% confidence intervals (CI) for dichotomous outcomes (need for RRT) and mean difference (MD) for continuous outcomes with 95% CI (e.g., changes in GFR, creatinine level at the end of intervention, etc.) (DerSimonian and Kacker, 2007). We used the I 2 statistics to assess for inconsistency across individual studies (DerSimonian and Kacker, 2007). An I 2 > 50% indicated a large inconsistency across studies (heterogeneity) not explained by chance (Higgins et al., 2003). All statistical analyses were performed using Review Manager Version 5.2 (The Cochrane Collaboration 2012). Additional prespecified subgroup analyses were conducted to explore the potential causes of heterogeneity for treatment effect on renal function. Treatment heterogeneity was analyzed also in relation with equations used to estimate the renal function. Subgroup analyses will be conducted for the following subgroups: CKD stages 3 to 5, kidney transplanted patients, and hyperfiltration patients (GFR > 110 ml min−1).

II.2.3. Results

Regarding the results we obtained at that time, a flow diagram proving the selection process of the included studies is depicted in **Fig. 43**. The initial search resulted in 1094 potentially relevant articles. A thorough analysis of the abstracts led to the exclusion of 1044 articles due to search overlap, non-relevance, renal function not reported, clinical studies other than observational, case reports, editorials, reviews, or meta-analyses. Fifty articles were studied full text, from which 27 were excluded due to non-relevance or lack of sufficient information. After an in-depth review, 23 observational studies were included in the present systematic review (Navarro-Diaz et al., 2006) (Serpa-Neto et al., 2009) (Friedman et al., 2014) (Saliba et al., 2010) (Chagnac et al., 2003) (Luaces et al., 2012) (Navaneethan et al., 2010) (Navaneethan and Yehnert, 2009) (Agrawal et al., 2008) (Mohan et al., 2012) (Fenske et al., 2013) (Hou et al., 2013) (Palomar et al., 2005) (Getty et al., 2012) (Jose et al., 2013) (Ruiz-Tovar et al., 2015) (Schuster et al., 2011) (Ngoh et al., 2016) (Reid et al., 2014) (Serra et al., 2006) (Amor et al., 2013) (Imam et al., 2017) (Golomb et al., 2014) (**Fig. 43**).

The main characteristics of the 23 studies included in the meta-analysis are presented in **Table 18**. A total of 3015 patients were included, with a mean BMI ranging from 39.5 ± 9.7 to 57.3 ± 12.6 kg m^2. The mean follow-up period generally varied between 6 and 24 months with only one study reporting a very short follow-up period of 30 days (Mohan et al., 2012) and another study reporting data 3.9 years after the weight loss surgical intervention (Jose et al., 2013) (**TABLE 18**). Studies reported used various surgical techniques (malabsorptive, restrictive, and hybrid procedures) for achieving weight loss, both laparoscopic or by open surgery (**TABLE 18**): the most commonly used technique was Roux-en-Y gastric bypass (RYGB) - performed in more than 50% of cases, followed by sleeve gastrectomy (SG) and adjustable gastric banding (AGB). Other types of gastric bypass (GB) techniques (Fobi Pouch GB, Salmon GB, mini GB) (Navarro-Diaz et al., 2006) (Hou et al., 2013) (Serra et al., 2006) as well as gastroplasty (Chagnac et al., 2003) have also been reported. Biliopancreatic diversion (BPD) was performed in two studies (Palomar et al., 2005) (Jose et al., 2013). With two exceptions (Serpa-Neto et al., 2009) (Friedman et al., 2014), all studies reported obesity related

Comorbidities - the most prevalent being hypertension (46%), diabetes (36%), and CKD (29.4%), followed by dyslipidemia, metabolic syndrome, obstructive sleep apnea, and cardiovascular/cerebrovascular disease. The quality of the observational studies ranged from 5 to 9, with a mean quality score of 6. This corresponds to a moderate overall risk of bias, mostly due to the absence of a control group (only 4 studies included controls drawn from the same community as the exposed cohort) and lack of control for confounders (only 11 studies performed adequate control for both the most important confounder and additional factors, while 6 studies did not include any confounder adjustment). However, selection bias was low (all studies included representative cohorts with certainty of exposure), outcome assessment was adequately performed in 22 out of 23 studies, follow-up was long enough in most studies (18 out of 23 studies reported outcomes within at least 12 months of follow-up), and there was only one study that lost subjects to follow-up.

Overall Study Analysis showed that Serum/Plasma Creatinine Overall, serum/plasma creatinine decreased in 11 (Navarro-Diaz et al., 2006) (Serpa-Neto et al., 2009) (Friedman et al., 2014) (Navaneethan et al., 2010) (Navaneethan and Yehnert, 2009) (Fenske et al., 2013) (Getty et al., 2012) (Jose et al., 2013) (Ruiz-Tovar et al., 2015) (Reid et al., 2014) (Golomb et al., 2014) out of 18 studies (Navarro-Diaz et al., 2006) (Serpa-Neto et al., 2009) (Friedman et al., 2014) (Saliba et al., 2010) (Luaces et al., 2012) (Navaneethan et al., 2010) (Navaneethan and Yehnert, 2009) (Agrawal et al., 2008) (Fenske et al., 2013) (Hou et al., 2013) (Getty et al., 2012) (Jose et al., 2013) (Ruiz-Tovar et al., 2015) (Schuster et al., 2011) (Ngoh et al., 2016) (Reid et al., 2014) (Imam et al., 2017) (Golomb et al., 2014) in which this parameter was assessed, irrespective of BS technique. In six of the remaining studies, baseline creatinine values were in the normal range and creatinine did not differ significantly before versus after surgery (Saliba et al., 2010) (Luaces et al., 2012) (Agrawal et al., 2008) (Hou et al., 2013) (Ngoh et al., 2016) (Imam et al., 2017).

Creatinine was reported to increase only in the study of Schuster et al. (Schuster et al., 2011) in ten patients with baseline moderate kidney impairment, after more than 24 months of follow-up following BS (see CKD subgroup analysis). A decrease in serum/plasma creatinine concentrations was also observed in the overall group after BS when a meta-analysis on 17 out of 23 studies was performed (MD, − 0.08 mg dl−1; 95% CI, − 0.10 to − 0.06 mg dl−1) GFR GFR was assessed in 17 out of 23 studies (Navarro-Diaz et al., 2006) (Serpa-Neto et al., 2009) (Friedman et al., 2014) (Saliba et al., 2010) (Chagnac et al., 2003) (Luaces et al., 2012) (Navaneethan and Yehnert, 2009) (Fenske et al., 2013) (Hou et al., 2013) (Getty et al., 2012) (Ruiz-Tovar et al., 2015) (Ngoh et al., 2016) (Reid et al., 2014) (Serra et al., 2006) (Imam et al., 2017) (Golomb et al., 2014): Friedman et al. (Friedman et al., 2014) and Chagnac et al. (Chagnac et al., 2003) directly measured GFR through plasma iohexol clearance and inulin clearance while the other authors reported estimated GFR (eGFR) by determining: 24-h creatinine clearance (Navarro-Diaz et al., 2006) (Serpa-Neto et al., 2009) (Saliba et al., 2010) (Getty et al., 2012) (Serra et al., 2006), Modification of Diet in Renal Disease (MDRD) equation (Friedman et al., 2014) (Navaneethan and Yehnert, 2009) (Fenske et al., 2013) (Hou et al., 2013) (Getty et al., 2012) (Jose et al., 2013) (Ruiz-Tovar et al., 2015), Cockcroft-Gault and lean body weight-adjusted Cockcroft-Gault (CG-LBW) formulae (Luaces et al., 2012) (Getty et al., 2012) (Ngoh et al., 2016) (Reid et al., 2014), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, respectively (Friedman et al., 2014) (Ngoh

et al., 2016) (Imam et al., 2017). eGFR significantly improved in all studies, 6 months or more after BS, irrespective of the surgical method performed and irrespective of the baseline values (hyperfiltration or impaired eGFR), with three exceptions where eGFR either did not change significantly (Luaces et al., 2012) or it decreased (Reid et al., 2014) (Serra et al., 2006) (Amor et al., 2013) (Imam et al., 2017) (Golomb et al., 2014) (**TABLE 18**)

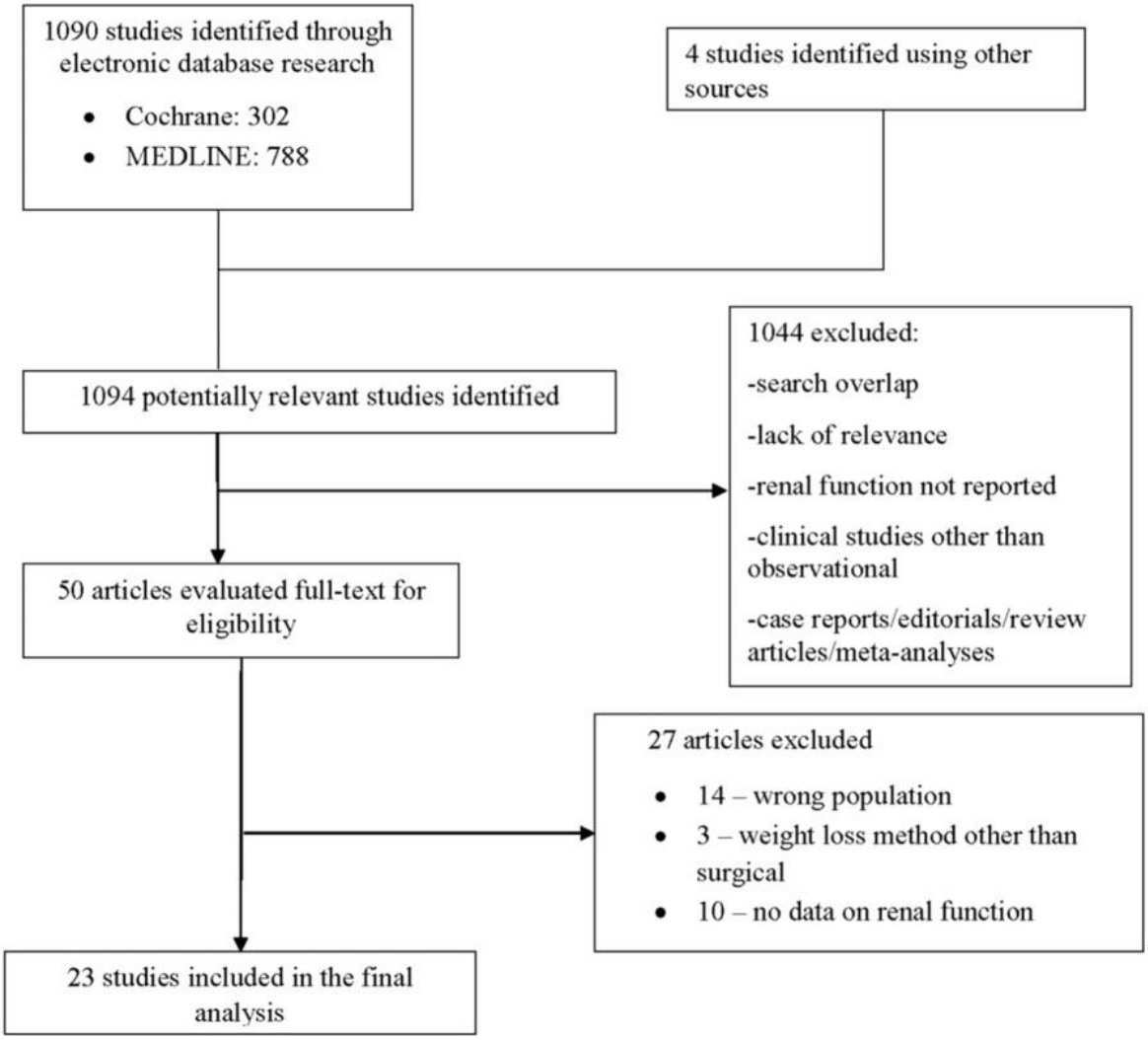

Fig. 43. *Flowchart of the selection process*

TABLE 18 *General characteristics of included studies*

Study	Year	Country	Design	Year of follow-up	Follow-up	Surgical technique	N	Age (years)	Baseline BMI (kg/m^2)	Comorbidities	CKD	Renal parameters assessed	GFR method
Navarro-Diaz et al.	2006	Spain	Prospective controlled study	12.2001-01.2004	24 mo	27-Fobi Pouch GB 34-Vertical banded gastroplasty with distal GB	61 patients (+ 24 controls)	41.10±9.07	53.62±9.65	Hypertension: n=22 T2DM: none	No*	Creatinine eGFR Proteinuria Albuminuria	Creatinine clearance (24-h urine creatinine, plasma creatinine)
Serpa Neto et al.	2009	Brazil	Retrospective	NA	8 mo	RYGB	140	18-60 y	46.17±5.44	NA	Exclusion criteria**	Creatinine eGFR Proteinuria Albuminuria	Creatinine clearance (24-h urine creatinine, plasma creatinine)
Friedman et al.	2014	USA	Prospective	2004-2011	Mean follow-up: 10 mo (296±103 days)	Not mentioned	36	50±11	46±9	NA	Exclusion criteria: serum creatinine >1.3 mg/dl in women and >1.5 mg/dl in men or dialysis	Creatinine Cystatin C mGFR eGFR	mGFR: plasma iohexol clearance eGFR: MDRD, $CKD\text{-}EPI_{creat}$, $CKD\text{-}EPI_{cystC}$, $CKD\text{-}EPI_{creat\text{-}cystC}$
Saliba et al.	2010	USA	Prospective	NA	12 mo	RYGB	35: 19-T2DM 16-non-T2DM	T2DM: 45±9 Non-T2DM:42±10	T2DM: 47±8 Non-T2DM: 48±8	T2DM: n=19 Hypertension: n=19 (12 T2DM, 7 non-T2DM)	No	Creatinine eGFR Proteinuria Albuminuria	Creatinine clearance (24-h urine creatinine, plasma creatinine)
Chagnac et al.	2003	Israel	Prospective	NA	12-17 mo	Gastroplasty	8 patients (+ 9 controls)	36±2	48.0±2.4	Hypertension: n=5 Renal disease: none T2DM: none	Exclusion criteria	mGFR RPF Albumin excretion fraction Fractional clearance of albumin	Inulin clearance
Luaces et al.	2012	Spain	Prospective	NA	12 mo	51-RYGB 10-Tubular gastrectomy	61	41.1±9.8	47.4±5	Hypertension: n=16 (44.1%) Dyslipidaemia: n=21 (35.6%)	n=5/61	Creatinine eGFR	CG-LBW equation

										T2DM: n=6 (10.2%) Impaired kidney function (eGFR<60ml/min): 8.3%			

Navaneethan et al.	2010	USA	Prospective	NA	6 mo	9-RYGB 4-LSG 2-LAGB	15	51.2±14.3	48.8±9.4	T2DM: all Hypertension: 53%	eGFR not assessed	Creatinine Cystatin C UACR	NA
Navaneethan et al.	2009	USA	Retrospective	2002-2005	12-24 mo	Any form	25	51.5±7.4	49.8±7.5	T2DM: 72% Hypertension: 96% Hyperlipidemie: 60% Coronary artery disease: 20% Cerebrovascular disease: 10%	Yes: CKD 3:96% (n=24) CKD2: 4% (n=1)	Creatinine eGFR	MDRD 4-variable formula
Agrawal et al.	2008	USA	Retrospective	12.2002-12.2003	12 mo	RYGB	94	45.6±10.5	49.1±8.0	T2DM: n=32 Metabolic syndrome: n=37 Hypertension: n=37	No Exclusion criteria: Overt renal disease (DN with proteinuria, glomerulonephritis, renal artery stenosis, CKD 3 or more, renal transplant.)	Creatinine UACR	NA
Mohan et al.	2012	USA	Retrospective	01.2006-07.2008	30 days	RYGB	38	41±10.3	46±8	Hypertensive: n=11 T2DM: excluded	NA	UACR	NA
Fenske et al.	2012	UK	Prospective	NA	12 mo	13-LAGB 10-RYGB 11-LSG	34	35-54 y	44.6±0.9	Hypertension: n=19	Exclusion criteria: eGFR<60ml/min/1.73 m^2 n=15: cystatin C>0.8 mg/l (corresponding to CKD 2)	Creatinine Cystatin C eGFR UACR	MDRD
Hou et al.	2013	China	Retrospective	12.2008-10.2010	12 mo	129-mini GB 55-RYGB 32-LSG 14-AGB	233	33.1±9.7	39.5±9.7	T2DM: n=209 Hypertension: n=110	CKD 2: n=39 CKD 3: n=6	Creatinine eGFR	MDRD-4 variable formula
Palomar et al.	2005	Spain	Prospective	NA	12 mo	BPD	35	40.1±11.6	46.9±6.3	Hypertension: 54.5% T2DM: 18%	No	Proteinuria Albuminuria	NA

										Hyperlipidaemia: 21% Obstructive sleep apnoea: 26.7%			
Getty et al.	2012	USA	Prospective	NA	6 mo	RYGB	37	47±11	47.6±6.3	Hypertension: n=27 T2DM: n=14 Metabolic syndrome: n=8 Dyslipidaemia: n=18	Exclusion criteria: CKD 3 or greater	Creatinine eGFR	MDRD Creatinine clearance (24-h urine creatinine, serum creatinine) Cockcroft-Gault equation
Jose et al.	2013	UK	Retrospective	2002-2005	Mean follow-up: 3.9 years (2-6)	BPD	25	42.8±11.3	57.3±12.6	Hypertension: n=9 T2DM: n=5 Dyslipidaemia: n=3 CKD 3 or greater (eGFR≤60 ml/min/m^2): n=7	Yes: CKD 3 or greater: n=7	Creatinine eGFR	MDRD
Ruiz-Tovar et al.	2015	Spain	Prospective	02.2009-05.2013	12 mo	LSG	50	49.2±6.4	48.4±7.7	Hypertension: n=28 T2DM: n=19 Dyslipidaemia: n=20 Obstructive sleep apnoea: n=17	Exclusion criteria: CKD 3 or greater (eGFR<60/ml/min/1.73 m^2)	Creatinine eGFR	MDRD-4
Schuster et al.	2011	USA	Retrospective	01.2003-12.2009	≥24 mo	RYGB	813 (56 with renal impairment: creatinine1.3-1.6 mg-dl n=40, creatinine>1.6 mg/dl-n=16)	All:45±10 Creatinine 1.3-1.6 mg/dl n=40): 54.5±7.5 Creatinine > 1.6 mg/dl (n=16): 49.5±10.69	All: NA Creatinine 1.3-1.6 mg/dl (n=40): 50.7±10.8 Creatinine > 1.6 mg/dl (n=16): 53.1±8.4	Creatinine 1.3-1.6 mg/dl (n=40): -Hypertension: 77.5% -T2DM: 67.5% Creatinine > 1.6 mg/dl (n=16): -Hypertension: 87.5% -T2DM: 68.8%	Yes: Creatinine 1.3-1.6 mg/dL-n=40 Creatinine > 1.6 mg/dl -n=16	Creatinine	NA
Ngoh et al.	2015	Singapore	Retrospective	07.2010-06.2013	12 mo	55-SG 13-GB	68	40.7±10.8	41.9±5.7	T2DM: 28(43%) Hypertension:	Yes: CKD 3- n=7	Creatinine eGFR	CKD-EPI aGFR(

										41(60%) Hyperlipidaemia: 39(57%)	CKD 4 – n=2 CKD 5- n=1		absolute GFR)=eGFR by CKD-EPIxBSA CG-LBW
Reid et al.	2014	USA	Retrospective	2004-2011	12 mo	117-RYGB 41-SG	158	40.8 ±0.9	47.0±0.6	T2DM : 28.5% Hypertension: 43.0%	Exclusion criteria: CKD 3 or greater	Creatinine eGFR UACR	CG formula modified for obese subjects using lean body weight
Serra et al.	2006	Spain	Prospective	12.2001-12.2004	12 mo	30- Fobi pouch GB 40-Salmon GB	70 (+ 24 controls)	41.6±9.1	53.3±9.6	T2DM : 17.4 % Hypertension (high systolic blood pressure): 54.3% Hypercholesterolemia 44.9% Hypertriglyceridemia :21.7%	No	eGFR Proteinuria Albuminuria	Creatinine clearance (24-h urine creatinine, plasma creatinine)
Amor et al.	2013	Spain	Prospective	NA	24 mo	RYGB/SG	255	45.6±10.6	47.7±6	Hypertension: 43.5% CV disease : 5.5% T2DM: 37.64 %	Exclusion criteria: -eGFR <60 ml/min -Renal transplantation at baseline -Glomerulonephritis -Nephrotic range proteinuria	UACR	NA
Imam et al.	2016	USA	Retrospective	01.2008-05.2015	36 mo	RYGB/SG	714 (+714 controls)	58.1±8.46	44.3±6.60	Hypertension: 90.8% T2DM: 65.8%	Yes (CKD 3 and 4: n=714)	Creatinine eGFR	CKD-EPI
Golomb et al.	2014	Israel	Retrospective	11.2011-07.2013	14 mo	LSG	10	Median: 57	41.6 [37-49]	Hypertension: 7 T2DM: 5 Dyslipidaemia: 10	Yes Transplant recipients: all (N=10)	Creatinine Proteinuria eGFR	NA

*Chronic kidney disease patients not included

**Chronic kidney disease mentioned as exclusion criteria

AGB= adjustable gastric banding surgery, BPD=biliopancreatic diversion, BSA=body surface area, CG= Cockcroft-Gault formula, CG-LBW= lean body weight- adjusted Cockcroft-Gault formula, CKD= chronic kidney disease, CKD-EPI= Chronic Kidney Disease Epidemiology Collaboration equation, $CKD\text{-}EPI_{creat}$=CKD-EPI equation using serum creatinine, $CKD\text{-}EPI_{creat\text{-}cyst}$= CKD-EPI equation using both serum creatinine and cystatin C, $CKD\text{-}EPI_{cystC}$= CKD-EPI equation using serum cystatin C, GB=gastric bypass, GFR= glomerular filtration rate (aGFR=absolute GFR, eGFR= estimated GFR, mGFR=measured GFR), LAGB=laparoscopic adjustable gastric banding, LSG=laparoscopic sleeve gastrectomy, MDRD= Modification of Diet in Renal Disease formula, mo=months, N/n=number, NA=not available, RYGB=Roux-en-Y gastric bypass, SG=sleeve gastrectomy, T2DM=type 2 diabetes mellitus, UACR=urine albumin creatinine ratio.

TABLE 19 *Targeted outcomes—summary of results*

Study	Number of participants	Study design	Study duration	Surgical technique	Outcomes	Results
Navarro-Diaz et al.	61 (plus 24 controls)	Prospective controlled study	24 mo	27-Fobi Pouch GB 34-Vertical banded gastroplasty with distal GB	**1.Cr(µmol/l)** (n=61) (mean ± SD)	**81.18±11.60** (baseline)
						72.92±12.72 (12 mo) (vs. baseline: p<0.001)
						73.91±11.37 (24 mo) (vs. 12 mo: NS)
					2.ClCr(ml/min) (n=61) (mean ± SD)	**139.51 ±41.90** (baseline)
						119.59 ±44.24 (12 mo) (vs. baseline: p=0.001)
						117.96 ±33.99 (24 mo) (vs. 12 mo:NS)
					3.Proteinuria (g/24 h) (n=61) Median (25^{th} and 75^{th} percentiles)	**0.14 (0.09 to 0.32)** (baseline)
						0.11 (0.08 to 0.14) (12 mo) (vs. baseline: p<0.004)
						0.11 (0.07 to 0.13) (24 mo) (vs. 12 mo: NS)
					4.Albuminuria (mg/24 h) (n=61) Median (25^{th} and 75^{th} percentiles)	**14.20 (7.95 to 92.2)** (baseline)
						13 (9.25 to 25.25) (12 mo) (vs. baseline: p<0.001)
						12.55 (6.47 to 19.92) (24 mo) (vs. 12 mo: p=0.006)
SerpaNeto et al.	140	Retrospective study	8 mo	RYGB	**1. Cr (mg/dl)** (mean ± SD)	**0.83±0.20** (baseline)
						0.69±0.13 (8 mo) (p<0.0001)
					2.ClCr (ml/min) (mean ± SD)	**148.75±35.27** (baseline)
						113.8±31.7 (8 mo) (p<0.0001)
					3. Proteinuria (g/24 h) (mean ± SD)	**0.15±0.09** (baseline)
						0.11±0.07 (8 mo) (p<0.05)
					4. Albuminuria (mg/24 h) (mean ± SD)	**14.13±12.24** (baseline)
						13.21±8.23 (8 mo) (p<0.05)
Friedman et al.	36	Prospective study	10 mo	BS technique not mentioned	**1.Cr (mg/dl)** (mean ± SD (25^{th}, 75^{th} percentiles))	**0.81±0.24 (0.64,0.93)** (pre-op)
						0.72±0.17 (0.59,0.83) (post-op) (p value NA)
					2.Cystatin C (mg/L) (mean ± SD (25^{th}, 75^{th} percentiles))	**1.12±0.36 (0.91,1.16)** (pre-op)
						1.09±0.29 (0.90,1.23) (post-op) (p value NA)
					3. mGFR (ml/min) (mean ± SD (25^{th}, 75^{th} percentiles))	**117±40 (82,136)** (pre-op)
						100±35 (80,115) (post-op) (p value NA)
					4. mGFR(ml/min/1,73m^2) (mean ± SD (25^{th}, 75^{th} percentiles))	**87±29 (61,105)** (pre-op)
						87±30 (67,107) (post-op) (p value NA)
					4.eGFR by MDRD (ml/min/1.73m^2)	**87±20 (73,104)** (pre-op)
						98±21 (84,111) (post-op) (p value NA)

					(mean ± SD (25th, 75th percentiles))		
					5. eGFR by CKD-EPI$_{creat}$(ml/min/1.73m^2) (mean ± SD (25th, 75th percentiles))	**98±14(88,108)** (pre-op)	
						100±17(92,110) (post-op) (p value NA)	
					6.eGFR by CKD-EPI$_{cystC}$(ml/min/1.73m^2) (mean ± SD (25th, 75th percentiles))	**67±22 (57,78)** (pre-op)	
						68±20 (54,80) (post-op) (p value NA)	
					7. eGFR by CKD-EPI$_{creat-cystC}$(ml/min/1.73m^2) (mean ± SD (25th, 75th percentiles))	**82±20(72,94)** (pre-op)	
						86±19 (74-101) (post-op) (p value NA)	
Saliba et al.	35	Prospective study	12 mo	RYGB	**1. Cr (mg/dl)** (mean ± SD)	***T2DM (n=19)***	***Non-diabetic (n=16)***
						0.64±0.11(baseline)	**0.72±0.12** (baseline)
						0.63±0.09 (6 mo) (vs. baseline: NS)	**0.68±0.15** (6 mo) (vs. baseline: NS)
						0.63±0.09 (12 mo) (vs. baseline: NS)	**0.71±0.11**(12 mo) (vs. baseline: NS)
					2. **ClCr (ml/min)** (mean ± SD)	***T2DM (n=19)***	***Non-diabetic (n=16)***
						155±57 (baseline)	**148±37**(baseline)
						131±29 (12 mo)(vs. baseline: p=0.02)	**117±29** (12 mo)(vs. baseline: p=0.03)
					3. Proteinuria (mg/24h) (mean ± SD)	***T2DM (n=19)***	***Non-diabetic (n=16)***
						181±165 (baseline)	**122±53** (baseline)
						109±68 (6 mo) (vs. baseline: NS)	**80±39** (6 mo) (vs. baseline: NS; vs. 12 mo: p<0.05)
						133±67 (12 mo) (vs. baseline: NS)	**125±58** (12 mo) (vs. baseline: NS)
					4.Albuminuria (mg/24 h) (mean ± SD)	***T2DM (n=19)***	***Non-diabetic (n=16)***
						26±50 (baseline)	**10±6** (baseline)
						18±33 (6 mo) (vs. baseline: NS)	**5±2** (6 mo) (vs. baseline: p<0.05; vs. 12 mo: p<0.05)
						15±29 (12 mo) (vs. baseline: NS)	**14±20** (12 mo) (vs. baseline: NS)
Chagnac et al	8 (+ 9 controls)	Prospective controlled study	12-17 mo	Gastroplasty	**1.mGFR (ml/min)** (N=8) (mean ± SEM)	**145±14** (pre-op)	
						110±7 (post-op) (p=0.01)	
					2.RPF (ml/min) (N=8) (mean ± SEM)	**803±39** (pre-op)	
						698±42 (post-op) (p<0.02)	
					4. Albumin excretion rate (ug/min) (N=8) (median (range))	**16 (4, 152)** (pre-op)	
						5 (3, 37) (post-op) (p<0.01)	
					5. Fractional clearance of albumin (N=8) (median (range))	**3.2x10^{-6} (1.1x 10^{-6} , 23x10^{-6})** (pre-op)	
						1.2 x10^{-6} (0.5x10^{-6} , 6.8 x10^{-6}) (post-op) (p<0.02)	

Luaces et al.	61	Prospective study	12 months	51-RYGB 10-Tubular gastrectomy	**1.Cr (mg/dl)** (mean ± SD)	**0.71±0.15** (pre-op)				
						0.69±0.11 (post-op) (p=0.63)				
					2.eGFR(ml/min) (mean ± SD)	**92.7±25.1** (pre-op)				
						95.7±23.4 (post-op) (p=0.28)				
Navaneethan et al.	15	Prospective study of T2DM cohort	6 mo	9-RYGB 4-LSG 2-LAGB	**1.Cr (mg/dl)** (mean ± SD)	***RYGB(n=9)***		***Other surgery (n=6)***		
						0.75±0.13 (pre-op)		**0.81±0.30** (pre-op)		
						0.65±0.07 (post-op) (p=0.007)		**0.62±0.13** (post-op) (p=0.02)		
					2.Cystatin C (ng/ml) (mean ± SD)	***RYGB(n=9)***		***Other surgery (n=6)***		
						1426.10 ± 389.29 (pre-op)		**1184.96±192.91**(pre-op)		
						1360.72± 209.03 (post-op) (p=0.32)		**1093.33±194.12** (post-op) (p=0.17)		
					2. UACR (mg/g Cr) (median (25 percentile-75 percentile))	***RYGB(n=9)***		***Other surgery (n=6)***		
						36 (7-94) (pre-op)		**8 (5.7-25.2)** (pre-op)		
						27 (5.5-42.5) (postop) (p=0.01)		**13.5 (4.7-56.2)** (post-op) (p=0.11)		
						Patients with pre-existing microalbuminuria (n=7-RYGB or other surgery)				
						65 (61-126) (pre-op)				
						39 (27-56) (post-op) (p=0.04)				
Navaneethan et al.	25	Retrospective study of cohort with stage 3 CKD	12-24 mo	Any form of BS	**1. Cr (mg/dl)** (mean ± SD)	**1.4±0.4** (baseline)				
						1.2±0.4 (6 mo) (vs. baseline: p<0.001)				
						1.1±0.3 (12 mo) (vs. baseline: p<0.001)				
					2. eGFR (ml/min/1.73m^2) (mean ± SD)	**47.9 ± 10.2** (baseline)				
						56.6±10.4 (6 mo) (vs. baseline: p<0.001)				
						61.6±16.7 (12 mo) (vs. baseline: p<0.001)				
Agrawal et al.	94	Retrospective study	12 mo	RYGB	**1.Cr (mg/dl)** (mean ± SD)	***Whole group***	***Microalbuminuria patients (30-300 mg/g) (n=21)***	***Obesity alone (n=25)***	***Metabolic syndrome (n=37)***	***Diabetes (n=32)***
						0.9±0.2 (baseline)	-	**0.9±0.2** (baseline)	**0.9±0.2**(baseline)	**1.0±0.3**(baseline)
						0.8±0.2 (12 mo) (p=0.128)	-	**0.8±0.2**(12 mo) (p=0.900)	**0.9±0.2**(12 mo) (p=0.624)	**0.9±0.2**(12 mo) (p=0.105)
					2.UACR (mg/g Cr) (median withinterquartile range)	***Whole group***	***Microalbuminuria patients (30-300 mg/g) (n=21)***	***Obesity alone (n=25)***	***Metabolic syndrome (n=37)***	***Diabetes (n=32)***
						9.5(5-28)	**66.0 (39-106)**	**6.5 (4-13)**	**8.0 (5-16)**	**16.5 (5-67)**

						(baseline)	(baseline)	(baseline)	(baseline)	(baseline)
						5.5(3-10)(12 mo) (p<0.001)	**13.0 (8-21)** (12 mo) (p<0.0001)	**4.5 (3-8)** (12 mo) (p=0.270)	**6.0 (3-13)** (12 mo) (p=0.012)	**6.0 (4-11)** (12 mo) (p=0.001)
					3.Microalbuminuria (%)	***Whole group***	***Microalbuminuria patients (30-300 mg/g) (n=21)***	***Obesity alone (n=25)***	***Metabolic syndrome (n=37)***	***Diabetes (n=32)***
						22.2 (baseline)	-	**10** (baseline)	**18.2** (baseline)	**35.7** (baseline)
						6.2 (12 mo) (p=0.004)	-	**5** (12 mo) (p=0.456)	**6.1** (12 mo) (p=0.289)	**7.1** (12 mo) (p=0.008)
Mohan et al.	38	Retrospective study	30 days	RYGB	**UACR (mg/g Cr)** (mean ± SD)	***UACR> 20 mg/g (n=15)***			***UACr ≤ 20 mg/g (n=23)***	
						80.5±90 (pre-op)			**5.8±3.4** (pre-op)	
						18±.8.1 (post-op) (p=0.01)			**8.1±9.8** (post-op) (p=0.2934)	
Fenske et al.	34	Prospective study	12 mo	13-LAGB 10-RYGB 11-LSG	**1.Cr (µmol/l)** (mean±SEM)	***Overall (n=34)***	***LAGB (n=13)***	***RYGB (n=10)***	***LSG (n=11)***	***Cystatin C >0.8 mg/l (n=15) (corresponding to eGFR 60-89 ml/min/1.73m^2)***
						74.4±2.2 (baseline)	**75.0±2.0** (baseline)	**81.1±2.4** (baseline)	**69.4±1.2** (baseline)	**72.2±1.5** (baseline)
						68.3±1.8 (1 mo)(vs. baseline: NS)	**67.6 ±1.3** (1mo) (vs. baseline: NS)	**71.4±1.5** (1mo) (vs. baseline: NS)	**67.0±1.1** (1mo) (vs. baseline: NS)	**62.9±1.3 (1 mo)** (vs. baseline: NS)
						60.6±1.1 (12 mo) (vs. baseline: p<0.001)	**61.1±1.0** (12 mo) (vs. baseline: p<0.001)	**64.9±0.9** (12 mo) (vs. baseline: p<0.001)	**57.3±.6** (12 mo) (vs, baseline: p<0.01)	**58.4±1.2** (12 mo) (vs, baseline: p<0.01)
					2.Cystatin C (mg/l) (mean±SEM)	***Overall (n=34)***	***LAGB (n=13)***	***RYGB (n=10)***	***LSG (n=11)***	***Cystatin C >0.8 mg/l (n=15) (corresponding to eGFR 60-89 ml/min/1.73m^2)***
						0.76±0.004 (baseline)	**0.75±.004** (baseline)	**0.76±.0001** (baseline)	**0.78±0.0005** (baseline)	**0.94±0.0005** (baseline)
						0.76±0.003	**0.71±.0003**	**0.76±.0001**	**0.84±0.0006**	**0.89±0.0004** (1

						(1 mo) (vs. baseline: NS)	(1mo) (vs. baseline: NS)	(1mo) (vs. baseline: NS)	(1mo) (vs. baseline: NS)	mo) (vs. baseline: NS)
						0.79±0.005 (12 mo) (vs. baseline: NS)	**0.80±.0004** (12 mo) (vs. baseline: NS)	**0.74±0001** (12 mo) (vs. baseline: NS)	**0.81±0.0004** (12 mo) (vs. baseline: NS)	**0.72±0.0004** (12 mo) (vs. baseline: p<0.01)
					3.eGFR(ml/min/1.73m^2) (mean±SEM)	***Overall (n=34)***	***LAGB (n=13)***	***RYGB (n=10)***	***LSG (n=11)***	***Cystatin C >0.8 mg/l (n=15) (corresponding to eGFR 60-89 ml/min/1.73m^2)***
						67.4±1.0 (baseline)	**77.1±1.5** (baseline)	**86.3±1.5** (baseline)	**44.1±1.0** (baseline)	**78.2±2.8** (baseline)
						86.1±2.1 (1 mo) (vs. baseline:NS)	**87.8±2.0** (1mo) (vs. baseline:NS)	**88.3±1.8** (1mo) (vs. baseline: NS)	**82.8±2.3** (1mo) (vs. baseline: NS)	**86.9±0.9** (1 mo) (vs. baseline: NS)
						85.0±2.0 (12 mo) (vs. baseline: p<0.001)	**85.4±2.1** (12 mo) (vs. baseline:NS)	**90.0±2.3** (12 mo) (vs. baseline:NS)	**81.4±1.8** (12 mo) (vs. baseline: p<0.001)	**86.7±0.9** (12 mo)(vs. baseline: p<0.01)
					4. UACR (mg/mmol Cr) (mean±SEM)	***Overall (n=34)***	***LAGB (n=13)***	***RYGB (n=10)***	***LSG (n=11)***	
						4.1±0.3 (baseline)	**4.8±1.1** (baseline)	**3.1±.3**(baseline)	**2.5±0.1** (baseline)	
						1.3±0.1 (1mo) (vs. baseline: NS)	**1.1±.13** (1mo) (vs. baseline: NS)	**0.5±0.1** (1mo) (vs. baseline: NS)	**2.5±0.1** (1mo) (vs. baseline: NS)	
						0.9±0.04(12 mo) (vs. baseline: p<0.01)	**0.9±.1** (12 mo) (vs. baseline: NS)	**1.0±0.1** (12 mo) (vs. baseline: NS)	**0.4±.04** (12 mo) (vs. baseline: p<0.001)	
Hou et al.	233	Retrospective study	12 mo	129-mini GB 55-RYGB 32-LSG	**1.Cr (mg/dl)** (mean ±SD)	***Hyperfiltration (eGFR>125 ml/min) (n=61)***	***Normal eGFR (eGFR=125-90 ml/min) (n=127)***	***CKD stage 2 (eGFR=89-60 ml/min) (n=39)***	***CKD stage 3 (eGFR=59-30 ml/min) (n=6)***	
						0.58±0.1	**0.75±0.1**	**0.9±0.1**	**1.4±0.2** (baseline)	

				14-AGB		(baseline)	(baseline)	(baseline)	
						0.61±0.13 (12 mo) (NS)	**0.78±0.7** (12 mo) (NS)	**0.8±0.1** (12 mo) (NS)	**1.2±0.3** (12 mo) (NS)
					2.eGFR(ml/min) (mean ±SD)	***Hyperfiltration (eGFR>125 ml/min) (n=61)***	***Normal eGFR (eGFR=125-90 ml/min) (n=127)***	***CKD stage 2 (eGFR=89-60 ml/min) (n=39)***	***CKD stage 3 (eGFR=59-30 ml/min) (n=6)***
						146.4±17.1 (baseline)	**105.7±9.6** (baseline)	**76.8±16.7** (baseline)	**49.5±6.6** (baseline)
						133.9±25.7 (12 mo) (p<0.05)	**114.2±22.2** (12 mo) (NS)	**93.3±20.4** (12 mo) (p<0.05)	**66.8±19.3** (12 mo) (p<0.05)
Palomar et al.	35	Prospective study	12 mo	BPD	**1. Proteinuria (mg/24h)** (mean ±SD)	**735** (baseline)			
						<200 (12 mo) (p<0.01)			
					2. Albuminuria (mg/24h) (mean ±SD)	**21.37**(baseline)			
						11 (12 mo) (p<0.01)			
Getty et al.	37	Prospective study	6 mo	RYGB	**1.Cr (mg/dl)** (mean ±SD)	**0.83 ±0.21**(baseline)			
						0.72±0.16 (6 mo) (p<0.001)			
					2. eGFR by MDRD (ml/min/1.73m^2) (mean ±SD)	**91.6±29.7**(baseline)			
						104.9±23.5 (6 mo) (p<0.01)			
					3. eGFR by CG (ml/min) (mean ±SD)	**197.1±88** (baseline)			
						158.04±54 (6 mo) (p<0.001)			
					4. ClCr (ml/min) (mean ±SD)	**136.5±53** (baseline)			
						139.4±52 (6 mo)(NS)			
Jose et al.	25	Retrospective study	3.9 y (2-6)	BPD	**1.Cr (µmol/l)** (mean ±SD)	**86.71±15.57** (baseline)			
						70.48±14.28 (end) (p<0.001)			
					2.eGFR (ml/min/m^2) (mean)	**71.0** (baseline)			
						81.6 (end) (p=0.048)			
					3. Change ineGFR (ml/min/m^2) (mean ±SD)	***Whole-group (n=25)***	***GFR>60 ml/min/m^2 (n=18)***	***GFR≤60 ml/min/m^2 (n=7)***	
						10.6±15.5	**3.8±10.5**	**28±10.5 (p<0.001)**	
Ruiz-Tovar et	50	Prospective	12 mo	LSG	**1.Cr (mg/dl)** (mean ±SD)	**0.89±0.17** (pre-op)			
						0.71±0.14 (post-op) (p=0.01)			

al.		study			**2.eGFR (ml/min/m^2)** (mean ±SD)	**62.5±14.6** (pre-op)		
						77.6±15.2 (post-op)(p<0.001)		
Schuster et al.	813	Retrospective study	≥ 24 months	RYGB	**1. Cr (mg/dl)** (mean ±SEM)	***Mild renal impairment (Cr-1.3-1.6mg/dl) (statistical significance NA)***		***Moderate renal impairment (Cr>1.6mg/dl) (statistical significance NA)***
						1.42±0.14 (baseline) (n=40)		**2.19±0.19** (baseline) (n=16)
						1.21±0.41 (6 mo) (n=40)		**2.24±0.42** (6 mo) (n=16)
						1.12±0.5 (12 mo) (n=24)		**2.04±0.38** (12 mo) (n=13)
						1.2±0.6 (>24 mo) (n=21)		**2.67±0.69** (>24 mo) (n=10)
Ngoh et al.	68	Retrospective study	12 mo	55-SG 13-GB	**1.Cr (µmol/l)** (mean± 1SD)	***Whole-group (n=68)***	***eGFR>90mL/min/1.73m² (n=58)***	***eGFR <90mL/min/1.73m² (n=10)***
						65±27 (baseline)	**58±12** (baseline)	**110±27** (baseline)
						64±25 (12 mo)(p=0.405)	**59±12** (12 mo) (p=0.422)	**96±25** (12 mo) (p value NA)
					2.eGFR by CKD-EPI (mL/min/1.73m^2) (mean± 1SD/median withinterquartile range)	***Whole-group (n=68)***	***eGFR>90mL/min/1.73m² (n=58)***	***eGFR <90mL/min/1.73m² (n=10)***
						108±19 (baseline)	**115±12** (baseline)	**69 (44-86)**
						102±19 (12 mo) (p=0.930)	**113±13** (12 mo) (p=0.082)	**79 (59-100)** (p value NA)
					3. **eGFR by CG-LBW (ml/min)** (mean± 1SD/median withinterquartile range)	***Whole-group (n=68)***	***eGFR>90mL/min/1.73m² (n=58)***	***eGFR <90mL/min/1.73m² (n=10)***
						172±65 (baseline)	**204±22** (baseline)	**104** (71-146)
						107±47 (12 mo) (p<0.001)	**122±19** (12 mo) (p<0.001)	**121 (94-165)** (p value NA)
					4.**a GFR(ml/min)** (=eGFRxbody surface area) (mean± 1SD/median withinterquartile range)	***Whole-group (n=68)***	***eGFR>90mL/min/1.73m² (n=58)***	***eGFR <90mL/min/1.73m² (n=10)***
						135±31 (baseline)	**143±22** (baseline)	**89 (59-117)**
						117±25 (12 mo) (p<0.001)	**122±19** (12 mo) (p<0.001)	**86 (62-113)** (p value NA)
Reid et al.	158	Retrospective study	12 mo	117-RYGB 41-SG	**1.Cr (mg/dl)** (mean±SE)	**0.72±0.1 (range 0.4-1.2)** (baseline)		
						0.67±0.1 (range 0.4-1.1) (12 mo) (p<0.0001)		
					2. eGFR (ml/min) (mean±SE)	***Overall group (n=158)***	***Hyperfiltration subgroup (eGFR>140 ml/min) (n=13)***	***Overall group after exclusion of hyperfiltration patients***

						97.5±2.2 (baseline)	**164.0±4.7** (baseline)	**91.6±1.6** (baseline)
						87.1±2.0 (12 mo) (p<0.0001)	**137.6±8.5** (12 mo)(p=0.0015)	**82.6±1.6** (12 mo) (p<0.0001)
					3.UACR (mg/g Cr) (mean±SE)	**21.5±3.2** (baseline)		
						10.2 ±1.2 (12 mo) (p<0.0001)		
Serra et al.	70 (+ 24 controls)	Prospective study	12 mo	30- Fobi pouch GB 40- Salmon GB	1. **ClCr (ml/min)Gom** (n=70) (median with interquartile range)	**125 (110-170)**(baseline)		
						112 (89-143)(12 mo)(p<0.001)		
					2.**Proteinuria (g/24h)** (n=70) (median with interquartile range)	**0.14 (0.09-0.32)**(baseline)		
						0.11(0.08-0.14)(12 mo) (p<0.001)		
					3.**Albuminuria (mg/24h)** (n=70) (median with interquartile range)	**14.8 (8.0-61)**(baseline)		
						12.8 (9.2-24.6)(12 mo) (p<0.001)		
Amor et al.	255	Prospective study	24 mo	RYGB/SG	**1. UACR (mg/g Cr)** (mean ±SD)	***Whole cohort (n=255)***	***T2DM subgroup (n=96)***	
						55.0 ± 139.1 (baseline)	**85.7±171** (baseline)	
						31.55 ± 108.5 (12 mo)(vs. baseline: p<0.001)	**42.2±142.8** (12 mo) (vs. baseline:p<0.005)	
						30.35 ± 153.7 (24 mo)(vs. 12 mo: p = 0.710)	**44.4±227.7** (24 mo) (vs. 12 mo: p=0.862)	
Imam et al.	714 (+714 controls)	Retrospective study	36mo	RYGB/SG	**1. Cr (mg/dl)** (mean ± SD)	**1.4±0.50 (baseline)**		
						NA		
					2.eGFR by CKD-EPI (mL/min/1.73m^2) (mean ±SD)	**48.2±10.12** (baseline)		
						58.9 (36 mo) (vs baseline p= 0.01)		

Golomb et al.	10	Retrospective study	14 mo	LSG	**1. Cr (mg/dl)** (median with interquartile range)	**1.44** [0.78-1.88] (baseline) **1.25 [NA]** (12 mo) (vs baseline p=0.04)
					2. ClCr (ml/min) (n=5)	**98 [NA]** (preoperative) **76 [NA]** (postoperative)
					3. Proteinuria (mg/24 h)	**391 [140-1197]** (baseline) **207 [95-336]** (postoperatively) (p=0.05 vs. baseline)

AGB= adjustable gastric banding surgery, BS=bariatric surgery, BPD=biliopancreatic diversion, CG= Cockcroft-Gault formula, CG-LBW= lean body weight- adjusted CG formula, CKD= chronic kidney disease, CKD-EPI= Chronic Kidney Disease Epidemiology Collaboration equation, $CKD\text{-}EPI_{creat}$=CKD-EPI equation using serum creatinine, $CKD\text{-}EPI_{creat\text{-}cyst}$= CKD-EPI equation using both serum creatinine and cystatin C, $CKD\text{-}EPI_{cystC}$= CKD-EPI equation using serum cystatin C, ClCr= 24-hours creatinine clearance, Cr=creatinine, GB=gastric bypass, GFR= glomerular filtration rate (aGFR=absolute GFR, eGFR= estimated GFR, mGFR=measured GFR), LAGB=laparoscopic adjustable gastric banding, LSG=laparoscopic sleeve gastrectomy, MDRD= Modification of Diet in Renal Disease formula, mo=months, n=number, NA=not available, NS=non-significant, post-op=post-operatory, pre-op=pre-operatory, RPF=renal plasma flow, RYGB=Roux-en-Y gastric bypass, SD=standard deviation, SE=standard error, SEM=standard error of mean, SG=sleeve gastrectomy, T2DM=type 2 diabetes mellitus, UACR=urine albumin creatinine ratio.

TABLE 20 *GFR evolution after surgery by method of estimation*

Study or Subgroup	After bariatric surgery Mean (Median)	SD (Interquartile range)	Total	Control-Before surgery Mean (Median)	SD (Interquartile range)	Total
Creatinine clearance (ml/min) - using a 24-hour urine sample						
Navarro-Diaz 2006[14]	117.96	33.99	61	139.51	41.90	61
Serpa-Neto 2009[15]	113.8	31.7	140	148.75	35.27	140
Saliba 2010[17]	131	29	19	155	57	19
Getty 2012[27]	139.4	52	37	136.5	53	37
Serra 2006[33]	112	89-143	70	125	110-170	70
MDRD(ml/min/1.73m^2)						
Friedman 2014[16]	98	21	36	87	20	36
Navaneethan 2009[21]	61.6	16.7	25	47.9	10.2	25
Fenske 2012[24]	85	2	34	67.4	1	34
Getty 2012[27]	104.9	23.5	37	91.6	29.7	37
Jose 2013[28]	81.6		25	71		25
Ruiz-Tovar 2015[29]	77.6	15.2	50	62.5	14.6	50
Hou 2013[25]						
Hyperfiltration patients	133.9	25.7	61	146.4	17.1	61
Normal eGFR	114.2	22.2	61	105.7	9.6	61
CG/CG-LBW equation						
Luaces 2012[19]	95.7	23.4	61	92.7	25.1	61
Getty 2012[27]	158.04	54	37	197.1	88	37
Reid 2014[32]	87.1	2	158	97.5	2.2	158
Ngoh 2015[31]	107	47	68	172	65	68
CKD-EPI						
Ngoh 2015[31]	102	19	68	108	19	68
Friedman 2014[16]	100	17	36	98	14	36
Imam 2016[35]	58.9		714	48.2	10.12	714

CG= Cockcroft-Gault formula, CG-LBW= lean body weight- adjusted Cockcroft-Gault formula, CKD-EPI= Chronic Kidney Disease Epidemiology Collaboration equation, GFR=glomerular filtration rate, MDRD= Modification of Diet in Renal Disease formula

Otherwise, GFR was significantly reduced in hyperfiltration patients (Navarro-Diaz et al., 2006) - (Chagnac et al., 2003), (Hou et al., 2013) (Getty et al., 2012) (Ngoh et al., 2016) - (Serra et al., 2006) and at the same time significantly increased in patients with eGFR < 90 ml min−1 ((Navaneethan and Yehnert, 2009) (Fenske et al., 2013) (Hou et al., 2013) (Jose et al., 2013) (Ruiz-Tovar et al., 2015) (Imam et al., 2017) (see CKD subgroup analysis below). When overall meta-analysis was performed on 13 out of 23 studies, we noticed a lack of significant differences for eGFR changes after surgery in the overall group (MD, − 3.07 ml min; 95% CI, − 13.89 to + 7.74 ml min−1). However, for the hyperfiltration group, a 31.87-ml min−1 reduction was observed (95% CI, 38.15 to 25.59 ml min−1).

Proteinuria/Albuminuria Albuminuria and/or proteinuria were reported by 13 out of 23 studies (Navarro-Diaz et al., 2006) (Serpa-Neto et al., 2009) (Saliba et al., 2010) (Chagnac et al., 2003) (Navaneethan et al., 2010) (Agrawal et al., 2008) - (Fenske et al., 2013), (Palomar et al., 2005) (Reid et al., 2014) - (Imam et al., 2017); it significantly improved in all but one exception (Saliba et al., 2010), where average baseline albuminuria was normal. Reductions in albuminuria and/or proteinuria were seen after various surgical techniques, including RYGB (Navarro-Diaz et al., 2006) (Serpa-Neto et al., 2009) (Navaneethan et al., 2010) (Agrawal et al., 2008) (Mohan et al., 2012) (Reid et al., 2014) - (Amor et al., 2013), SG (Fenske et al., 2013) (Reid et al., 2014) (Amor et al., 2013) (Golomb et al., 2014), BPD (Palomar et al., 2005), or gastroplasty (Chagnac et al., 2003) (***TABLE 19***). An overall reduction in albuminuria and/or proteinuria was seen when meta-analysis on 2 out of 23 studies was performed. After adjustment for confounders, improvement in albuminuria/proteinuria was both weight- and blood pressure-independent in most studies (Navaneethan et al., 2010) (Agrawal et al., 2008) (Mohan et al., 2012) (Reid et al., 2014). Only Amor et al. (Amor et al., 2013) reported normalization of albumin excretion as being associated with a larger decrease in waist circumference and BMI in type 2 DM patients. Predictors of albuminuria reduction in the studies included in this review are baseline albuminuria, insulin sensitivity/change in HbA1c levels, and adiponectin (Navaneethan et al., 2010) (Agrawal et al., 2008) (Mohan et al., 2012) (Amor et al., 2013).

Nephrolithiasis

The occurrence of nephrolithiasis was assessed only by Palomar et al. (Palomar, et al. 2005) which found a decrease in calcium, phosphate, uric acid, and citrate urinary excretion and a tendency toward an increase in oxalate urinary excretion but no increase in renal stone production (Palomar et al., 2005) (**TABLE 21**). Need for RRT The need for RRT was not reported in the included studies with the exception of Palomar et al. (Palomar et al., 2005) that did not find any cases of kidney failure after BPD.

Regarding the Transplantation Subgroup Analysis, only the study of Golomb et al. (Golomb et al., 2014) included kidney transplant patients (ten patients with normal eGFR after transplantation). Creatinine values and proteinuria significantly decreased 12 months after BS in the ten patients included, while in five out of ten subjects, their median creatinine clearance had the tendency to decrease. The incidence of nephrolithiasis and the need for RRT were not assessed/reported.

Thus, the present systematic review shows an improvement in renal parameters after BS: (1) although creatinine did not change in some studies after surgery, it significantly decreased in most of them, (2) eGFR profile improved in almost all studies (decreased in patients with hyperfiltration and increased in patients with reduced eGFR), and (3) proteinuria/albuminuria decreased significantly in all studies with one exception (Saliba et al., 2010). Obese patients initially develop kidney hyperfiltration with increased eGFR. As kidney structural damage occurs, eGFR progressively declines to CKD values. BS tends to stabilize eGFR across different categories of kidney function in bariatric patients, with reduction toward the normal range in hyperfiltration and increase toward the normal range in CKD, respectively. The apparent deterioration of renal function in some studies (Reid et al., 2014) - (Golomb et al., 2014) was accompanied by improvement of the other renal parameters

TABLE 21 *Incidence of nephrolithiasis and need for renal replacement therapy among the included patients*

Author	N	Surgical technique	Lithiasis after BS (number of patients)	Type of calculi	Need for RRT
Navarro-Diaz 2006[14]	61	27-Fobi Pouch GB 34-Vertical banded gastroplasty with distal GB	Not reported	Not reported	Not reported
Serpa Neto 2009[15]	140	RYGB	Not reported	Not reported	Not reported
Friedman 2014[16]	36	Not mentioned	Not reported	Not reported	Not reported
Saliba 2010[17]	35	RYGB	Not reported	Not reported	Not reported
Chagnac 2003[18]	8	Gastroplasty	Not reported	Not reported	Not reported
Luaces 2012[19]	61	RYGB/Tubular gastrectomy	Not reported	Not reported	Not reported
Navaneethan 2010[20]	15	RYGB/LSG/LAGB	Not reported	Not reported	Not reported
Navaneethan 2009[21]	25	Any form	Not reported	Not reported	Not reported
Agrawal 2008[22]	94	LRYGB	Not reported	Not reported	Not reported
Mohan 2012[23]	38	RYGB	Not reported	Not reported	Not reported
Fenske 2013[24]	34	LAGB/RYGB/LSG	Not reported	Not reported	Not reported
Hou 2013[25]	233	LGB/RYGB/AGB/SG	Not reported	Not reported	Not reported
Palomar 2005[26]	35	BPD	No (N=0)	Urinary study for lithiasis decrease in Ca, P, UA and Citrate excretion	No cases of kidney failure
Getty 2012[27]	37	RYGB	Not reported	Not reported	Not reported
Jose 2013[28]	25	BPD	Not reported	Not reported	Not reported
Ruiz-Tovar 2015[29]	50	LSG	Not reported	Not reported	Not reported
Schuster 2011[30]	813	RYGB/RYGB	Not reported	Not reported	Not reported
Ngoh 2015[31]	68	SG/GB	Not reported	Not reported	Not reported
Reid 2014[32]	158	RYGB/SG	Not reported	Not reported	Not reported
Serra 2006[33]	70	Fobi pouch GB/Salmon GB	Not reported	Not reported	Not reported
Amor 2013[34]	255	RYGB/SG	Not reported	Not reported	Not reported
Imam 2016[35]	714	RYGB/SG	Not reported	Not reported	Not reported

Golomb 2014[36]	10	LSG	Not reported	Not reported	Not reported

AGB= adjustable gastric banding surgery, GB=gastric bypas, BPD=biliopancreatic diversion, BS=bariatric surgery, Ca=calcium, LAGB=laparoscopic adjustable gastric banding, LSG=laparoscopic sleeve gastrectomy, N=number, P=phosphate, RRT=renal replacement therapy, RYGB=Roux-en-Y gastric bypass, SG=sleeve gastrectomy, UA=uric acid,

(decrease in creatinine levels and albuminuria) and rather reflects a weight loss-induced reduction of ultrafiltration and not a real kidney injury (possible confounders include ethnic minorities, cohorts composed mainly of females, and unknown differences in duration of comorbidities) (Reid et al., 2014) - (Golomb et al., 2014).

Also, the apparent increase in creatinine values in moderate CKD patients in the study of Schuster et al. (Schuster et al., 2011) rather reflects the natural course of the disease in patients with a more severe baseline kidney disease stage, especially as creatinine tended to decrease in mild CKD patients in the same study (Schuster et al., 2011). With regard to these discrepancies, one must take into account that creatinine is only a crude indicator of eGFR, due to its variable tubular secretion and reabsorption especially in kidney disease (Perrone, Madias and Levey, 1992); also, it is difficult to accurately estimate GFR using formulae in obese patients, due to body size confounders. Unfortunately, equations that properly account for obesity have not yet been established. MDRD significantly underestimates, while Cockcroft-Gault highly overestimates GFR when compared with 24-h creatinine clearance (Getty et al., 2012). However, 24-h creatinine clearance determination is burdensome, may be hampered by 24-h urinary output collection and also exceeds true GFR due to tubular secretion (Getty et al., 2012). Also, measured GFR does not seem to significantly correlate to body surface area or weight in obese individuals (Friedman et al., 2014). According to Friedman et al. (Friedman et al., 2014), the best predictor in obese patients is the CKD-EPI-derived equation that uses both serum creatinine and cystatin C, which estimates GFR within 30% of its value more than 80% of the time (Friedman et al., 2014).

eGFR improvement did not correlate with BMI reduction/ weight loss per se (Friedman et al., 2014) (Luaces et al., 2012) (Reid et al., 2014) but was rather a consequence of lower blood pressures values (Serpa-Neto et al., 2009) (Chagnac et al., 2003) and improved metabolic parameters (e.g., glycemia) (Chagnac et al., 2003). On the contrary, improvement in albuminuria was weight independent and blood pressure independent in most studies in which adjustment for BMI and blood pressure as possible confounders was performed (Navaneethan et al., 2010) (Agrawal et al., 2008) (Mohan et al., 2012) (Reid et al., 2014). Reduction of albuminuria seems to be influenced by baseline albuminuria, insulin sensitivity/change in HbA1c levels, and adiponectin (Navaneethan et al., 2010) (Agrawal et al., 2008) (Mohan et al., 2012) (Amor et al., 2013). Of particular interest are the beneficial effects of BS in patients with overt CKD. The resolution of comorbidities such as hypertension, metabolic dysfunctions, and sleep apnea as a result of BS is the main contributor to renal function enhancement (Buchwald et al., 2004), (Piche et al., 2015).

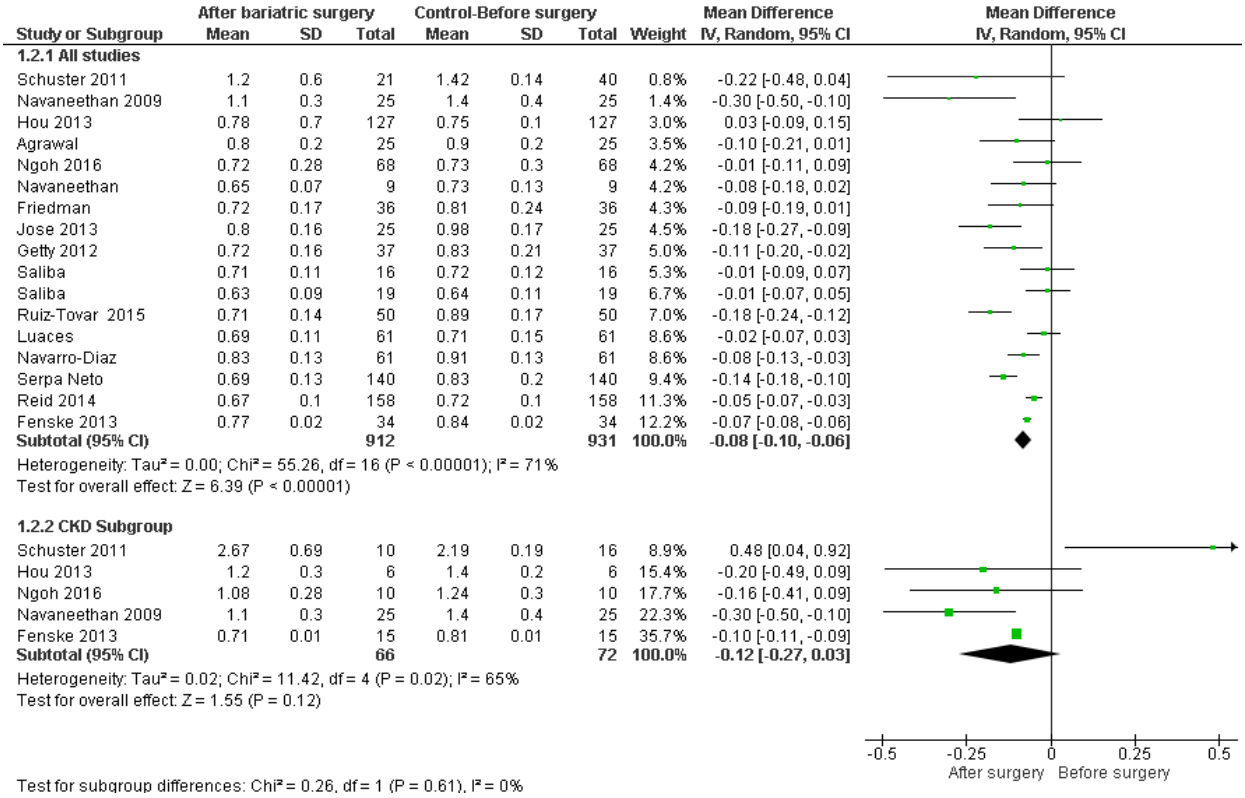

Fig. 44. *Forest plot comparing creatinine values before surgery versus after surgery*

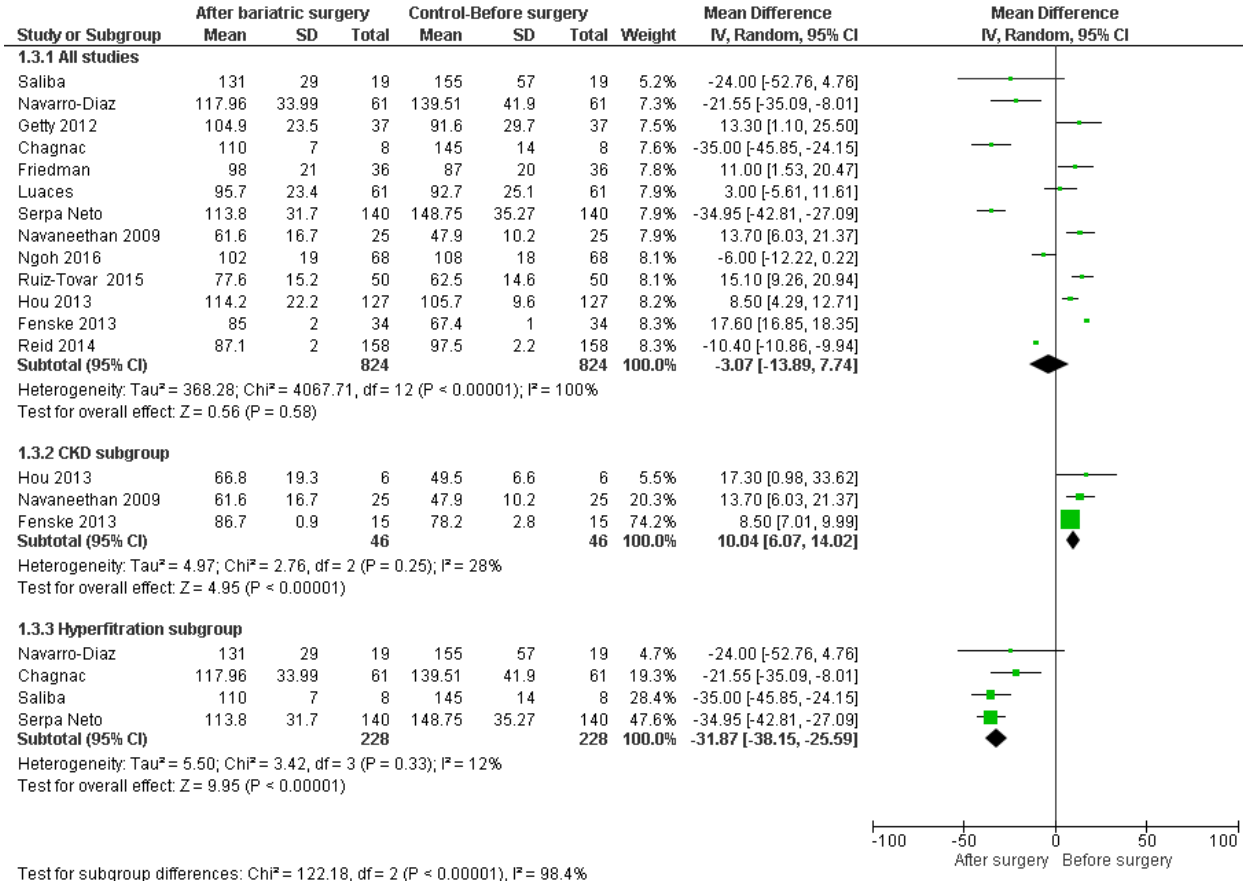

Fig. 45. *Forest plot comparing GFR before surgery versus after surgery. GFR, glomerular filtration rate*

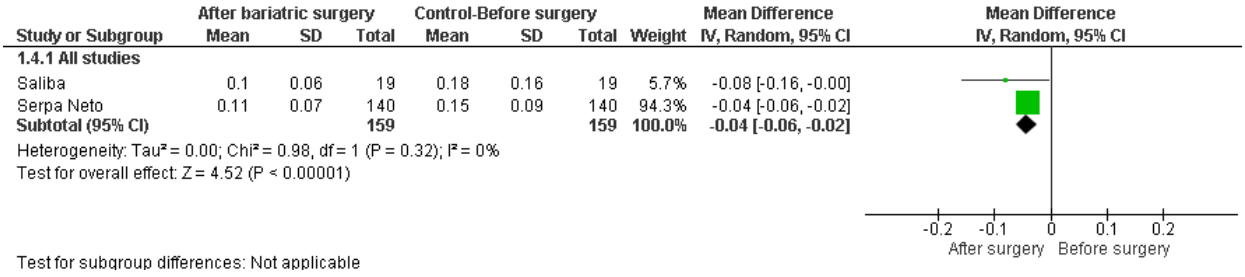

Fig. 46. *Forest plot comparing proteinuria before surgery versus after surgery*

Nonetheless, BS also attenuates renal inflammation and fibrosis via weight loss: the serum and urinary levels of macrophage migration inhibitory factor (MIF), monocyte chemoattractant protein-1 (MCP-1), and chemokine-ligand 18 (CCL18)-proinflammatory and profibrotic major mediators of renal damage- significantly decrease after BS procedures (Fenske et al., 2013). Renal tissue expression of transforming growth factor beta (TGF-β) is also attenuated in animal models of diabetic nephropathy after Roux-en-Y esophagojejunostomy (Wang et al., 2016).

II.2.4. Discussion

Weight gain is a very common problem in kidney transplant recipients that increases the risk for kidney dysfunction, graft loss, and complications (Chan, Garneau and Hajjar, 2015). Bariatric surgery is a much more efficient weight loss procedure compared with medical treatment in CKD patients (MacLaughlin et al., 2014), but there is currently little knowledge about it in renal transplant recipients. Golomb et al. (Golomb et al., 2014) showed a favorable effect of LSG on renal outcomes up to 14 months after BS in transplanted patients. However, the creatinine clearance reduction in five patients in this study urges for assessment of long-term outcomes.

The results of BS in CKD patients are encouraging and give rise to the following question: is BS appropriate and safe for all stages of CKD? Turgeon et al. (Turgeon et al., 2012) have demonstrated a positive trend between CKD severity and the incidence of complications after BS, even after controlling for diabetes and hypertension. However, the 30-day overall mortality of 0.12% and the absolute incidence of complications of less than 10% in both open surgery (associated with a higher risk) and laparoscopic procedures combined (Turgeon et al., 2012) is comparable with that of the general population (0.3 and 4.1%, respectively) (Flum et al., 2009). Potential renal pitfalls include an increased risk for oxalate nephropathy (Duffey et al., 2010) and for acute kidney injury in the CKD population (Rao, Bhattacharya and Agrawal, 2014).

On the long term, BS is associated with nephrolithiasis, with incidence rates as high as 3% and a rate of recurrence of more than 30%. The major cause is hyperoxaluria, which is maintained 2 years or more after GB (Duffey et al., 2010). Although the only study that assessed incident nephrolithiasis after BS (Palomar et al., 2005) did not report any modifications in oxalate excretion after BS (BPD) and no increase in the incidence of lithiasis, oxalate nephropathy may accelerate CKD progression in patients with pre-existing CKD, leading even to initiation of dialysis (data mainly from case reports or case series, therefore incidence could not be quantified) (Nasr et al., 2008). The need for RRT was not reported in the described studies (only Palomar et al. (Palomar et al., 2005) reported no

incident kidney failure after BPD) especially as, with few exceptions (Luaces et al., 2012), (Navaneethan and Yehnert, 2009), (Fenske et al., 2013), (Hou et al., 2013), (Jose et al., 2013), (Schuster et al., 2011), (Ngoh et al., 2016), (Imam et al., 2017), (Golomb et al., 2014), CKD was an exclusion criteria or not mentioned at all.

Our findings regarding the positive impact of BS upon renal function are in concordance with the meta-analysis of Navaneethan et al. (Navaneethan et al., 2009) and the systematic reviews of Afshinnia et al. (Afshinnia et al., 2010) and Bolignano and Zoccali (Bolignano and Zoccali, 2013) which globally investigated the renal effects of various weight loss interventions (surgical and non-surgical). Regarding non-surgical weight-loss methods, diet and medical interventions are not without caveats in CKD: lowcarbohydrate diets are usually rich in proteins and therefore have a negative impact upon kidney function (Knight et al., 2003); at the same time, no weight-loss medication has been adequately tested in overt CKD (Kang and Park, 2012). As BS is an emerging option for weight loss in renal patients (MacLaughlin et al., 2014), our review focused only on the impact of BS upon kidney function. Likewise, we only found one meta-analysis that specifically addressed the effects of BS on renal function, recently published in 2016: Li et al. (Li et al., 2016) have confirmed the improvement of measured GFR and/or eGFR in both hyperfiltration and CKD stage 2 obese patients and also reported reductions in albuminuria/proteinuria after BS. However, the meta-analysis did not assess comorbidities or confounders and also the occurrence of adverse renal effects such as nephrolithiasis or the need for RRT. Moreover, their study focused on CKD stage 2, while we included all stages of CKD. More long-term prospective studies that evaluate overall complications and renal complications after different BS procedures are needed. Also, studies that evaluate the effect of BS in ESRD patients on dialysis patients and/or in CKD patients that are kidney transplant recipients or candidates for transplantation are necessary. Thus, the strengths of this study include the systematic approach and extensive review of literature, with data extraction and appraisal performed by two independent reviewers. We assessed the overall kidney function by evaluating the effect of BS upon creatinine values, eGFR, and albuminuria/ proteinuria and also the possible adverse renal effects associated with BS, such as nephrolithiasis and need for RRT. We also reviewed the results from all-stage CKD patients independently of populations with normal kidney function. However, most cohorts were very small and the available evidence, mostly observational, is at moderate risk of bias and limited by heterogeneity among studies with regard to the effect of BS upon creatinine levels (although reported results regarding GFR and proteinuria were rather homogenous, thus providing reliable results), indirect comparisons and inconsistency for some outcomes (e.g., proteinuria, nephrolithiasis).

Our review could not exclude publication bias of original studies, as probably authors that have not found positive effects of BS or did not find any effect at all are less likely to publish their results.

Conclusion: BS seems to have positive effects on the kidney function, including creatinine values, GFR, and albuminuria/ proteinuria. BS tends to normalize GFR across different categories of renal impairment such as hyperfiltration and reduced GFR patients. Finally, future studies specifically addressing CKD subpopulations that also investigate CKD progression and need for RRT would allow for more precise and firm conclusions to be drawn with regard to the effects of BS on kidney disease.

II.3. The metabolic surgery and the liver function

II.3.1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is highly associated with obesity, metabolic syndrome (MS) and type II diabetes (DM II) and defines a spectrum of liver disease ranging from simple steatosis to steatohepatitis (NASH) – the progressive form of the disease; the latter may exhibit varying degrees of hepatic fibrosis and may progress to cirrhosis and end stage liver disease; it also associates an increased risk of developing hepatocellular carcinoma (Calzadilla, Bertot and Adams, 2016). NAFLD affects 15% of the non-obese population and 65% of grade I and II obese people; a higher percentage of the disease (more than 85%) is found in morbidly obese people, making it one of the most important modern public health issues in the world. The prevalence of the progressive form of NAFLD (NASH) ranges between 20 and 40% in obese patients (Fabbrini et al., 2010), which should be worrying for public health systems.

Hepatic biopsy is the gold diagnostic method in NAFLD as it remains nowadays the only way to differentiate between the simple and the progressive forms of the disease, which involves lobular inflammation, hepatocyte ballooning, accompanied or not by hepatic fibrosis. Though, it cannot be used for the screening of NAFLD not only because it is an invasive, expensive, requiring qualified staff and risk-taking method (Grant and Neuberger, 1999) but also because it should be applied to a very large number of patients, given that NAFLD is a pandemic condition. Therefore, continuous and intense efforts are being made to identify noninvasive markers that could participate in NAFLD diagnosis. Among the directions with increasing interest in their use as non-invasive markers are the elastometry tests which seem to be more and more reproducible and reliable nevertheless highly accepted by patients. Therefore, numerous noninvasive markers were studied over time in order to guide clinicians in assessing patients with obesity and NAFLD in terms of liver damage severity. Such an easy to dose and non-expensive marker is serum 25 (OH) vitamin D, the most stable of its circulating forms that also reflects the status of vitamin D in humans (Pappa et al., 2014). There is already evidence showing the link between low serum vitamin D, obesity and the presence of metabolic syndrome or any of its various components, which are further closely related to NAFLD (Ford et al., 2005).

Personal contribution – published papers:

Livadariu R, **Timofte D**, Trifan A, Dănilă R, Ionescu L, Sângeap AM, Ciobanu D. Vitamin D deficiency, a noninvasive marker of steatohepatitis in patitents with obesity and biopsy proven nonalcholic fatty liver disease. Acta Endocrinologica (Buc), 2018, 14(1): 76 – 84.

Livadariu R, Dănilă R, Ionescu L, Ciobanu D, **Timofte D.** Study of biochemical and clinical markers in steatohepatitis related to obesity. Rev Chim, 2018, 69(6):1501 – 1506.

The main objectives of the study were to evaluate clinical and biological data that can be used as noninvasive markers of the progressive form of NAFLD in obese patients and also to test the hypothesis that there is a significant association between vitamin D deficit and the severity of NAFLD.

II.3.2. Material and Methods

Thus, we performed a prospective study on 64 obese patients successively hospitalized for bariatric surgery in our Surgical Unit between November 2014 and November 2016. We included only patients aged over 18 years with medical indication of bariatric surgery. Exclusion criteria: patients with history of alcohol consumption (over 20g/ day in women and over 30g/day in men), chronic hepatitis B or C, other chronic liver disease or patients undergoing hepatotoxic drug use. We also excluded patients with renal, endocrine or other chronic diseases that could affect metabolic or cardiovascular functions.

All patients signed the Informed Consent approved by the Ethics Committee of the University of Medicine and Pharmacy Grigore T. Popa Iasi, according to the requirements of the Declaration of Helsinki and in accordance to some published models. All patients underwent full clinical evaluation including personal and medical history and complete clinical examination. Anthropometric measurements were noted: BMI (body mass index) was calculated by the formula G (kg)/T^2 (cm) and waist circumference was measured in centimeters, half point between the last rib and iliac spines.

Fasting blood samples were collected in the morning of surgical procedure to assess serum Chol, HDL-Chol, Triglycerides, Insulin, Gly, 25(OH) vitamin D. Insulin resistance was assessed by homeostatic model assessment-insulin resistance index (HOMA-IR = fasting glucose (mmol/L) X fasting insulin (mU/L)/22.5). CRP, Fibrinogen level, NLR and PLR scores (obtained by dividing the neutrophil counts and the platelet counts by the lymphocyte count respectively) were evaluated, with prognostic value for liver inflammation. 25 (OH) vitamin D serum level was measured using chemiluminescence assay. We used a single a value of serum 25 (OH) vitamin D, considering it to be the exact value that reflects vitamin D status when evaluating by liver biopsy the hepatic changes related to obesity. Of associated comorbidities, type II diabetes mellitus (DM) and arterial hypertension (HTA) were noted as components of the metabolic syndrome (MS).

All patients included in our study underwent liver biopsy during bariatric surgery consisting of laparoscopic sleeve gastrectomy. The biopsy specimens measured at least 1.4/0.4 cm and were evaluated by an experienced pathologist who had no access to the clinical or biological data of the patients; hepatic tissue was fixed in buffered formalin and stained with hematoxylin-eosin and van Gieson. NAFLD in its various forms was diagnosed using Kleiner score (Kleiner et al., 2005). The necroinflammatory activity score of NAFLD was also evaluated using the score system developed by Brunt (Brunt et al., 1999).

The database was completed using Microsoft Excel 2013 version and the statistically analysis was performed in SPSS V.19.0. Continuous variables were expressed using mean, median and standard deviation (SD) values. Significance level (p-value) was considered to be 0.05 (5%) with 95% probability (confidence interval). The t-Student test, the ANOVA test or chi-square test were used to verify the statistical significance. We also calculated the odd ratio (OR-the chances of those exposed to present a certain feature are OR higher than the chances of the unexposed ones). In this way, the age of the patients in our study ranged between 18 and 60 years, with an average of 41.33 years ± 11.9 SD. The mean BMI was 45.06 kg/m^2 ± 6.67 DS, ranging between 35 and 67 kg/m^2. The male patients were significantly fewer (23.4%) than the females. We noted the presence of MS in 46.9% of our

patients. DM II was present in 18.8% of cases, arterial hypertension in 32.8%, dyslipidemia in 92 % and OSA in 65.6 % of cases.

II.3.3. Results

Histopathological evaluation of liver biopsies revealed the presence of NAFLD in 100% of the patients, at it follows: hepatic steatosis (38%), steatohepatitis in which we noted two forms: with fibrosis (31%) and without fibrosis (20%), cumulating 51%; 11% of cases (7 patients) had NASH with vanished steatosis as the histopathological examination revealed specific NASH changes (lobular inflammation, hepatocyte ballooning with or without fibrosis) in the absence of macro vesicular steatosis, the condition for defining steatohepatitis. No necroinflammatory activity was noted in 50% of patients (NAS = 0), 43.75% of patients had mild necroinflammatory activity (NAS = 1) and 6.25% had moderate activity (NAS = 2). Liver fibrosis was present in 25 patients (39%), 18 patients had mild fibrosis, 4 medium fibrosis and 3 severe fibrosis; none of the patients had liver cirrhosis. BMI was higher in the steatohepatitis group compared to the simple steatosis group (which we considered the basis of comparison because it is the benign form of the disease) but the results did not show statistical significance. In contrast, waist circumference was significantly higher in the steatohepatitis groups (both with and without fibrosis) compared to the hepatic steatosis group (

TABLE *22*)

In patients diagnosed with MS, the proportion of histopathological form of NASH with fibrosis was nearly double (65%) compared to the proportion of simple steatosis (33.3%), statistically confirmed by the chi-square test ($\lambda 2 = 4.385$, $p = 0.036$). Data analysis also confirmed ($p = 0.023$) that the proportion of patients with NASH was about 3 times higher compared to patients with simple steatosis (45.5% versus 16.7%) in hypertensive patients.

TABLE 22 *Differences in anthropometric index mean values according to the constituted liver diseases in NAFLD*

Waist circumference	**Mean**	**C.I. 95%**		**SD**	**Min.**	**Max.**	**p**
		Min.	**Max.**				
Steatosis	125.29	117.84	132.75	17.657	89	148	
NASH	139.09	133.54	144.64	15.643	100	159	***0.003***
Simple NASH	136.92	126.93	146.91	16.530	103	159	***0.05***
NASH + fibrosis	140.50	133.34	147.66	15.306	100	158	***0.004***
NASH "vanished steatosis"	134.29	119.29	149.28	16.214	8	44	0.23

Regarding dyslipidemia, frequency analysis did not reveal statistically significant differences except for the comparison between the NASH with fibrosis group and simple steatosis group, the elevated levels of LDL Col correlating positively with the presence of NASH with fibrosis ($\lambda 2 = 4.227$, $p = 0.04$). We noted the statistically significant positive association between DM II and NASH with fibrosis when comparing this group with the group of patients diagnosed with hepatic steatosis alone ($\lambda 2 = 6.229$, $p = 0.01$).

Regarding OSA, our data proved that its presence was statistically associated with NASH, either with fibrosis (p = 0.01) and without fibrosis (p = 0.02).

Assessing the risk of waist circumference increase (in 10 cm thresholds) and BMI increase on the occurrence of specific liver disease within NAFLD, the results showed that each 10 cm increase in WC increased the risk of steatohepatitis 2.64 times (p = 0.007). The risk of NASH with fibrosis was increased by 3.7 times (p = 0.04) by the presence of MS and 6.8 times (p = 0.02) by the DM II; hypertension was associated with an increased risk of NASH (OR = 4.1, p = 0.02) and NASH with fibrosis (OR = 4.0, p = 0.04). Also, the presence of OSA was statistically associated with a significant increase in risk of NASH with fibrosis (OR = 9.4, p = 0.03) and simple NASH (OR = 5.5, p = 0.02), as showed in

TABLE *22*.

Regarding the serum inflammatory markers, we firstly observed that the mean value of serum Fb was normal in patients with steatosis (379.83 mg/dL ± 43.89 SD) and pathologically increased in patients with NASH (421 mg/ dL ± 44.84 SD), either in case of histopathological form with fibrosis (423.55 mg/dL ± 47.00 SD) or without fibrosis (417.08 mg/dL ± 42.86 SD) and in the NASH with vanished steatosis group (403.43 mg/dL ± 52 SD). Also, the mean value of serum CRP was normal (0.47 mg/dL ± 0.45 SD) in patients diagnosed with the benign form of NAFLD (steatosis) and pathologically increased in patients with NASH (1.07 mg/dL ± 0.94 SD), either with fibrosis (1.10 mg/dL ± 0.96SD) or without fibrosis (1.03 mg/dL ± 0.95 SD) and in the NASH with vanished steatosis group (1.48 mg/dL ± 1.08 SD). As presented in table 3, we found that the mean values of serum Fb and CRP were significantly higher in patients diagnosed with the progressive forms of NAFLD, namely NASH (both with and without fibrosis), but also in patients with NASH with fibrosis, when considered separately.

The risk analysis statistically confirmed that the increased serum value of Fibrinogen and CRP increases the risk of NASH and the specific subgroup of NASH with fibrosis (**TABLE 23**,

All patients included in our study had low levels of 25(OH) vitamin D. 84.37% had vitamin D deficit, with an average of 12.58 ng/mL and values ranging between 3.45 ng/mL and 19.78 ng/mL. 15.62% of the patients were vitamin D insufficient, with an average of 25.34 ng/mL. Table 35 summarizes the clinical and biological characteristics of patients, dividing them into two groups according to their plasma 25-OH D levels (vitamin D insufficiency: 20–30 ng/mL and vitamin D deficiency: <20 ng/mL). Aside from the significantly different plasma vitamin D levels among the 2 groups, and patients with vitamin D deficiency having a significantly higher prevalence of T2DM (p=0.033), patients were well-matched for age and clinical or biological metabolical parameters, including BMI, HOMA-IR, HDL-Chol, Chol, TG. The statistical analysis also revealed a significant correlation between vitamin D deficit and higher levels of PCR (p=0.001).

TABLE 24).

TABLE 23 *Analysis of clinical factor risks on NAFLD*

Risk factor	NAFLD	OR	95% CI		p
			Min.	Max.	

Waist circumference – each 10 cm increase	Simple steatosis	0.63	0.45	0.88	0.007
	NASH	2.64	1.14	2.36	0.007
	Simple NASH	1.51	0.74	3.08	0.25
	NASH with fibrosis	1.40	0.92	1.77	0.82
	NASH “vanished steatosis”	1.51	0.73	3.08	0.25
Increasing in obesity grade (II – III)	Simple steatosis	1.06	0.27	4.08	0.93
	NASH	0.90	0.22	3.61	0.88
	Simple NASH	1.20	0.11	12.88	0.88
	NASH with fibrosis	1.11	0.098	1.83	0.92
	NASH “vanished steatosis”	1.20	0.11	12.88	0.88
Metabolic syndrome	Simple steatosis	0.409	0.143	1.172	0.09
	NASH	2.714	0.909	8.105	0.07
	NASH with fibrosis	3.714	1.063	12.975	0.04
	Simple NASH	1.714	0.431	6.826	0.44
	NASH “vanished steatosis”	1.500	0.268	8.383	0.64
DM II	Simple steatosis	0.307	0.060	1.576	0.15
	NASH	4.397	0.468	9.831	0.12
	NASH with fibrosis	6.828	1.228	7.952	0.02
	Simple NASH	1.735	0.474	6.350	0.40
	NASH “vanished steatosis”	4.805	0.458	50.375	0.19
Hypertension	Simple steatosis	0.271	0.078	0.938	0.3
	NASH	4.167	1.166	4.890	0.02
	NASH with fibrosis	4.091	1.020	6.403	0.04
	Simple NASH	4.286	0.928	9.796	0.06
	NASH “vanished steatosis”	2.000	0.282	14.198	0.48
OSA	Simple steatosis	0.127	0.040	0.406	0.1
	NASH	7.500	2.235	9.165	0.01
	NASH with fibrosis	9.444	2.151	11.475	0.03
	Simple NASH	5.556	1.200	5.712	0.02
	NASH “vanished steatosis”	10.000	1.030	97.044	0.04

All patients included in our study had low levels of 25(OH) vitamin D. 84.37% had vitamin D deficit, with an average of 12.58 ng/mL and values ranging between 3.45 ng/mL and 19.78 ng/mL. 15.62% of the patients were vitamin D insufficient, with an average of 25.34 ng/mL. Table 35 summarizes the clinical and biological characteristics of patients, dividing them into two groups according to their plasma 25-OH D levels (vitamin D insufficiency: 20–30 ng/mL and vitamin D deficiency: <20 ng/mL). Aside from the significantly different plasma vitamin D levels among the 2 groups, and patients with vitamin D deficiency having a significantly higher prevalence of T2DM (p=0.033), patients were well-matched for age and clinical or biological metabolical parameters, including BMI, HOMA-IR, HDL-Chol, Chol, TG. The statistical analysis also revealed a significant correlation between vitamin D deficit and higher levels of PCR (p=0.001).

TABLE 24 *Inflammatory serum markers in NAFLD*

Serum	NAFLD	Mean	C.I. 95%	SD	Min.	Max.	p

inflammation marker			Min.	Max.				
Fibrinogen (mg/dL)	Steatosis	379.83	361.30	398.7	43.89	288.00	460.00	
	NASH	421.00	405.10	436.90	44.84	298.00	500.00	0.002
	NASH with fibrosis	423.55	401.55	445.55	47.00	298.00	493.00	0.003
	Simple NASH	417.08	391.18	442.98	42.86	359.00	500.00	0.01
	NASH "vanished steatosis"	403.43	355.34	451.51	52.00	315.00	482.00	0.8
CRP (mg/dL)	Steatosis	0.47	0.28	0.66	0.45	0.20	2.30	
	NASH	1.07	0.74	1.40	0.94	0.28	4.60	0.02
	NASH with fibrosis	1.10	0.65	1.55	0.96	0.36	4.60	0.01
	Simple NASH	1.03	0.45	1.60	0.95	0.28	3.20	0.06
	NASH "vanished steatosis"	1.48	0.48	2.48	1.08	0.46	3.00	0.05

TABLE 25 *Risk analyses of increased of serum inflammatory markers for NAFLD*

Risk factor	NAFLD	OR	95% CI		p
			Min.	Max.	
Increased Fibrinogen value	Steatosis	0.994	0.977	1.010	0.40
	NASH	1.022	1.007	1.038	0.05
	NASH with fibrosis	1.023	1.002	1.044	0.03
	NASH "vanished steatosis"	1.013	0.99	1.035	0.23
Increased CRP value	Steatosis	0.883	0.436	1.791	0.73
	NASH	8.849	1.501	52.158	0.01
	NASH with fibrosis	3.698	0.917	14.916	0.05
	NASH "vanished steatosis"	5.618	1.306	24.159	0.02

As summarized in **TABLE 27**, the only difference in histological severity of liver disease among the two groups was related to lobular inflammation that significantly correlated with vitamin D deficit (p=0.040). Despite having more lobular inflammation, but with no correlation with any of the other histological parameters, severe steatosis (p=0.790), hepatocyte ballooning (p=0.777), fibrosis (p=0.162), there was no significant correlation between plasma 25-OH D deficit and definite NASH. The multivariate analysis showed that fibrosis and steatohepatitis were independent predictors of low vitamin D concentration (**TABLE 28**).

TABLE 26 *Clinical and biochemical characteristics related to vitamin D level*

Parameter	**Vitamin D level** Deficiency (n=54)	Insufficiency (n=10)	**p-value for F_{ANOVA} test**
Age (year)	41.44 ± 12.15	40.70 ± 11.04	0.858
Sex* (M/F)	13/41	2/8	0.777

Diabetes* (%)	22.2	0.0	0.033
HTA* (%)	31.5	40.0	0.603
BMI (kg/m2)	45.34 ± 6.93	43.59 ± 5.12	0.452
HOMA-IR	5.38 ± 3.22	4.11 ± 2.05	0.238
IR-present * (%)	61.1	50.0	0.514
Total cholesterol (mg/dL)	197.21 ± 37.56	218.13 ± 46.65	0.171
HDL-cholesterol (mg/dL)	43.30 ± 10.21	47.50 ± 9.47	0.231
Triglycerides (mg/dL)	154.85 ± 87.17	151.20 ± 71.42	0.898
TG/HDL chol score	4.00 ± 3.82	3.47 ± 2.02	0.672
25(OH) vitamin D (ng/mL)	12.59 ± 4.39	24.34 ± 2.98	0.001
Fibrinogen (mg/dL)	407.78 ± 50.05	381.30 ± 32.92	0.114
PCR (mg/dL)	2.00 ± 0.68	2.07 ± 1.07	0.905
PLR	115.46 ± 32.07	118.07 ± 27.90	0.811
NLR	2.11 ± 0.68	2.07 ± 1.07	0.905

Results are shown as mean ± SD and *Kruskal-Wallis test

TABLE 27 *Relation between severity liver disease and plasma vitamin D level*

Parameter	**Vitamin D level** Deficiency (n=54)	Insufficiency (n=10)	**p-value for F_{ANOVA} test**
Mild/medium steatosis *(%)	63.0	60.0	0.859
Severe steatosis *(%)	25.9	30.0	0.790
Lobular inflammation *(%)	74.1	40.0	0.040
Hepatocyte ballooning *(%)	75.9	80.0	0.777
Fibrosis* (%)	42.6	20.0	0.162
Simple steatosis* (%)	33.3	60.0	0.116
HASH* (%)	55.6	30.0	0.134
HASH + "vanished steatosis" * (%)	11.1	10.0	0.917

Results are shown as mean ± SD and *Kruskal-Wallis test

TABLE 28 *Multiple logistic analysis of anthropometric data, clinical and biological markers and histopathological liver changes. Dependent Variable: Vitamin D Level*

Term	**B Coefficient**	**S.E.**	**95% CI**	**t**	**Sig.**
Constant	3.945	1.597	0.686÷7.204	2.518	0.020
Age	-0.004	0.008	-0.020÷0.012	0.504	0.619
Sex	-0.251	0.174	-0.613÷0.112	1.437	0.165
Diabetes	0.059	0.255	-0.471÷0.590	0.232	0.818
HTA	0.131	0.158	-0.198÷0.460	0.828	0.417
BMI	-0.011	0.011	-0.033÷0.011	1.029	0.315
Steatosis	-0.075	0.166	-0.420÷0.269	0.453	0.655
Inflammations	0.274	0.353	-0.460÷1.008	0.776	0.446
Hepatocyte	0.044	0.317	-0.615÷0.703	0.139	0.891
Fibrosis	-1.014	0.487	-2.026÷0.002	2.083	0.050
Steatohepatitis	-1.089	0.473	-2.073÷0.105	2.302	0.032
SH+Fibrosis	-0.151	0.675	-1.554÷1.252	0.224	0.825
HOMA-IR	-0.027	0.043	-0.116÷0.063	0.625	0.538
IR-present	0.097	0.234	-0.390÷0.583	0.414	0.683

Chol	0.001	0.003	-0.004÷0.006	0.445	0.661
HDL	0.027	0.018	-0.011÷0.065	1.497	0.149
Triglycerides	-0.008	0.006	-0.020÷0.004	1.345	0.193
TG/HDL	0.335	0.192	-0.063÷0.734	1.750	0.095
25(OH) Vit.D	0.002	0.014	-0.027÷0.030	0.121	0.905
Fibrinogen	0.002	0.002	-0.002÷0.005	0.944	0.356
PCR	-0.099	0.238	-0.593÷0.395	0.417	0.681
PLR	-0.001	0.004	-0.010÷0.008	0.171	0.866
NLR	0.031	0.160	-0.301÷0.363	0.194	0.848

II.3.4. Discussion

Vitamin D has long been regarded only as regulatory factor for phospho-calcium metabolism and bone homeostasis. However, many studies have shown more varied properties, namely immunomodulatory role (Chen et al., 2007), involvement in cellular differentiation and proliferation (Bikle, 2009), hormone secretion (Li et al., 2002) and anti-inflammatory and antifibrotic effects (Beilfuss et al., 2015) (Mellenthin et al., 2014). All these roles as well as the complex implications in other common pathologies to our days, such as metabolic syndrome and insulin resistance, support the importance of maintaining optimal vitamin D levels (Oros et al., 2012). Values below 20 ng/mL define vitamin D deficiency and its level between 20 and 30 ng/mL is recognized as insufficiency (Kennel et al., 2010). Vitamin D deficiency is pandemic, affecting more than one billion people worldwide, the mechanisms underlying this phenomenon being still unknown although intensely studied (Bouillon, 2010).

The results of our study did not show an inverse correlation between BMI and vitamin D level, as most previous publications found (Stein et al., 2009) (Cigerli et al., 2016). However, as 100% of the study subjects had abnormal low levels of vitamin D, with more than 80% deficiency, we can rally to the literature data showing intense association between obesity and low levels of vitamin D (Al Asoom, 2016). The 25 (OH) vitamin D deficit encountered in patients with morbid obesity and NAFLD may be explained by the increased amount of adipose tissue in which the vitamin D is distributed in obese patients; this will decrease the circulating serum of vitamin D level, given that vitamin D, synthesized in the skin or from food sources will either take the 25 hydroxylation hepatic pathway or be stored in adipocytes (Wortsman et al., 2000). Another explanation could be related to sedentary lifestyle, poor micro-nutrient nutrition and lack of exposure to sunlight of obese people (Targher et al., 2007) (Bradlee et al., 2010). Patients in our study were included throughout all the seasons, which gave homogeneity to our studied group. Even so, in our group of study we did not notice consistent differences between vitamin D status depending on the season in which the patient was enrolled.

Referring to the natural history of NAFLD we must take into account all pathological forms comprised in the spectrum of this disease, from liver steatosis to steatohepatitis the progressive and aggressive form of the disease, accompanied or not by liver fibrosis. The occurrence of hepatic fibrosis reaches 40-50% in patients with NASH and the results in our study support this data. This has a great importance, as a recent study of 619 patients followed for an average of 12.6 years shows that liver fibrosis, independent of the severity of any other liver histological modification and independent of the nonalcoholic fatty liver

disease activity score, is the most important histopathological finding associated with an increased rate of general and hepatic mortality, with an increased likelihood of developing a liver complication (Angulo et al., 2015).

Our results concerning the prevalence of NASH are concordant with literature data showing that up to 59.1% of NAFLD patients have the progressive form of liver disease (Younossi et al., 2016). Cases when the histopathological examination revealed important changes such as lobular inflammation, hepatocyte ballooning, accompanied or not by hepatic fibrosis but lacking macro vesicular liver steatosis, should be considered, especially when it comes to patients with an increased risk of NAFLD due to obesity, MS or type II DM. These patients were considered as NASH with vanished steatosis in agreement with other researchers in NAFLD, finding patients with the same histopathological condition (Bedossa et al., 2012). It is important to underline that patients with this special liver histopathology type associated abnormal mean values of inflammatory serum markers (CRP and fibrinogen). Anthropometric indices are valuable in medical practice primarily because of their ease of use. As our data also show, waist circumference, the index that reflects excessive visceral fat, may be a good predictor of NASH in obese patients. In our study, the mean waist circumference was significantly higher in NASH patients (with and without fibrosis) comparing with simple steatosis patients. The link between inflammatory hepatic changes (lobular inflammation and hepatocyte ballooning) and the excess of visceral adipose tissue can be explained by at least two pathological mechanisms: the ability of excess visceral fatty tissue to secrete proinflammatory factors (IL- 6, IL-8, TNFα, leptin) and the hepatic lipotoxicity caused by the increased free fatty acids flux directly into the liver following increased lipolysis in excess of visceral fat tissue affected by insulin resistance (Sharma et al., 2015). Nevertheless, both visceral and non-visceral fatty tissue accumulation are a common pattern in patients with NAFLD (Popa et al., 2015).

The results of our study also showed significant association between the progressive form of NAFLD (NASH) and the obesity associated comorbidities. OSA and hypertension were significantly associated with an increased risk of NASH with fibrosis but also with an increased risk for overall NASH, while type II DM and MS significantly increased the risk of NASH with fibrosis. These results can be found in the previously published literature (Ding et al., 2017) (Marchesini et al., 2003) (Cazzo et al., 2018) (Benotti et al., 2016) and underline once again the importance of not only evaluating the obese patient from all points of view but of evaluating NAFLD as a disease pathologically connected with all obesity comorbidities. The importance of comorbidity assessment is very important as in obese patients there are two categories described, the metabolically healthy and metabolically unhealthy (Gutierrez-Grobe et al., 2017) (Yoneda et al., 2007).

The inflammatory status in obese patient has some special features, as there are no autoimmune diseases or infectious pathologies involved. Our data confirmed first of all that in our patients, diagnosed with obesity and NAFLD, there were important imbalances in inflammatory status, quantified by serum values of CRP and fibrinogen. When compared to the benign form of NAFLD (steatosis), the high values of mean serum inflammatory markers correlated with its progressive forms (NASH with and without fibrosis). More, the increased values of serum CRP associated a significantly increased risk of NASH (OR = 8.84, p = 0.01) and NASH with fibrosis (OR = 3.69, p = 0.05) and it seems that the CRP value is a

more important marker for the NASH prediction than the serum fibrinogen value. A study of 100 patients with histopathological certified NAFLD underlines the importance of PCR assessment as a marker of steatohepatitis and, moreover, advanced liver fibrosis in patients with NASH. Not the least, NAFLD and the metabolic driven comorbidities pose serious problems related to the adherence and compliance of patients therefore would continue being a serious public health issue. The results of our study support the fact that the obese patients are in a state of chronic inflammation; more importantly, they show that the pathological changes of inflammatory serum markers mirror to some extent the status of hepatic impairment within NAFLD.

II.3.5. Conclusions

Thus, diagnosing the degree of hepatic impairment in NAFLD prior to the severe hepatic fibrosis development has a major importance, but liver biopsy cannot be used in NAFLD screening. Anthropometric indices as well as clinical data such as the presence of MS, DM II and OSA can be used to select a target group to be screened for NAFLD. Waist circumference, more than BMI, reflects the possible presence of NASH. Inflammatory markers such as serum CRP and fibrinogen values can serve as simple, reliable, noninvasive diagnostic markers for NASH, the progressive form of NAFLD. Simple clinical and biological data available to the practitioner in medicine can be used to identify obese patients at high risk of NASH, aiming to direct them to specialized medical centers.

II.4. The metabolic surgery and the psychological context

II.4.1. Introduction

Obesity is considered to be one of the most important health issues worldwide, and is associated with significant physical disorders and also with psychosocial morbidity (Dixon et al., 2003) (Triggermann, 2005) (van Hout, n.d.). For this reason, several modern treatments have been developed, including dietary interventions, prescribed medications, intragastric devices and bariatric operations. Many studies have shown that bariatric surgeries are a proven, effective therapeutic intervention in cases of severe obesity (Buddeberg-Fischer et al., 2006). However, because the surgery is considered to be an invasive method, some concerns rise about its potential negative effect on patients' psychological health (Dymek et al., 2002). As a result, to address these concerns, many experiments were conducted. Interestingly, the results of these studies showed that bariatric procedures significant reduce anxiety, depression scores and increases quality of life in general (Mathus-Vliegen and de Wit, 2007).

Anxiety and obesity are both major public health problems, affecting millions of people of all ages and cultures. Whereas anxiety is reported more in developed countries, where it is the most prevalent mental disorder, obesity is one of the most prevalent global public health disorders (Lykouras and Michopoulos, 2011). The prevalence of obesity globally is in a continuous rise and has been attributed to lifestyle factors, often arising from urbanization, that encourage the accumulation of excess calories through a sedentary lifestyle and an excessive food intake (Zhang and Wang, 2012). The connection between obesity and anxiety is a complex one. Emotional eating is one of the many plausible mediators that act between these two major health issues. The "emotional eating" term was first introduced in

the psychosomatic theory, which considered overeating as a coping mechanism to regulate and reduce negative emotions (Ganley, 1989) (Braet et al., 2008). This reaction, however, is not evolutionarily advantageous or biologically appropriate, given that a reduction in food intake, not an increase, in the face of stress is expected. In particular, the stress-induced flight-or-fight response of the hypothalamic pituitary-adrenal axis should decrease physiological hunger. In terms of anxiety, emotional eating may be a way of dealing with hyperarousal (Braet et al., 2008).

There are many options to treat severe obesity and associated comorbidities. First type of treatment includes lifestyle modification such increasing physical activities, changing the food choices, all to achieve a caloric deficit and to start to lose body fat. The second type of treatment includes psychosocial intervention, medications, and bariatric surgery. However, changes in the lifestyle of obese individuals rarely last. This is due to poor diet adhering, lack of education in the sports science area and not last due to a busy schedule and chaotic daily programs (Sacks et al., 2009) (Li et al., 2005). On the other hand, there is extensive evidence that bariatric surgery is safe and highly effective in reducing body fat and, therefore, obesity-associated comorbidities and mortality (Buchwald et al., 2004) (Sjostrom et al., 2012). Thus, the rise of the number of obese individuals who choose the bariatric treatment is due to the obesity epidemic, along with unsuccessful nonsurgical weight-reduction programs (Padwal et al., 2010). Despite the flourish of the bariatric surgery over the past two decades there is still no consensus on the criteria for psychological assessment of patients who sign up for this type of surgical intervention. Although there are relatively many studies on the subject, there is still no unanimity in the results on the influence of bariatric surgery on the psychological behavior of patients or how this type of procedure may influence the psychological traits of the treated patients (Herpertz et al., 2004) (Andersen et al., 2010). Thus, as the number of patients undergoing bariatric surgery increases, so does the need to understand how psychiatric symptoms may influence the results of the operation and the other way around, how the operation itself may influence the post surgery psychiatric health of the patients (Munoz et al., 2007) (Dymek et al., 2002).

As before mentioned, obese individuals suffer from psychosocial impairments. Studies have shown that individuals who suffer from obesity are more likely to have to deal with social discrimination, lower education, lower income and higher rates of unemployment when compared to the normal-weight population (Willett et al., 1999) (Sullivan et al., 1993). These individuals also report higher levels of anxiety and depressive symptoms, impairment of physical functioning, public distress and a low self-esteem (Wadden, Butryn, et al. 2006) (Moore et al., 1962) (Herpertz et al., 2006) (Wadden et al., 2001) (Kolotkin et al., 2001). Studies show that for individuals who suffer from obesity grade 3 bariatric surgery is the proven, most effective way of treatment (Monteforte and Turkelson, 2000). After the bariatric operation patients present considerable weight loss and improvement of somatic comorbidities (Christou et al., 2004). However, the weight loss present after the bariatric surgery also helps improving mental health and psychosocial functioning (Dixon et al., 2001) (Herpertz et al., 2003) (de Zwaan et al., 2002). That is the reason why the positive outcome of a bariatric procedure should be measured not only in terms of weight loss but also should include the psychological status benefits that this procedure has (Ballantyne, 2003).

Personal contribution – published papers:

Timofte D, Ciuntu B, Bulgaru Iliescu D, Hainarosie R, Pantea Stoian A, Mocanu V. Laparoscopic sleeve gastrectomy is associated with reduced depressive symptoms: a one-year follow-up study. Revista de Cercetare și Intervenție Socială, 2018, 61: 147 – 154. IF – 0,838

Timofte D, Ciuntu B, Iliescu DB, Hainarosie R, Neagoe R, Hristov I, Stoian AP, Mocanu V. The impact of bariatric surgery on anxiety symptoms. Revista de Cercetare și Intervenție Socială, 2018, 62: 185 – 195. IF – 0,838

Therefore, the objective of the present study was to evaluate the possible positive effect of laparoscopic sleeve gastrectomy on reducing depression symptoms, togheter with anxiety aspects mentioned above.

II.4.2. Material and Methods

- ***Anxiety symptoms in bariatric surgery patients***

Thus, the sample of this study was formed from 7 patients (4 men and 3 women), all Romanians, who were hospitalized for laparoscopic sleeve gastrectomy surgery in the Surgery Service, "Sf. Spiridon" Clinical Emergency Hospital in Iasi (Romania). Patients who participated in this study self-reported by telephone the patient administered version of the Zung Self Rating Scale for Anxiety. Despite its simplicity, the Zung Self Rating Scale for anxiety is widely used in the psychiatric field. It is not considered a replacement for a professional diagnosis, but has been proven internally reliable in many different tests and continues to be used in the clinical field. The rating scale is scored from 1 to 4 points. Most answers go in order of 1 (a little of the time) to 4 (most of the time). However, questions 5, 9, 13, 17, and 19 are scored in the opposite order, since they represent positive/nonanxiety statements. Scores are then calculated and individuals are given the following results: (1) 2044 Normal Range; (2) 45-59 Mild to Moderate Anxiety Levels; (3) 60-74 Marked to Severe Anxiety Levels; (4) 75-80 Extreme Anxiety Levels. This scale was self-administered before and 12 months after the laparoscopic sleeve gastrectomy. It should be noted that none of our participants scored in the Extreme Anxiety Levels range before or 12 months after the operation.

All patients from this study were operated through the laparoscopic sleeve gastrectomy procedure.

- ***Depressive symptoms in bariatric surgery patients***

As mentioned, the sample of this study was formed from 7 patients (4 men and 3 women), all Romanians, who were hospitalized for laparoscopic sleeve gastrectomy surgery in the Surgery Service, "Sf. Spiridon" Clinical Emergency Hospital in Iasi (Romania). Patients who participated in this study self-reported by telephone the patient-administered version of the Montgomery-Asberg Depression Rating Scale (MADRS-S). This scale consists of 9 items administered assessing patients' mood, feelings of unease, sleep, appetite, ability to concentrate, initiative, emotional involvement, pessimism and zest for life. Each item is scored between 0 and 3, with three intermediate levels (0.5, 1.5, and 2.5). The total

score is calculated by summing the answers of the nine items, ranging between 0 and 27 (higher scores indicate increased impairment). This scale was self-administered before and 12 months after the laparoscopic sleeve gastrectomy. All patients from this study were operated through the laparoscopic sleeve gastrectomy procedure. All patients treated by surgical intervention were given the same kind of dietary advice and were recommended to take a daily oral supplement containing vitamins and minerals.

II.4.3. Results

- ***Results of the study on anxiety symptoms in bariatric surgery patients***

At baseline, before patients underwent laparoscopic sleeve gastrectomy surgery, 13.3% patients scored below the cut-off score of the scale and were considered to be in the normal range of anxiety. The majority of our sample patients (46.6%) presented mild to moderate anxiety levels. Furthermore 40% scored in the severe anxiety levels range of the test. In addition, our analysis showed that there was no significant connection between the anxiety level and the Body mass index p= 0.982. The mean (±SD) BMI for anxiety groups was the following: For the normal range group 42.9 (±4.5) kg/m^2, for the mild to moderate group 43.3 (±6) kg/m^2 and for the severe group 43.2 (±7) kg/m^2 (please see Figure below).

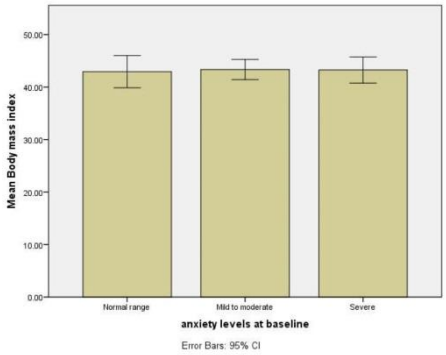

Fig. 47. *No significant differences between our three anxiety levels groups regarding BMI before laparoscopic sleeve gastrectomy.*

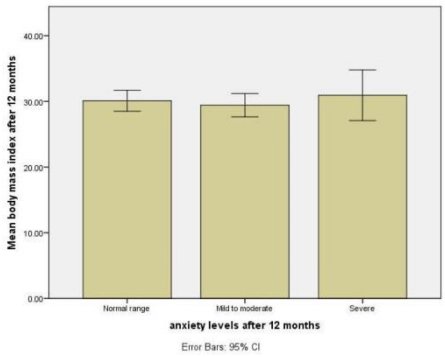

Fig. 48. *No significant differences between our three anxiety levels groups regarding BMI 12 month after laparoscopic sleeve gastrectomy*

12 months after the laparoscopic sleeve gastrectomy the distribution in the three anxiety groups changed. 42.2% scored in the normal range of the anxiety test, the majority of the patients still scored in the mild to moderate range. However, only 11.1% of the patients still presented severe symptoms of anxiety 12 months after the bariatric procedure. The percentage of patients with mild to moderate symptoms of anxiety remained equal at 46.6%. The same as at baseline, there was still no significant difference regarding the BMI in the three distinct anxiety level groups p=0.688. The mean (±SD) BMI for anxiety groups 12 months post-surgery was the following: For the normal range group 30.1 (±4.7) kg/m^2, for the mild to moderate group 29.4 (±5.4) kg/m^2 and for the severe group 30.9 (±5) kg/m^2 (**Fig. 52**). Furthermore, the paired sample T-test analysis showed a significant difference (p< 0.01) between the anxiety scores before and after laparoscopic sleeve gastrectomy.

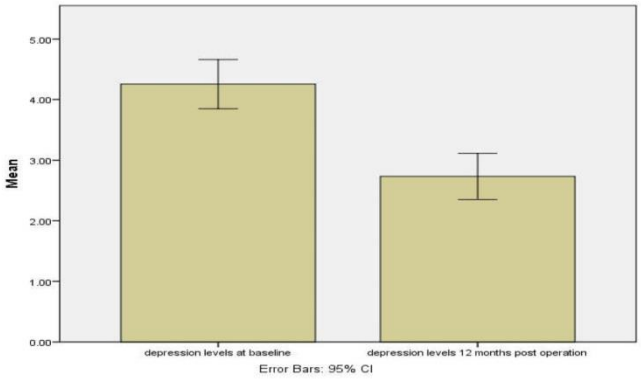

Fig. 49. *Changes in depression scores from baseline to 1-year follow-up in obese patients treated with laparoscopic sleeve gastrectomy surgery*

- ***Results of the depressive symptoms in bariatric surgery patients***

At baseline, before patients underwent laparoscopic sleeve gastrectomy surgery, the mean of the patients regarding the depression scale was 4.25, with SD of 1.94. From all participants 38.8% scored below the cut-off score of the scale and were considered to be clinically depressed.

12 months after the laparoscopic sleeve gastrectomy the score mean regarding the depression decreased to 2.73 (SD=1.82). Furthermore, the laparoscopic sleeve gastrectomy also influenced the number of patients who scored under the cut-off score: only 17.7% of the patients were diagnosed as being depressed 12 months after the laparoscopic sleeve gastrectomy. The paired sample t-test analysis showed a significant difference ($p< 0.01$) between the depression scores before and after laparoscopic sleeve gastrectomy.

II.4.4. Discussion

This study investigated the course and the prognostic significance of preoperative and postoperative depressive disorders in extremely obese bariatric surgery patients in a prospective design with a self-reported depression scale administered prior to the surgery and 1 year postoperatively.

The results showed that the prevalence point of depressive disorders decreased significantly after surgery ($p<0.01$). The score on the depression scale we used significantly decreased after the laparoscopic sleeve gastrectomy. Furthermore, a significant change in mental health status could be observed with regard to depressive symptoms. Depression scores decreased in the 1st year after surgery. 38.8% of the bariatric patients had depression scores of clinical relevance before surgery. One year after surgery, only 17.7% still suffered from depressive symptoms. These findings are in concordance to other studies found in the literature regarding on the possible effect of bariatric surgery on depression (Ryden and Torgerson 2006). In summary, our findings speak in favor of a considerable improvement in a psychological aspect such as depressive symptoms in the course of the 1st year after the laparoscopic sleeve gastrectomy. However, our results present some limitations. It should be taken into consideration that our investigation was limited to evaluating patients after a 12month interval. The data in the literature is clearly showing that psychosocial improvement generally reaches a plateau at 1 year postoperatively and then gradually decline (Dymek et al., 2002) (Burgmer et al., 2007). Further studies on our sample will concentrate on a follow

up on these patients for a more extended time period to determine whether improvement in depression symptoms stops, continues or regresses.

There are many studies on the relationship between obesity and comorbid psychiatric conditions (Mitchell et al., 2012). It is important that the bariatric programs include pre-surgical assessment and treatment of psychiatric conditions due to concerns of the possible negative impact of these conditions on bariatric surgery's outcomes (Tsuda et al., 2009) (Santry et al., 2006). Among various psychological behaviors, the relationship between mood and the outcome of the bariatric procedure, in term of weight loss, has received a great deal of attention (Legenbauer et al., 2009). The results of these studies on mood disorders show that mood symptoms are good predictors for post-surgical weight loss, quality of life, and poorer post-surgical outcomes (Kinzl et al., 2007) (Kinzl et al., 2006). Interestingly, the data shows that anxiety is present in up to 48% of pre-weight loss surgery candidates and it is identified as the most common psychological disorder present before the operation's psychological evaluation (Andersen et al., 2010) (Rutledge et al., 2012). Despite being more prevalent, the symptoms of anxiety in pre-surgical candidates are less understood and have received less attention in the literature than mood symptoms.

Currently, obesity is the most common preventable chronic disease in the world, as its prevalence has increased alarmingly. Bariatric surgery has emerged as a new and effective treatment of this disease. The effectiveness of bariatric surgery has been studied and proven in several meta-analyses. The results of these studies confirmed that surgery is clearly more efficient in inducing weight loss and improving comorbidities than any other nonsurgical therapy (Buchwald et al., 2009) (Shekelle et al., 2004) (Garb et al., 2009). However, it is important to mention that a small percentage of patients treated with bariatric interventions fail to lose significant amount of weight after the operation and one possible explanation may lie in the psychological factors and not necessarily in to the surgery's technical factors (Ning et al., 2010) (Sturm, 2003). From this hypothesis comes the importance of our study. It is essential to study the behavior changes that bariatric surgery can generate on patients, but also to measure in which way the psychological traits of an individual may influence the outcome of the operation (Buchwald et al., 2009) (Yusuf et al., 2005).

In our study the bariatric procedure influenced the anxiety levels in the all patients investigated. The number of patients who presented severe symptoms of anxiety decreased from 40% to 11.1%. Although the number of patients with mild to moderate anxiety remained the same 12 months after the operation, it is important to mention that none of the all patients increased their anxiety level in this period of time. All of the 11.1% of patients who still had severe symptoms of anxiety 12 months post laparoscopic sleeve gastrectomy, experienced the same level of anxiety symptoms at the beginning of the study, at baseline before the operation. Furthermore, 28.9% of our sample decreased their anxiety levels from sever to mild to moderate or even to a normal range. These results are in agreement with the results found in the majority of other similar studies. The available data reports a significant decrease in anxiety rating scores after any type of bariatric procedure. However, in the available literature, there is usually a positive association between the decrease in anxiety scores and the amount of weight loss (Karlsson et al., 2007). In our sample such an association was not found. Patients with different levels on anxiety did not lost weight in significant different amounts. The level of anxiety symptoms had no significant influence on

the amount of weight lost by all participants 12 months after the laparoscopic sleeve gastrectomy. Although, as before mentioned, the majority of the studies show a significant influence of anxiety on the bariatric surgery's outcome, there is not a consensus in these results. For example, a study on German obese patients showed a prevalence of anxiety disorders of 18.5% before the operation (Baumeister and Harter, 2007).

Interestingly, the prevalence of anxiety disorders did not decrease significantly after surgery in this study. There are relatively few studies that measure how anxiety symptoms alone influence bariatric procedure's outcome, or how anxiety levels increase or decrease after this type of surgical procedure. Most common are the studies which test these hypotheses in connection with other disorders such as depression or in connection with the patients' quality of life in the follow up after the bariatric surgery. This is important to mention for the reason that these studies usually show that after bariatric surgery the symptoms of depression show larger reductions than the symptoms of anxiety (Burgmer et al., 2007) (Andersen et al., 2010). For example, in a study with a long time follow up, the results reported by the authors showed that the overall decrease of the anxiety scores from baseline to the 10-year follow-up did not differ between surgery and control subjects. Furthermore, as opposed to the depression subscale scores, no significant long-term relationship was observed between the anxiety symptoms and body fat loss. Although, this study has a longer period of follow up, the results are similar to those in our sample. As before mentioned, the association between anxiety and obesity has been investigated less thoroughly compared to the association between depression and obesity, but there is evidence that weight loss may have a different effect on depression when compared to the effect on anxiety symptoms (Karlsson et al., 2007).

This difference may come from the diverse nature of anxiety symptoms, which may add further complexity to understanding the role of anxiety in the outcome of any bariatric procedure. Many of the clinical anxiety's symptoms such as cognitive, somatic, affective, and behavioral impairments are also common to various medical conditions, such as type II diabetes, cardiovascular disease, cancer, asthma, and osteoarthritis (Guh et al., 2009). The issue arises when these physiological symptoms overlap with symptoms of anxiety, such as fatigue, shortness of breath, heart palpitations, chest tightness, sweating, and pain. As a result, it is difficult to differentiate when patients report any of the above symptoms, if the symptoms are due to their current physiology, a co-morbid medical condition, an underlying psychological condition, or any combination of these variables. All this data highlights the unique role that anxiety may play in the outcome of bariatric procedures. Furthermore, it raises the need for a continuum exploration of cognitive and physiological anxiety symptoms in this population (Wedin et al., 2012).

In addition, oxidative stress could be also a contributing factor in the relations which are established between anxiety and obesity, with previous studies describing the importance of oxidative stress in obesity and metabolic disorders, as well as the connections between anxiety and affective disorders vs. the oxidative metabolism. In this context, we could also mention the importance of exercising performing, with studies showing that it can reduce anxiety-related manifestations (Stonerock et al., 2015), in both animal models and specific patients or in relation with other factors such as oxidative stress status modifications, as it was previously showed. Thus, although the results of our study do not support the idea that

individuals with anxiety symptoms loose less weight compared to their healthy counterparts, close surveillance of these patients is still needed to help identifying at-risk populations who would benefit from adjunctive interventions that target anxiety in patients who show no reduction in these symptoms after the procedure. The important results of our study showed a reduction in the number of patients with severe anxiety and in those with mild to moderate symptoms after the bariatric procedure which will likely result in a positive health outcome of these patients.

Our results also raise the question of the nature of the association between obesity and depression. Studies show that a reciprocal link between depression and obesity exists. Specifically, the results of one meta-analysis obesity showed that obesity increases the risk of depression, and on the other hand depression was found to be an important predictive factor of developing obesity (Luppino et al., 2010). The connection between depression and obesity may be explained involving both biological and psychological mechanisms. The most popular theory asserts that the inflammation as well as HPA-axis dysregulation typically found in obesity could mediate this relationship between obesity and depression. Other factors to be taken into the consideration may be the diabetes mellitus and insulin resistance, which have been shown increase the risk of depression. Finally, the psychological explanation asserts that weight related stigmatization (Chen et al., 2007), increased body dissatisfaction and decreased self-esteem might increase the risk of depression. To conclude, it is safe to assert that severe obesity might cause or aggravate depression. For this reason, some authors suggested that depression should be considered another co-morbidity of obesity (Dixon et al., 2001). Although before a bariatric surgery it is recommended that the patients undergo some kind of psychological evaluation (Bauchowitz et al., 2005), there is no clear consensus on how to determine suitability regarding psychological impairments of a patient seeking bariatric surgery. For example, only 53% of the bariatric programs agree that having depression symptoms prior to the operation may be a definite contraindication. However, 60% of the bariatric programs which were included in this analysis considered suicide attempt within in the past year as a definite contraindication. Interestingly, being clinically depressed prior to the operation may be associated with a significant weight loss after the bariatric procedure.

Some studies have been shown that depressed individuals lose more weight than their non-depressed counterparts after bariatric surgery (Averbukh et al., 2003) (Clark et al., 2003). Beside the weight loss which is expected after bariatric surgeries, a number of studies have described an improvement in mental health during the first year after surgery; depression symptoms were one of the psychological aspects which decreased at 1 year after the operation in this meta-analysis (Magallares and Schomerus 2015). Another systematic review of 40 studies from 1982–2002 showed that patients which underwent bariatric procedures presented a consistent improvement of axis I psychiatric disorders of the DSM, particularly depression and anxiety (Herpertz et al., 2003).

Although depression scores improve in the year first following bariatric surgery as before mentioned, some studies reported that these scores tend to decline after a longer period of time (Mitchell et al., 2014). Unfortunately, we do not have information about depression scores after a prolonged period of time in our registries and can thus we cannot evaluate whether the depression symptoms reoccur significantly after 2 years or more.

However, it is of a real concern that 37.7% of the participants of our study met criteria for current depressive disorder (32.7%). This percentage is alarming when compared to the 4week prevalence rates in obese in patients who showed that only 9.1% of the patients were depressed (Baumeister and Harter, 2007). Even though depressive symptoms improved after the laparoscopic sleeve gastrectomy, our data show that not all patients were free from depressive disorders after surgery and these patients still need medical attention. In addition, the rates of depressive disorders post-surgery still appear to be elevated (17.7% at 1-year post surgery) compared to the 4- week prevalence rates in the obese general population (7.6%) or, as before mentioned, in obese patients (9.1%) (Burgmer et al., 2007). That is the reason why bariatric program should expand well beyond the operation itself, with a long period of careful follow up, education classes with nutrition and sport science advices and with psychological counseling. In this context, we could also mention the importance of exercising performing, with studies showing that it can reduce depression-related manifestations (Craft and Perna, 2004), in both animal models and human patients or in relation with other factors such as oxidative stress status modifications, as our group previously showed. In this way, our findings highlight the positive impact of bariatric surgery on patients' psychological well-being, further strengthening its place as an effective treatment approach not only for weight reduction but also as an important tool to fight against depression.

SECTION II. FUTURE PROJECTS IN THE ACADEMIC, PROFESSIONAL AND RESEARCH FIELD

The leading aim of my feature research plans regards ***bariatric surgery***. It is the most efficacious treatment for obesity, type 2 diabetes mellitus, and other obesity-related comorbidities. Medical outcomes of bariatric procedures together with long-term management of bariatric patients are currently reviewing today's guidelines for nutritional support.

In order to approach this we are going to perform *genetic studies on the expression of adipocyte biomarkers*. We suggest that adipose tissue biology in the period from birth to weaning is not just a staging platform for the emergence of adult white fat but that it has properties designed to serve the unique needs of energy metabolism in the newborn.

Excess fat mass accumulation can lead to a wide range of metabolic and cardiovascular complications resulting from dysfunctional adipose tissue. The latter includes lower storage capacity, adipocytes hypertrophy, immune cell infiltration and altered secretion of anti- and proinflammatory mediators. The increased adipose tissue mass in obesity mainly leads to an expansion of the size of the prevailing adipose cells.

However, there is also a continuous turnover of the adipose cells, and thus recruitment of new adipocytes. Recent studies have shown that in vitro subcutaneous adipose derived stem cells (ASCs) or preadipocyte differentiation is negatively associated with visceral obesity, visceral adipocyte hypertrophy, and a dysmetabolic state.

The bariatric surgery may improve obstetrical outcomes, including preeclampsia, gestational diabetes, and macrosomia, insulin resistance, and hypertension. Because of thiese considerations, another direction of our future research regards *endocrine response to bariatric surgical procedures*. We plan to explore this in short, medium and long-term cohort studies.

The development of insulin-resistance and its complications known as metabolic syndrome are associated with the abdominal distribution of body fat and particular pathophysiological mechanisms. Metabolic syndrome is represented by a group of interrelated disorders, including obesity, hyperglycaemia, hyperlipidaemia, and hypertension. It is also a significant risk factor for cardiovascular disease and increased morbidity and mortality.

Bariatric surgery has been shown to improve obesity-related comorbidities and significantly improve quality of life. Several mechanisms have been proposed to explain improved cardiometabolic alterations after bariatric surgery, and likely multiple changes are responsible for the beneficial effects.

The study of *the shifts in the intestinal microbiota after bariatric surgery* is the third domain to be explored by us in the nearest future. The intestinal microbiota favors an increased capacity to harvest energy from the diet, thus links the microbiome to obesity. Obesity is associated with reduced microbial diversity and richness and an altered composition. After bariatric surgery, the gut microbiome changes significantly and may contribute to weight loss. It is unclear whether these changes are the result of changes in diet or weight loss or are purely surgically driven.

A permanent focus of ours is to develop new and better types of bariatric surgeries because the main procedures are viewed as restrictive, malabsorptive or a combination of both. The least invasive procedure, laparoscopic adjustable gastric banding, is an exclusively restrictive procedure. An adjustable gastric band is Laparoscopic Sleeve Gastrectomy. This procedure also represents the restrictive part of the biliopancreatic diversion. Finally, the Roux-en-Y gastric bypass consists of creating a small gastric pouch (restriction) anastomosed to the jejunum (slight malabsorption).

Obesity is characterized by low-grade, chronic and systemic inflammation resulting, in part, from altered AT immune responses (Labrecque et al., 2017). In the obese state, expression of several proinflammatory adipokines is increased, suggesting significant contribution of AT circulating levels of these mediators. In many cases, the quantitative contribution of AT to elevated plasma levels of these molecules in obesity remains to be fully established.

Under a positive energy imbalance, AT expansion occurs through hypertrophy (increase in size of existing adipocytes) and/or hyperplasia (increase in adipocyte number through adipogenic adipocyte differentiation). The inability of preadipocytes to differentiate and store lipids may result in excessiveadipocyte hypertrophy. This, in turn, is associated with altered endocrine and immune responses, such as adverse adipokine secretion. The AT of obese individuals is also characterized by macrophage infiltration, which is viewed as both a cause and a consequence of this AT immune response and leads to chronic inflammation.

Current literature suggests that bariatric surgery may improve inflammatory status in morbidly obese individuals (Appachi et al., 2013, Cancello et al., 2005) .

Patient provide evidence about the weight loss surgery (WLS) experience, regarding achievement of weight goals, adherence to rules, and improved health status. Participants must be encouraged by educators to identify expected outcomes of educational programming, monitor holistic transformations, foster peer support, and use technology in *weight management centers (WMC) programming and promoting*. Our results validate the need for the Metabolic and Bariatric Surgery Accreditation and Quality Improvement Program's education requirement in our country. Future educational research could help develop best practices in WLS patient education and assess associations between education and clinical outcomes.

The study about the *modifications of adipose tissue macrophage after bariatric surgery* is also taken into account for perspective research. Accumulating evidence suggests that bariatric surgery leads to lower macrophage numbers in. In morbidly obese women, the percentage of macrophages was significantly higher compared to that of lean women, and macrophages formed crown-like structures around single adipocytes. These are formed with macrophages predominantly presenting the M1 phenotype. A shift in the distribution of the remaining macrophages was also observed, including two features:

1) disappearance of CLS;
2) macrophages located near blood vessels.The expression of monocyte chemotactic proteinsdecreased after the bariatric surgery.

Anti-inflammatory factors

Adiponectin is an anti-inflammatory adipokine secretedalmost exclusively by adipocytes. Plasma and genetic expression levels of adiponectin are reduced in obese

compared with lean individuals. Bariatric surgery appears to improve systemic adiponectin levels, especially in the long term. Studies that have examined this adipokinein the short term are less conclusive.

Interleukin 10 *(*IL-10) is an anti-inflammatory cytokine secreted by adipocytes and M2 macrophages. IL-10 is expressed and released by visceral and SC AT, but it is expressed at higher levels in visceral fat. It is known to inhibit the production of IL-1, IL-6, IL-8, TNF-a. Circulating IL-10levels were reported to be elevated in obese compared to normalweight women, and these results are consistent with theincreased expression of IL-10 observed in the AT of obese individuals

Proinflammatory factors

Tumour necrosis factor alpha (TNF-a) is a well-known proinflammatory cytokine expressed mainly by monocytes and macrophages. This cytokineis related to insulin resistance and has deleterious effects on adiponectin and IL-6. TNF-a is increased both in AT and in theblood of obese individuals as well as in insulin resistance.Although it could be expected that TNF-a levels decline afterweight loss induced by bariatric surgery, results are far from unanimous.

Interleukin 6 is similar to TNF-a, IL-6 is a highly studied proinflammatory mediator. IL-6 induces fever and the production of acute-phase proteins by the liver and is also involved in chronic inflammatory responses. This cytokine is secreted by a diversity of cell types, including monocytes, adipocytes, endothelial cells and fibroblasts. It is overexpressed in the AT of obese patients, and adipocytesas well as macrophages are both responsible for AT-derived IL-6.Studies that have investigated circulating IL-6 concentrations overshort periods of time have generated divergent results, whereas thelong-term decline in this cytokine seems more apparent.

C-reactive protein (CRP) is a proinflammatory marker inducedby IL-6 and expressed by hepatocytes and mature adipocytes. Elevated circulating CRP levels are observed in obese individuals. Although a large number of studies have showed that bariatricsurgery decreases circulating CRP levels, some failed to demonstratethis relationship at various follow-up times. At about 3 months,many reports have shown that circulating levels of CRP are significantly decreased after LAGB, whereas others failedto demonstrate any change after RYGB or LSG.

Chemerin is a chemoattractant protein derivedfrom adipocytes and is implicated in the regulation of adipogenesisand adipocyte metabolism. In obese individuals,circulating and visceral AT gene expression levels of chemerin are elevated compared with lean subjects. A relatively small number of studies have evaluated the effectof bariatric surgery on chemerin and found that circulating chemerin was significantly lower after bariatric surgery.

Our future study about *adipose depot type and adipocyte size after bariatric surgery* will consider that hypertrophic adipocytes increase production of pro-inflammatory adipokines and also saturated fatty acids in the extracellular space have the capability to initiate a direct inflammatory response in macrophages through activation of pattern recognition receptors.

The most obvious effect of bariatric surgery is loss of up to half of total adipose tissue mass within the first year after surgery along with improvements in systemic metabolism within the first few months after surgery.

Very few studies have successfully measured local inflammation within adipose tissues after surgery in animal studies. However, these limited findings do indicate that inflammation decreases within the distinct adipose depots as assessed by TNF-alpha and IL-6 mRNA expression as well as number of macrophages and T-cells residing within the mesenteric depot in particular. These observations support the contention that bariatric surgery reduces inflammation associated with obesity.

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