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aesthetic medicine

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Journal article – online *if there is no DOI, provide the URL for the specific article	Coppinger T, Jeanes YM, Hardwick J, Reeves S. Body mass, frequency of eating and breakfast consumption in 9-13-year-olds. *J Hum Nutr Diet*. 2012; 25(1): 43-49. **doi: 10.1111/j.1365-277X.2011.01184.x**
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Newspaper article – online	Pollack A. FDA approves new cystic fibrosis drug. *New York Times*. January 31, 2012. http://www.nytimes.com/2012/02/01/business/fda-approves-cystic-fibrosis-drug.html?ref=health. Accessed February 1, 2012.
Websites	Outbreak notice: Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://wwwnc.cdc.gov/travel/notices/outbreak-notice/haiti-cholera.htm Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.
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Book chapter – in print	Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. *Allergens and Allergen Immunotherapy*. 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

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Example Article

1. Zoellner J, Krzeski E, Harden S, Cook E, Allen K, Estabrooks PA. Qualitative application of the theory of planned behavior to understand beverage consumption behaviors among adults. *J Acad Nutr Diet*. 2012;112(11):1774-1784. doi: 10.1016/j.jand.2012.06.368.

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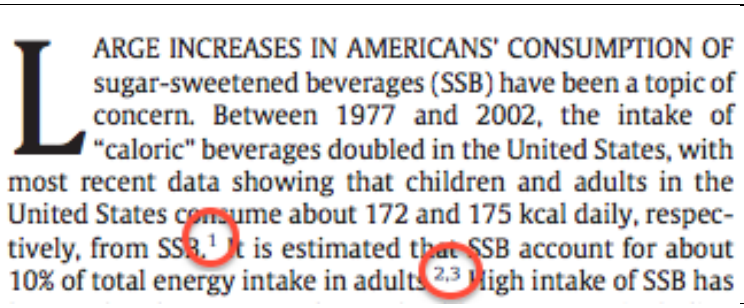
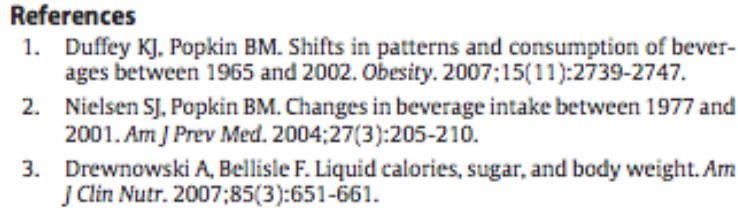


LARGE INCREASES IN AMERICANS' CONSUMPTION OF sugar-sweetened beverages (SSB) have been a topic of concern. Between 1977 and 2002, the intake of "caloric" beverages doubled in the United States, with most recent data showing that children and adults in the United States consume about 172 and 175 kcal daily, respectively, from SSB.[1] It is estimated that SSB account for about 10% of total energy intake in adults.[2,3] High intake of SSB has

References Section Example



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1. Duffey KJ, Popkin BM. Shifts in patterns and consumption of beverages between 1965 and 2002. *Obesity*. 2007;15(11):2739-2747.
2. Nielsen SJ, Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med*. 2004;27(3):205-210.
3. Drewnowski A, Bellisle F. Liquid calories, sugar, and body weight. *Am J Clin Nutr*. 2007;85(3):651-661.

Use commas to separate multiple citation numbers in text, like you see between references 2 and 3. Unpublished works and personal communications should be cited in the text (and not on the reference list).[1] Superscript numbers are placed outside periods and commas, and inside colons and semicolons. When citing the same source more than once, give the number of the original reference, then include the page number (in parentheses) where the information was found. See pages 41-44 of the *AMA Manual of Style* for more information.

References

Citing AMA guide website. http://libguides.stkate.edu/content.php?pid=99799&sid=749106. Updated April 2011. Accessed October 24, 2012.

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AMA Manual of Style: A Guide for Authors and Editors. 10th ed. Oxford: Oxford UP.

EDITORIAL

In modern years, aesthetics has become quite important in every aspect of everyday life: following the hundreds of journals, magazines, blogs and websites pointing their attention towards this interesting and fascinating topic, the request for aesthetic medicine has increased manifolds.

Aesthetic Medicine is a new field of medicine, in which different specialists share the aim of constructing and reconstructing the physical equilibrium of the individual. Treatment of physical aesthetic alterations and unaesthetic sequel of illnesses or injuries, together with the prevention of aging, are perhaps two of the most iconic areas of intervention for Aesthetic Medicine. However, in order to prevent frailty in the elderly, a program of education is similarly important. Furthermore, the line between health and beauty is extremely thin: psychosomatic disorders resulting from low self-esteem due to aesthetic reasons are frequent and cannot be ignored by a clinician.

It is therefore clear that there is no figure in the field of medicine which is not involved in Aesthetic Medicine: endocrinologists, gynecologists, angiologists, psychologists and psychiatrists, plastic surgeons, dermatologists, dieticians, physiotherapists, orthopedists, physical education instructors, massophysiotherapists, podologists, and rehabilitation therapists are just some of the specialists who are sooner or later going to have to answer their patients' needs for aesthetic interventions. The involvement of all these specialists fits the description of health as defined by the WHO: "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" for which, undeniably, a team of different physicians is required.

The number of patients requiring medical consultation for esthetic reasons is rapidly increasing: in order to be able to provide adequate feedback, medical and paramedical specialists should be trained and, more importantly, should be taught how to work together. Existing Societies of Aesthetic Medicine from different countries share the aim of creating such teams and provide constant updates to the literature: the creation of an international network of specialists from all around the world under the flag of Aesthetic Medicine represents a challenge, but at the same time it is the proof of the widespread interest in this topic.

The first issue of this Journal represents the results of the efforts of the many national Societies and of the *Union Internationale de Médecine Esthétique,* now together as one; it is our hope that in years to come this Journal might improve our knowledge in this field, and provide adequate scientific advancement in the field of Aesthetic Medicine.

Francesco Romanelli, MD
Editor-in-chief
Associate Professor at "Sapienza"
University of Rome

EDITORS' NOTES

Aesthetic Medicine, the booming medical activity

Aesthetic Medicine was born in France 40 years ago. The French Society of Aesthetic Medicine was the first of its kind in the world, followed by Italy, Belgium and Spain. Starts were rather difficult as aesthetic procedures in those early years were only surgical. At that time aesthetic doctors and cosmetic dermatologists had very few real medical procedures to offer to their patients for treating aesthetic problems on face and body.

At the beginning of the '80s, viable medical procedures started to emerge in Europe for aesthetic and cosmetic purposes. Mostly, at that time, they were imported from the United States: those included collagen injections for wrinkles (Zyderm by Dr. Stegman), and chemical peels (phenol by Dr. Baker, TCA by Dr. Obagi). But, subsequently, European research on Aesthetic Medicine gained momentum. Hyaluronic acid appeared on the market, as it was discovered that it could be used as a dermal filler for wrinkles.

During the '90s, the use of lasers offered aesthetic doctors and cosmetic dermatologists new possibilities. The "beam revolution" started with CO2 laser for facial resurfacing. Today, CO2 resurfacing is not used as much anymore, because of the long and difficult post-op. CO2 laser was replaced with the gentler Nd-YAG and Erbium lasers and more recently with non-invasive photonic devices for facial rejuvenation, including IPL, US and radiofrequency. These new technologies allow today's aesthetic doctors and cosmetic dermatologists to offer their patients procedures with low risk of post-op complications.

Then, Botulinum Toxin has "invaded" both sides of the Atlantic Ocean. Today, Botox injections are the most popular treatment for facial expressive wrinkles. Botox injections are now so common everywhere that many cosmetic surgeons have given up their bistouries for syringes.

Last but not least, development in Aesthetic Medicine is shown by mesotherapy and adipolipolysis. About lipolysis, new data and recent publications have explained that radiofrequency, ultrasounds and cryolyse could have positive action to dissolve fat and to improve some unaesthetic disorders like cellulite. These non invasive procedures intend to replace the surgical liposculpture with success.

Nowadays, Aesthetic Medicine has the necessary tools to address all major disorders within the aesthetic field.

After 40 years, Aesthetic Medicine is now active in 27 countries in the world (France, Italy, Spain, Belgium, Morocco, Poland, Russia, Switzerland, Romania, Kazakhstan, Algeria, Brazil, Argentina, Uruguay, Venezuela, Colombia, Chile, Mexico, U.S.A, Canada, South Korea, and recently Ecuador, China, South Africa, Turkey, Ukraine and Georgia). All 27 national Societies are members of the *Union Internationale de Médecine Esthétique* (U.I.M.E.).

Aesthetic Medicine is taught in 8 countries (France, Italy, Spain, Brazil, Argentina, Mexico, Venezuela, Kazakhstan) in universities that deliver UIME's diplomas after 3 to 4 years of studies.

What is the future of Aesthetic Medicine?

In the last few decades, patients' desires to look and feel younge, have fueled Aesthetic Medicine and Cosmetic Dermatology: many different procedures have been developed to satisfy the demands.

As life-span have increased, patients today are not only asking about aesthetic procedures, they are also asking for a way to stay in good physical conditions in the last decades of their lives.

As a direct result, Anti-Aging Medicine, which covers skin aging and general aging, has recently emerged and expanded very quickly.

Anti-Aging Medicine can offer senior patients better nutrition, dietary supplementation with vitamins, minerals, antioxidants, and eventually hormone replacement therapy, but only when needed.

Today, and in the near future, both Aesthetic Medicine and Anti-Aging Medicine will offer to our patients, who now live longer, better wellness with aesthetic treatments for skin aging and anti-aging treatments for general aging.

Aesthetic Medicine is booming, but all medical practitioners should be correctly trained, so its future will be bright.

Jean-Jacques Legrand, MD
General Secretary of UIME

Aesthetic Medicine: a bioethic act

When in 1977 the Italian Society of Aesthetic Medicine published the first issue of the magazine "La Medicina Estetica" Carlo Alberto Bartoletti, the Founder, wrote an editorial in which traced the pathway of the discipline and of the Scientific Society, still valid and projected into the future.

Today from that Editorial Board arise an International Journal, which wants to be indexed, in order to give to the doctors practicing Aestehetic Medicine all around the world a solid basis of shared knowledge.

In the late '60s, what was called in Italy Aesthetic Medicine, moved its first steps thanks to "remise en forme and anti aging projects" imported from the experience the "Institutul de geriatrie Bucuresti", directed by Dr. Ana Aslan.

For this reason,there is the bioethical imperative that the Discipline should be first prevention, then return to physiology and finally correction.

The worldwide diffusion and the efforts of Industries born on the wave of the phenomenon have often led to choose the fastest route to achieve and maintain the physical aspect in the myth of beauty at all costs, without considering that aesthetic is not synonymous of beauty, but it is a balance between body and mind, and the role of the doctor is to take care of the Person globally and not only focusing on the correction of "a badly accepted blemish".

Faithful to the teaching of my Master had almost 50 years ago, this new journal will have the task of elevating the human resources, aligning and validating methodologies, but above all affirming the *humanitas* of the medical art in its purest sense to pursue the good and the graceful for the person who relies on it.

Fulvio Tomaselli, MD
Honorary President of the Italian
Society of Aesthetic Medicine

Aesthetic Medicine needs science. All over the world.

All Aesthetic Doctors know that science is the basis for safety. Safety is the most important issue in our discipline.

Unfortunately, Aesthetic Medicine is more often surrounded by marketing than by science, despite the hard work done by Scientific Societies all over the World. And, too often doctors working in this field are dealing with sellers that promote products with insufficient scientific studies. However, they sell it anyway. I think that doctors must learn that the first thing to ask about a medical device is the scientific background regarding that product: patients treated, follow up period, adverse events and, most of all, publications.

With this new International Journal completely dedicated to Aesthetic Medicine, proposed by the Italian Society of Aesthetic Medicine, endorsed by UIME and shared by all the National Societies of Aesthetic Medicine belonging to UIME, World Aesthetic Medicine wants to stimulate scientific production in this discipline to increase safety and quality in aesthetic medical procedures.

Another important goal of the Journal is to catalyze the proposal of new protocols and guidelines in Aesthetic Medicine, with the consensus of the entire Aesthetic Medicine Scientific Community.

What this Journal should achieve in the near future is to improve the number and quality of scientific production in Aesthetic Medicine, in order to allow this discipline to grow in the field of evidence based medicine, not only in the rationale field.

I hope this can be the start of a new era for Aesthetic Medicine, with the commitment of all Scientific Societies all over the world.

Emanuele Bartoletti, MD
Managing Editor
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The nodule of discord: the unresolved diatribe on the pathogenesis of cellulite in the light of the ad-ipocyte pathophysiology

Part 1 – adipose tissue physiophatology

Ferdinando Terranova

MD, International School of Aesthetic Medicine - Rome

ABSTRACT

The studies about cellulite published by indexed journals are limited in number and reach antithetical conclusions. Consequently, it is not still possible to resolve the serious discord on the nature of this disease, its origin and even the most basic aspects of its histopathology. Fortunately, in recent years, this understanding gap was partially filled thanks to the findings about the complex pathophysiology of adipose tissue. Different fat deposits distributed in various regions of the body show extremely variable dimensions, in relation to caloric balance, gender and age, and manifest very diverse biological activities.

The upper body adipose fat, when in excess, triggers a series of pathogenic mechanisms that provoke, in the tissue, a deep inflammatory remodelling, and, at systemic level, the onset of a complex interweaving of disease states, named metabolic syndrome and including insulin resistance, diabetes, hypertension, dyslipidaemia and cardiovascular atherosclerotic illness.

The subcutaneous tissue of the lower limbs is well-developed in women, becoming the elective cellulite location; even when in excess, it does not entail an increased risk of systemic complications, against which it seems, in fact, to perform a protective function.

Keywords
Cellulite, adipose tissue, fibrosis, inflammation, metabolic syndrome

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Introduction

Every doctor is bound to experience deep discomfort when he is forced to treat any disease on the basis of scarce and contradictory knowledge; unfortunately, a similarly embarrassing situation is carried out all the time when a female patient asks the attending physician to help her resolve the problem of cellulite.

This condition, almost exclusively feminine, compromises the figures (and good humour) of millions of women all over the world.

All media that target women under 50 make much of this disorder and its remedies.

Countless measures (surgery[1], drugs[2], herbal medicine[3], homeopathy[4], cosmetics, electromagnetic devices,[5,6,7,8] physiotherapy[9,10], massage[11], etc.) achieved ephemeral glory before proving to be ineffective; this does not stop beauty salons and beautyfarms continuing to collect huge amounts of money by passing off unlikely panaceas.

Faced with a muchfelt problem, the progress of medical science appears remarkably small; questioning the Medline, it turns out that studies on this subject published by international level journals are very limited in number. Those who take the trouble to read them will discover that they reach antithetical conclusions. Even more surprising is the lack of interest in the "cellulite problem" which is expressed not only by the University Institutes but also by the research laboratories of the big companies in the cosmetic field, that, nevertheless, have provided and continue to provide valuable contributions to the advancement of knowledge about skin pathophysiology.

Consequently, it is still not possible to resolve the serious differences of opinion that have been dragging on for years concerning the nature of this disease, its origin, and even its most elementary histopathologic aspects. It also lacks a universally recognised name: most of the authors who touch on the subject tend to begin the discussion with an introduction that explains that the word "cellulite", which came into international use, is inappropriate and inadequate, as it suggests that the affliction is inflammatory. However no better name has been found. Indeed, the term "cellulitis" is used in English medical science to indicate an altogether different disease: the gangrenous suppurative infection of the subcutaneous fat[12] . The chapters on panniculitis[13], liposclerosis[14], and lipodystrophy[15], include morbid forms clearly not comparable to common cellulite.

Finally, the other designations employed from time to time (EFSP, lipoedema, *adiposis oedematosa, dermopanniculosis deformans*, *status protrusus cutis*, etc.) contain morphological and pathogenic conceptions not shared by everyone.

There may be several causes of this continuing knowledge deficit: on the one hand, the enormous amount of pseudo-scientific junk circulating on the topic of cellulite makes the argument daunting for any serious study group; on the other hand, in English-speaking countries, where a large part of biomedical research is carried out, the theory has prevailed that denies nosological dignity to cellulite, considering it a "normal" expression of female trochanteric adiposity. Finally, the solubilisation which the adipocyte lipid content undergoes during the fixation process of histological samples alters the morphology of the cells, making the reconstruction of the three-dimensional tissue organisation difficult and controversial.

Fortunately, in recent years, this disastrous gap in understanding one of the most common female blemishes has been partially filled in what we might call an indirect way: the ever-rising prevalence of obesity and related diseases has produced an incentive for a growing interest by researchers around the world to study the pathophysiology of the adipose tissue. Therefore, thousands of articles have been published which, alt-hough not taking any account of the "cellulite problem", have provided an enormous wealth of information on the morphological and functional characteristics that adipose tissue may exhibit, based on body area, gender, age, BMI, etc. Singling out, from such works, the histological and histochemical aspects identified in the female gluteal-femoral subcutaneous, and also taking due note of those tissue changes which, not being described in any of these publications, likely cannot be part of the histopathology of such a commonly seen condition as cellulite, you may be able, albeit with difficulty, to obtain the necessary information to unravel the jumbled tangle of theories on the pathogenesis of this blemish.

The adipose tissue

Cellulite is a condition that, in women, electively affects subcutaneous fat, with a prevalence for some of its locations.

It is necessary, therefore, to begin the discussion about the pathogenesis of this complaint with a brief mention on the morphology of adipose tissue and on the anatomical and functional features that it takes depending on the gender and the anatomical site.

Morphological aspects

Adipose tissue is a special type of connective tissue, whose constitutive element is represented by specialized cells (adipocytes or fat cells), containing bulky lipid droplets; these represent energy reserves, which can be used by the body in response to specific hormonal and nerve signals in the intervals between

meals and during periods when the calorie intake is lower than the metabolic requests. In adipose tissue, apart from adipocytes, there are other cell types, which constitute the stromal-vascular support structure: multipotent stem-cells, preadipocytes, endothelial cells, perivascular cells, fibroblasts, immune system cells (macrophages, neutrophils, lymphocytes). It is estimated that one gram of fat tissue contains, in addition to 4-6 million other cells, about 1-2 million adipocytes which, despite being numerically inferior, represent, thanks to their large size, about 90% of the tissue volume[16].

Adipose tissue is now considered a "widespread organ", the largest of the whole body: in a male with medium physical shape, it represents 15-20% of the weight; in females it arrive at 20-25%[17].

Adipocytes, isolated or in small groups, are scattered throughout the connective tissue, especially near the vessels; it is possible to speak, more properly, of adipose tissue when it forms large clusters of cells, macroscopically visible.

The largest of those deposits are in subcutaneous and in abdominal cavity: in the latter, distinction must be made among the retroperitoneal fat (whose effluent blood flows in the large circulation) and splanchnic fat (omental, mesenteric and epiploic), whose venous blood is drained from the portal vein and passes, then, into the liver.

In subcutaneous, below the integumentary system, are disposed layers, named adipose pannicula or cushions, of variable thickness, depending on the adequacy of food intake compared to the metabolic needs; they contribute to determine the body silhouettes and perform tasks of energy reserve, thermal insulation and mechanical protection.

The gluteal-femoral panniculus manifests different functional characteristics than those in the upper body. In the subcutaneous fat is stored more than 80% of the total lipid material, while abdominal and retroperitoneal deposits account for 10~20% of body fat in men and 5~10% in women.

Adipose tissue is also present in many other somatic regions, such as female mammary gland, palms, soles of the feet, orbital, inguinal and axillary cavities, mediastinum and thymus.

In some of these areas, adipose tissue performs functions of mechanical support.

According to a series of morphological and functional characteristics, firstly including the manners of fat storing in the cytoplasm and the metabolic activities, two different histological types can be distinguished.

White adipose tissue

Unilocular adipose tissue is often referred to as "common" or "yellow" or "white", hence the acronym WAT. The reference to the colour is justified by the chromatic tone, determined by the type of lipids that there accumulate: for the 90-99% triglycerides (TG), with small amounts of carotenoids, free fatty acids, di- and monoglycerides, phospholipids, cholesterol and its esters.

The white adipose tissue is made up of large cells with a rounded or polyhedral shape. The diameter is 60 µm, on average, but can reach 120 µm. The term "unilocular" is motivated by cell volume being occupied almost entirely by a single lipid droplet, which crushes the scant cytoplasm and the nucleus in a thin peripheral rim; in section, are observed, therefore, aspects "signet ring-like".

Electron microscopy reveals tiny secondary lipid droplets, next to the main one, a small Golgi apparatus, few filamentous mitochondria, an endoplasmic reticulum and many free ribosomes.

The lipid droplets are today considered true organelles; they are covered by a phospholipid monolayer, which binds specific proteins that play an indispensable role in the management of the contained energy (see Fig. 1): in addition to the PAT family proteins, including the perilipin A[18,19], it has been recently detected the presence of peptides used for moving, docking and fusing the vesicular elements (Rabs, SNAREs, etc.)[20,21].

Figure 1

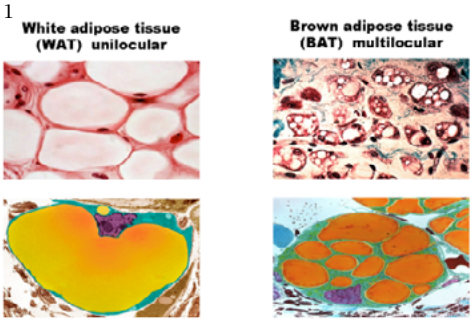

Figure 1 - White and brown adipose tissue

Each fat cell is surrounded by a thin basal lamina[22,23], in which are present proteoglycans, non-fibrillar collagen (predominantly type IV and VI), laminins, entactins, fibronectin, etc. Proteomic techniques have shown that the adipocytes actively intervene in the synthesis and maintenance of this extracellular matrix, whose size and composition change depending on the functional state[24,25].

Each adipocyte is in contact with at least one capillary. The blood flow is high in relation to the cytoplasmic volume: it exceeds the striated muscle perfusion and further increases in prolonged fasting.

The cellular elements of adipose tissue are densely pushed together to form clusters divided into lobules by connective tissue septa, where blood vessels can be found.

In subcutaneous, these branches, disposed in a direction roughly perpendicular to the epidermis, form a fibrous network (retinacula cutis) which connects the deep dermis with the muscles or the periosteal.

The connective band of Camper is parallel to the surface and separates the higher subcutaneous layer from the deeper, which, according to some, holds certain metabolic differences[26].

Brown adipose tissue

The multilocular adipose tissue, also called brown adipose tissue (from which the acronym BAT) is formed by cells of medium size (not exceeding 50 µm), densely juxtaposed, polygonal shaped, containing multiple, small lipid droplets scattered in the cytoplasm, beside the nucleus and the cell organelles[27]. The dark colour (which justifies the tissue name) is due to the high number of mitochondria and to the rich vascularity.

The triglycerides contained in the tiny lipid droplets are not intended to be released to become energy substrates to other cells, but are subjected to β-oxidation directly in the mitochondria of brown adipocytes. Here the presence of the uncoupling protein 1 (UCP1) enables cells to uncouple the respiratory chain from oxidative phosphorylation; then, the energy released is employed to produce ATP, but is dispersed in the form of heat.

In small mammals and in some other animal species that hibernate during the winter season, the BAT is very abundant, especially between the shoulder blades and in the armpits, and serves to maintain the homeothermy. When the body temperature tends to drop, endocrine stimuli (especially thyroid hormones and catecholamines acting on β3-adrenergic receptors) increase thermogenesis in mitochondria of multilocular cells.

In the human species the brown adipose tissue is present, in small quantities, in foetuses and new-borns, in whom it is mainly localized in the scapular site; with the growth, it is transformed, for the most part, in white fat. Until a few years ago, it was generally agreed that, in adults (where the higher weight/surface ratio makes the thermoregulation less critical) the BAT was virtually absent. Conversely, recent studies, based on the use of positron emission tomography (PET-CT) after infusion of F^{18}-fluorodeoxyglucose, have shown that clusters of functionally active BAT, UCP1-immuno-positive, are commonly detected at all ages[28], scattered in an area that extends from the neck up to the lateral cervical, supraclavicular and, to a lesser extent, thoracic, paraspinal and suprarenal zones; they are more abundant in females, and decreases in the elderly.

Further observations have emphasized the importance of these findings; it was noticed that the amount of brown adipose tissue and its functionality increase with prolonged exposure to low-temperatures[29]. In addition, it was found an inverse relationship between the BAT volume and the BMI (Body Mass Index); in other words, in overweight subjects brown adipose tissue appears less expanded than in controls; therefore it can be assumed that a deficient activity of the BAT can be one of the causes of obesity. It was indeed calculated that 50 grams of brown adipose tissue, subjected to strong hormonal stimulation, can bear an energy expenditure equal to 20% of the entire body expenditure in rest conditions[30].

The histogenesis of the brown adipocytes is a long debated matter, being not clear whether they constitute elements distinct from white fat cells or, rather, derive from the latter. Paradoxically both opposing arguments have received confirmation.

On the one hand, it is proved that the cellular elements of BAT and WAT originate from distinct mesenchymal precursors. Indeed, the white adipocytes differentiate from the preadipocytes. Brown adipocytes conversely, derive from the same stem cells (marked by MYF-5 protein positivity) which generate the muscle fibres; their development as BAT cells is triggered by the transcription factor PRDM-16 and by the protein BMP-7 (bone morphogenic protein 7)[31].

On the other hand, it was demonstrated that, outside of the brown adipocyte clusters, specific stimuli can induce the common white adipocytes to turn into elements (by some denominated "beige"[32], by others "brite" adipocytes) which, without expressing factor MYF-5, behave like the true BAT cells[33]; in fact, they are UCP1-positive and increase the energy expenditure through the uncoupling of the oxidative phosphorylation[34] (Fig. 2).

Figure 2

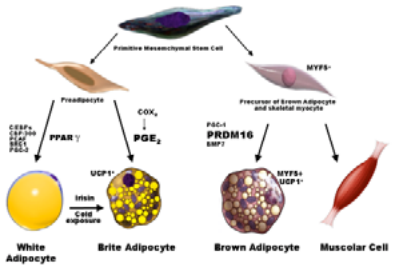

Figure 2 - Histogenesis of white, brown and "beige-brite" adipocytes

Between the stimuli that can induce the "browning" of the WAT there are the long cold exposure, the action of PGC1α (peroxisome proliferator-activated receptor-γ-coactivator 1alpha) or the activation of the cyclooxygenase-2, which results in the synthesis of prostaglandin PGE_2[35].

A prolonged physical exercise stimulates the secretion, by the muscle tissue, of irisin[36], a miokine with endocrine-like activity; it is capable of inducing, in white adipocytes, the activation of the PGC1α factor, which triggers the UCP1 expression and the acquisition of morphological and functional features of beige cells[37,38]. More recent observations tend, however, to indicate that the irisin activity is, in fact, relatively modest[39].

Experimental studies in mice in which UCP1 expression and BAT development were inhibited, as well as research on human UCP1 polymorphisms, suggest that brown adipose tissue likely plays an important role in preventing the accumulation of visceral fat and the insulin resistance[40].

The rising incidence, in Western countries, of obesity and related diseases makes the demand for effective drug therapies increasingly urgent.

The difficulties associated with maintaining highly restricted food intake and the so far disappointing results on clinical use of anorectic drugs explain the current interest in the biological processes that can increase calorie expenditure.

Unfortunately, the attempts to develop selective β3-adrenergic receptors agonists have not yet achieved the desired success.

The recent identification of factors that induce brown and beige adipocyte differentiation and activation has opened new perspectives in the search for an obesity cure[41].

Functional aspects

Adipogenesis

The white adipocytes originate from mesenchymal precursors, morphologically indistinguishable from fibroblasts, probably located near the vessels.

In the past it was believed that the proliferation of these stem cells occurred only in the prenatal, childhood and adolescent growth phases and that, instead, in the adult, in case of superabundant food intake, fat mass enlarge mainly through a volume increase of resident adipocytes.

In the contrary, today we know that, even after the development age, fat tissue holds staminal elements able to proliferate and differentiate into adipocytes[42,43,44]; it is estimated that the immature forms (mesenchymal totipotent precursors and committed but not yet differentiated preadipocytes) constitute, on average, between 15% and 50% of the total tissue volume, with large loco regional differences[45].

Until an equipoise is maintained between nutritional input and caloric needs, the consistency of fat cell population remains unchanged; this does not mean that it is static, nor that the elements that compose it are immortal. Their cell number is kept constant thanks to a dynamic balance between apoptosis[46,47,48] and neo-adipogenesis. In other words, an equilibrium exists between the parallel processes of death and regeneration: the newly formed fat cells replace those that disappear, with a turnover of around 10 percent a year[49].

If, however, the energy substrates introduced with the food exceed the requirements, the surpluses are initially stored through an increase in the size of the pre-existing adipocytes. Soon after, surpluses begin to be reversed also within new elements, generated from preadipocytes[50].

The neoadipogenesis entails an initial phase in which precursors proliferate, followed by the process of differentiation: the fusiform cells take on a rounded shape and accumulate triglycerides within gradually more and more voluminous lipid droplets. In parallel, other dramatic changes involve all cytoplasmic components, the cytoskeleton and even the structures of the surrounding extracellular matrix[51], which undergoes an intense remodeling[52], accompanied by the formation of new blood vessels[53,54]. Nuclear receptors PPARγ (targets of the oral antidiabetic drugs tiazolinediones) are the main neoadipogenesis inducers[55]. The natural ligands of these receptors appear to be n3-polyunsaturated fatty acids from food and eicosanoids derived therefrom.

The rate between the processes of hypertrophy and hyperplasia can move in one direction or another, according to many factors, including individual genetically determined variability, gender, age and, especially, the body site[56,57].

In principle, the increase in adipocyte number prevails in young subjects, in females and in the gluteal-femoral subcutaneous district: when the lipid material in excess is stored through neoadipogenesis the average cell size does not undergo significant increases and the local and systemic metabolic activities are not compromised.

Conversely a predominant tendency towards adipocyte hypertrophy, typical especially of visceral fat in middle and old aged obese man, predisposes the tissue to morphological and functional alterations, able to favour the occurrence of systemic diseases that are now included within the so-called metabolic syndrome.

An excessive cell volume growth rate (especially concerning visceral adipocytes) seems, therefore, to be one of the main triggers of the metamorphosis that brings cells normally engaged in tasks essential to the organism survival to become “sick fat”[58], a serious nuisance for general homeostasis[59].

The change in fat cell size is an expression of the equilibrium between the processes that lead to the gather of triglycerides (lipogenesis) and those that determine their hydrolysis (lipolysis). This balance

is defined by nutritional status and is regulated by endocrine factors, including catecholamines, insulin, steroid hormones, thyroxine and some adipokines.

Lipogenesis

The first step of the process is represented by the uptake from blood of free fatty acids (FFAs), while their neo-synthesis into adipocytes from glucose is very limited in the human species.

The lipoproteinlipase (LPL), synthesized by adipocytes and transferred to the endothelial cells, removes FFAs from triglycerides carried by plasma lipoproteins, i.e. chylomicrons and VLDL (very-low-density lipoprotein). The FFAs hydrolysed enter within the adipocytes, by passive diffusion and active transport. FFAs are, then, converted into acylCoA and, finally, within the endoplasmic reticulum, they are linked to glycerol-3-phosphate, obtained from glucose metabolism. The triple esterification of glycerol leads back to the formation of triglycerides that are stored within the lipid droplets.

As already mentioned, these are not simple molecular accumulations but are real organelles, surrounded by a phospholipids' single layer[60], on the surface of which is bound an entire pool of specific proteins: the PAT family[61], so called from the initials of the most representative species. Among these, the most abundant, on the coat of the mature lipid droplets, is perilipin A[62].

The PAT proteins play an essential role in the sequence of events leading to the birth and to the development of lipid droplets, also participating actively in the process of lipolysis.

Insulin exerts a powerful stimulating action on the activity of LPL and, therefore, on the uptake of FFAs and the subsequent liposintesis[63].

Lipolysis

In humans, the major hormones involved in lipolysis activation are the catecholamines (epinephrine and norepinephrine) that, at the cellular level, interacts with four varieties of adrenergic receptors: β1, β2, β3 and α2.

The three β subtypes, and particularly the β2 one, transmit a lipolytic stimulus, while α2 units send off a contrary signal; the adipocyte is the only cell that hosts simultaneously, on its membrane, agonist and antag-onist adrenoceptors. The amount of triglycerides hydrolyzed depends on the local balance between these opposite inputs[64].

The β receptors are coupled to an excitatory G protein, which activates the membrane enzyme adenylate cyclase, leading to an increase of the pool of cAMP (cyclic adenosine monophosphate), which, in turn, triggers the protein kinase A (PKA).

he 2 receptors have opposite effects: being coupled to an inhibitory G protein, block the adenylate cyclase, reducing the availability of cAMP and, therefore, the PKA activity[65] (see Fig. 3).

Figure 3

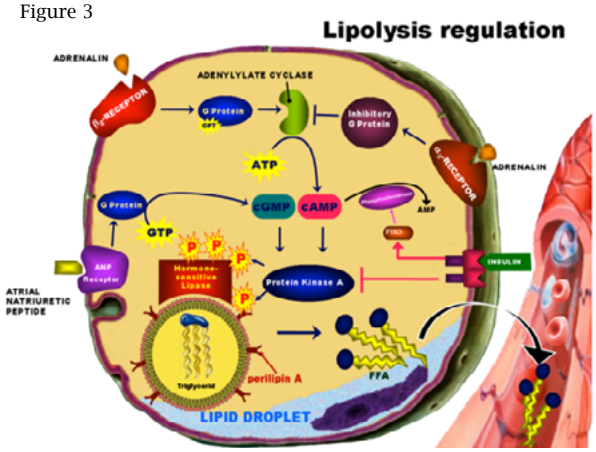

Figure 3 - Mechanisms of regulation of adipocyte lipolysis

Under normal conditions, the β2 receptor is the most important promoter of the lipolytic activity and the adrenaline is its main ligand. The same hormone, however, also excites the inhibitory α2 adrenergic receptor. The balance between lipolytic and antilipolytic catecholamine stimulation depends, in a determined district, from the numerical ratio between β2 and α2 receptors and / or from local variations in their sensitivity.In addition to catecholamines, other substances may influence, in a positive or negative sense, the hydrolysis of triglycerides, for the most part via post-receptorial mechanisms, which modify the cAMP concentration.

Among these, insulin, which represents the most powerful antilipolytic hormone: its binding to the specific receptor increases the function of phosphodiesterase 3B; this constitutionally active enzyme, forms part of a system of counter-regulation, used to degrade cAMP as it is being formed[66]. Such an action mode explains how phosphodiesterase inhibitors 3, for example aminophylline and theophylline, blocking the removal of cAMP, maintain its high availability, in order to determine a prolongation of the lipolytic stimulus[67]. Another mechanism of modulation of triglyceride hydrolysis is put in place by adenosine: by acting on a specific receptor, it exerts a strong antilipolytic effect, by inhibition of the adenilitatocyclase enzyme and, therefore, of the cAMP production. Caffeine and theophylline are adenosine antagonists (see Table 1). Finally a strong lipolytic agent is constituted by atrial natriuretic peptide: it excites a specific receptors, coupled with a G protein that, in turn, activates the guanylate cyclase enzyme. The result is the production of cGMP, similar, as regard the action, to the cAMP[68].

ADRENERGIC RECEPTORS AGONISTS AND POST-RECEPTOR MODU-LATORS	
noradrenalin	non-selective beta and alpha-adrenergic agonist
isoprenaline	non-selective beta-adrenergic agonist
propanolol	beta-adrenergic antagonist
clonidine	selective alpha-2 agonist
yohimbine	selective alpha-2 antagonist
forskolin	direct adenylate cyclase activator
aminophylline	selective inhibitor of phosphodieste-rase
dibutiril-AMP	stimulator of protein kinase A
adenosine	adenylate cyclase inhibitor
N6-(l-2-fenilisopropil) adenosine	adenosine receptor agonist
caffeine	adenosine antagonist

Table 1 - Adrenergic receptor agonists and antagonists and post-receptor modu-lators capable of interfering on lipolysis

Until a few years ago it was believed that the hydrolysis of triglycerides in adipose tissue was made solely by sensitive lipase hormone (HSL).

This assumption has proved inaccurate when it was discovered that, in mice genetically devoid of HLS, basal lipolysis is not compromised.

In fat cells of these animals do not show a growing accumulation of unhydrolysed triglycerides: rather the content of diglycerides (DG)[69] raises; this can be explained taking into account that the HLS is much more efficient in the lipolysis of DG than of TG.

In 2004, three research groups identified in adipocytes another lipolytic enzyme, called Adipose Triglyceride Lipase (ATGL), endowed with specific capacity for the hydrolysis of the TG[70]. Subsequent studies have recognized the presence of additional enzymes (for example, a monoglyceride-lipase), and a large number of cofactors. Based on this set of acquisitions, it now seems clear that the adipocyte lipolysis is a much more complex and articulated process than previously thought.

The sequence of events leading up to the release of FFAs in blood circulation, based on current knowledge, it seems to follow the pattern summarized in Figure 4.

The catecholamine stimulation leads, as has been said, to the synthesis of cAMP and to the activation of PKA; the latter determines the phosphorylation of perilipin A.

This allows the cofactor CGI-58 to detach from the perilipin A, in order to go to tie to the ATGL: the complex ATGL/CGI-58 is fixed on the surface of the lipid droplet and start the lipolysis, unplugging a first fatty acid chain by TG. In this way molecules of DG are obtained.

At this point, the PKA phosphorylate also the HLS:

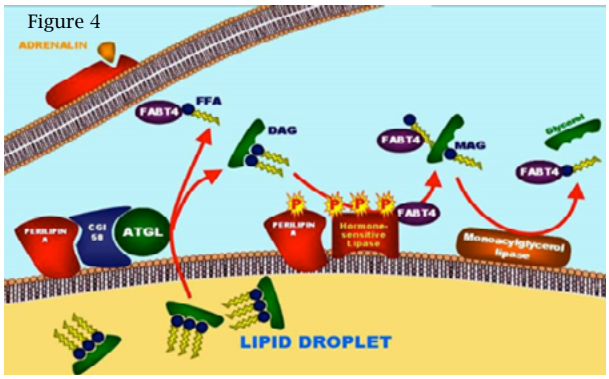

Figure 4 - Various lipase work in sequence to hydrolyze the three molecules of fatty acids from triglycerides

this allows this enzyme, constitutionally inactive, to move from its normal cytoplasmic position and to bond on the surface of the lipid droplet[71].

Here its main task is to promote the separation of a second fatty acid chain by DG, turning them into monoglycerides (MG). The terminal phase of lipolysis is entrusted to a monoglyceride lipase which hydrolyses the last-fatty acid chain, generating FFAs and glycerol.

In this complex sequence of events, also other protein cofactors participate, including the lipotransin, which helps to promote the translocation of HLS from the cytoplasm to the surface of the lipid droplet, and the fatty acid-binding protein 4 (FABP4) which acts as an intra cytoplasmic FFA transporter.

Insulin and adipose tissue

Adipose tissue is a major target of insulin and demonstrates, under normal conditions, an extreme sensitivity to this hormone.

Insulin stimulates the uptake of glucose by adipocytes, causing the translocation towards the membrane of the GLUT4 receptors; starting from the glucose, adipose cell can, thus, achieve glycerol and synthesize new fatty acids that are then esterified into triglycerides.

FFAs, however, in the human species, are primarily taken from the circulation, since the neo-lipogenesis capacity of adipose tissue seems to be much smaller than it is observed in rodents.

The sharp increase in fatty acid synthesis induced by insulin is, usually, almost all, done in the liver, where the hormone increases the uptake of glucose, which is employed for the production of FFAs; these, in turn, are used to synthetize triglycerides which are finally assembled in the VLDLs.

Lipoproteins transport to the adipose tissue the lipids produced by the liver, together with those coming from the diet. As mentioned above, the

triglycerides are not absorbed directly by adipocytes, but they are first hydrolyzed by an extracellular lipoprotein lipase. Then, enter the cell as fatty acids, in order to be, again, esterified to glycerol, in the endoplasmic reticulum.

Insulin exerts a powerful stimulating action on the LPL, increasing, thus, the storage of FFAs in the fat.

At the same time, the hormone inhibits lipolysis, triggering the phosphodiesterase: the consequent reduction in the availability of cAMP prevents the phosphorylation processes required to activate the lipolytic enzymes.

Other hormones, such as glucocorticoids, are able to increase the activity of the LPL[72].

Abnormalities in adipocyte differentiation, proliferation and biological functions, such as those that occur in conditions characterised by a fat tissue expansion or excessive (obesity) or inadequate (lipodystrophy) or altered in its regional distribution are frequently associated with insulin resistance, resulting, therefore, in disorders of metabolic homeostasis and in predisposition to type 2 diabetes.

Adipokines

Until a few years ago, the adipocytes were only considered deposit for energy reserves, stored as triglycerides in the postprandial phase and mobilized in the form of free fatty acids (FFAs) during post absorptive and starvation periods.

In fact, already more than twenty years we have begun to understand that adipose tissue is not a mere stock, but is an active endocrine organ, the largest and most versatile of the whole body, capable of producing important molecules, called adipokines[73], which are involved in the regulation of all phases of the calorie substrates management, by tuning the sense of hunger and thus the food intake, the energy expenditure, the fat transport, storage and oxidation, the carbohydrates synthesis and utilization, the insulin sensitivity[74].

Data were acquired which indicate that the adipose tissue is able to realize significant systemic actions going beyond the energy metabolism, by the means of its influence on the functions of circulatory system, immune system[75], kidney, gonads and scaffold bone, with important impacts even on cell proliferation, on inflammatory processes[76,77], on reproduction and on the pathophysiology of ageing[78].

Adipokines exert both autocrine and paracrine actions, as they modulate the biological functions of adipocytes themselves and of stromal cells (e.g., macrophages), both a true endocrine activity, since they, once discharged into the circulatory flow, affect distant organs. The adipokines targets are numerous and include central nervous system, pancreatic islets, liver, skeletal and cardiac muscle, endothelium and blood.

A particular importance is assumed by the hormones involved in the maintenance of adipocyte metabolism through modulation of insulin action: some adipokines increase insulin sensitivity (such as adiponectin and leptin)[79], while others reduce it, triggering insulin resistance (e.g., resistin, TNF-α, IL-6)[80,81].

Among the substances with endocrine and/or paracrine activity released from adipose tissue, are included: leptin[82,83], adiponectin[84,85], resistin[86,87], IL-6[88], TNF-α[89,90,91,92], and soluble receptor for TNF-α[93], FIAF (fasting-induced adipose factor)[94], MCP-1 (monocyte chemoattractant protein-1), osteopontin[95], ASP (acylation-stimulating protein)[96], visfatin[97], apelin[98], RBP4 (retinol binding protein 4)[99], adipsin[100], vaspin (visceral adipose tissue-derived serine protease inhibitor)[101], chemerin[102], prostaglandins[103,104,] lipoprotein lipase[105], angiotensinogen and angiotensin II[106],FGF(fibroblastgrowthfactor)[107],TGF-β(transforming growth factor-beta)[108], PAI-1 (plaminogen activator inhibitor-1)[109], VEGF (vascular endothelial growth factor)[110,111], MMP (matrix metalliproteinases)[112,113].

It is important to note that some of these substances, in addition to being directly released by adipocytes, are also (and, in some cases, mostly) secreted by the stromal cells or by macrophages, which, as it will be said later, infiltrate extensively visceral fat of obese people. Adipose tissue is the main site of leptin synthesis.

Serum levels are higher in obese subjects, showing a positive correlation with BMI. The production and secretion of leptin are regulated by calorie intake, reaching a peak within 12 hours after the meal, but also from various substances: sex hormones, insulin, glucocorticoids, TNF-α and IL-6 increase them, while catecholamines, thyroid hormones, GH and testosterone inhibit them. In the brain, particularly in the arcuate nucleus of the hypothalamus, leptin has the task of en-hancing energy expenditure and of inducing satiety, through the release of anorectic agents, such as α-MSH (α-melanocyte-stimulating hormone) and CART (cocaine and amphetamine-regulated transcript) and the inhibition of neurotransmitters that stimulate hunger, as NPY (neuropeptide Y)[114]. The strains of mice carrying mutations in the genes for leptin (ob/ob) or for its receptor (db/db) show massive obesity.

In humans, obese individuals probably have a leptin resistance condition in the brain.

The receptors of leptin are found, however, in many other locations: liver, fat, muscle, pancreas, spleen, lung, ovaries, adrenal glands, immune system and endothelial cells. This indicates that leptin is a pleiotropic molecule, not just a satiety hormone; indeed, many mouse leptin-deficient strains, in addition to hyperphagia and severe obesity, also display alterations in the reproductive, immune, hormonal and nervous functions[115].

Adiponectin is a protein belonging to the family of complement factor C1q and is secreted exclusively by adipocytes: circulating levels are very high in the healthy, while are significantly reduced in obese and diabetic people. Recently it was shown that for the adiponectin synthesis and secretion it is essential that the cells retain a good mitochondrial function.

There are two types of specific receptors, localized, respectively, in the liver and in the muscle.

Adiponectin elevates insulin sensitivity in adipose tissue, muscle and liver; it favours lipid oxidation and reduces the expression of adhesion proteins on endothelial cells, in which it stimulates the production of NO and counteracts the effects caused by TNF-α and by oxidized LDL.

Adiponectin also blocks the monocyte differentiation and the formation of foam cells.

It inhibits the activity of the MMP enzymes, protecting the atherosclerotic plate from breaking; it has also an antithrombotic action, reducing platelet aggregation[116].

As mentioned above, in obese patients, especially with visceral adiposity, circulating levels of adiponectin are lower than normal; this condition is associated with increased risk of diabetes, reduced peripheral glucose uptake and decreased muscle fatty acids oxidation[117].

Visfatin has similar effects to insulin; it appears to activate the insulin receptor, binding it in a different site than insulin. Since circulating levels are significantly lower than required by its modest affinity for the receptor, it is likely that visfatin may act via paracrine or autocrine, rather than endocrine way. The visfatin production appears to be specific of abdominal fat depots; in fact, visfatin plasma concentrations correlate with the WHR index[118].

The activities of resistin are not yet fully known; most of the information comes from studies on rats, in which resistin seems to have inflammatory and diabetogen effects. In rodents, the circulating levels of resis-tin are proportional to the degree of obesity and its role in the development of insulin resistance has been amply demonstrated[119].

However, differences of opinion remain, on the role that such adipokine takes in humans, where its production is made not only by adipocytes, but also from blood monocytes, macrophages and neutrophils. While some suggested that the plasma levels of resistin[120] and/or individual variations in its amino acid composition[121] can affect the onset

Main Adipokines		
Adipokine(s)	**Site of Action**	**Function**
Leptin	Hypothalamus	Represses hunger, increases energy metabolism
	Immune system	Keeps immune system up-regulated
	Cardiovascular system	Anti-inflammatory effect
	Endocrine system	Regulates puberty and reproduction
	Skeletal muscle	Improves insulin sensitivity
Adiponectin	Immune system	Decreases the release of inflammatory molecules
	Skeletal muscle	Increases fatty acid oxidation, glucose uptake, and lactate production
	Liver	Reduces the levels of molecules involved in gluco-neogenesis, increases free fatty acid metabolism
	Cardiovascular system	Anti-atherosclerotic effect
Resistin	Immune system	Stimulates inflammation
	Cardiovascular system	Impairs vascular relaxation
Retinol-binding protein 4	Plasma	Transports vitamin A
	Skeletal muscle	Impairs insulin signalling
Tumor necro-sis factor alpha (TNF-α)	Skeletal muscle	Impairs insulin signalling
Visfatin	Skeletal muscle	Binds to insulin receptors and mimics insulin
	Immune system	Causes release of TNF-α and interleukins (inflam-matory signals)
Interleukin 6	Skeletal muscle	Impairs insulin signalling
Angiotensinogen and angiotensin II	Vascular system	Induces smooth muscle cell contraction and raises blood pressure
	Adipose tissue	Pro-inflammatory effect
Free fatty acids	Skeletal muscle	Promotes insulin resistance
	Liver	Promotes insulin resistance

Table 2 - Main substances produced by adipocytes (sometimes also by stromal cells)

of obesity and diabetes, others do not confirm these observations[122].

Recent data suggest that resistin is involved in inflammatory processes. In adipose cells and in monocytes it stimulates the production of TNF-α and IL-6; furthermore, the expression of this adipokine is associated with other inflammatory markers, such as PCR, rising in patients with inflammatory gut disease and coronary heart disease.

Besides the production of adipokines, a further significant endocrine activity recognized in the adipose tissue is related to the presence of an aromatase involved in sex steroids bioconversion[123].

Anatomical and functional differences according to the site

In the human species lipid deposits show morphological and physiological disparities, depending on body region and gender.

From the anatomical and physiological point of view, in adipose organ two main areas can be identified: visceral and subcutaneous fat[124].

In the context of both, some districts can be further distinguished by not completely overlapping characteristics[125,126].

For example, within subcutaneous tissue, significant functional differences appear to exist between the depots of the upper body (and, in particular, of abdominal wall) and those of the gluteal-femoral zone[127].

Visceral adipose tissue, in turn, comprises omental and mesenteric fat, tributary of the portal venous system, and the retroperitoneal and perirenal fat, whose effluent blood is drained from the veins of the systemic circulation. Metabolically similar, in some ways, to the visceral adipose tissue are the perivascular, pericardial, mediastinal, cervical and pelvic (gonadal, epesididymal, urogenital) localizations[128].

Researches conducted in vitro (on adipocytes taken from different areas) and in vivo (using microdialysis experiments or metabolic studies with labelled tracers) indicate that both lipogenesis, both lipolysis are regulated in a dissimilar way in men compared to women and in splanchnic compartment compared to subcutaneous[129].

The biological specificity that the adipocytes assume in different locations seems to be determined by a combination of factors, including cell morphology, innervation, vascularization, nature and amount of membrane receptors, intracellular signal transduction pathways, gene expression patterns[130], secretory capacity, etc.

Women generally show a greater adiposity than men; in addition, they are characterised by a reduced amount of visceral fat and by an higher proportion of subcutaneous adipose tissue, mainly localized in the lower body ("gynoid" or "pear-like" habitus)[131,132,133].

In contrast, men accumulate more ample deposits of fatty tissue in the central or abdominal site, assuming, therefore, an "android" or "apple-like" habitus, which is associated, statistically, with a higher risk of metabolic complications[16,134,128].

These two opposing models of fat distribution occur from puberty, making clear the role that, in their genesis, sex hormones play[135,136].

In particular, it is clear that the female prevailing deposition of subcutaneous fat in the gluteal-femoral region is related to the ovarian estrogen production[137].

In menopausal women, characterised by a drop in the levels of circulating estrogen, there is an increase of visceral adiposity, which results in the gradual development of an android fat distribution; this change is prevented or restricted in those taking an hormone replacement therapy[138].

In genetically male transsexuals who want to take on female secondary sexual characteristics, after one year of treatment with estrogens it is evident a predominant localization of fat in the subcutaneous of the lower limbs[139,140].

The androgens, however, determine, on the adipose tissue distribution, different effects, depending on gender[141]. In men, testosterone declines with age and this decay is accompanied by an increase of body fat, which is mainly localized in the abdomen[142,143]. In elderly males the testosterone replacement therapy causes a decrease in visceral fat deposits and an increase in lean muscle mass[144].

Conversely, in females, testosterone administration induces a significant increase in splanchnic fat[145] that is also found in women with polycystic ovary syndrome (PCOS), characterized by an overproduction of androgens[146,147].

Finally, animal studies show that a prenatal testosterone administration to female rats causes, in adulthood, a male pattern of fatty deposits distribution[148].

Numerous studies have shown that, in both sexes, the adipocytes express receptors for gonadal steroids; in particular, they are equipped with the androgen receptors and with the various types of estrogen receptors: both nuclear receptors (ERα, ERβ and its variants), both membrane G protein-coupled receptors[149.] Their expression and activity differs, however, depending on gender and on site: in general, the ERβ receptors are most represented in females. In both sexes, the presence of ERβ1, ERβ4 and ERβ5 (evaluated by determination of mRNA and protein) is much greater in subcutaneous than in visceral adipose tissue[150]. Conversely, the abdominal fat contains more ERα proteins than the gluteal-femoral one[151]. Consequently, the ratio between

waist and hips circumferences (WHR) appears correlated to the ERα/ERβ ratio, pointing out that estrogen receptors play a role in modulating the regional fat distribution. Overall, estrogen activity is more in-tense at level of the female lower limbs subcutaneous, also by the means of an high expression, in this area, of P450 cytochrome, one of the components of aromatase, the enzyme complex that converts circulating androgens (androstenedion and testosteron) into estrone and estradiol, respectively[152].

Estrogens stimulate the proliferation of preadipocytes; this mitogen effect has been demonstrated both in visceral, both in subcutaneous fat, but it appears much more pronounced in the latter, especially in females[153].

It is also possible that sex steroids influence adipose tissue biology primarily by means of effects on the central nervous system, rather than through direct action on adipocytes.

For example, in animal models, estrogens act on neurons of the ventromedial hypothalamic nucleus, increasing thermogenesis in brown adipose tissue, so as to limit the accumulation of lipids in visceral adipose tissue[154].

The visceral adipocytes are greater in men than in women[155]; in obese male they can achieve diameters larger than 120 μm.

In the subcutaneous, adipocytes sizes exhibit gender differences and local variation which vary depending on body weight: in lean subjects, generally, it is observed that the female gluteal-femoral adipocytes are larger than male's and are larger even than adipocytes in abdominal subcutaneous (whose dimensions are comparable in men and women). In overweight individuals, the relationship between cell volumes in different subcutaneous deposits tend to change: in obese women it is observed that the adipocyte diameter in the abdominal panniculus grows in proportion to the BMI, while in gluteal-femoral fat it remains almost constant[156].

To determine these cell size differences, the diverse capacity of neo adipogenesis in the various regions assumes a considerable importance.

Fat cells are subject to a continuous turnover: every year about 10% undergoes apoptosis and is replaced by new-born elements[49].

The production of new adipocytes is also employed to cope with any increase in fat stores due to an excesses in food intake; in this respect, however, visceral adipose tissue differs, in behaviour, from subcutaneous, within which abdominal panniculus shows a further different answer mode compared to the gluteal-femoral fat. While, in fact, overeating determines in the adipose tissue of the upper body (i.e. in visceral fat and, to a lesser extent, in the abdominal subcutaneous) an expansion mainly due a diameter rising of the pre-existing fat cells, the lower limbs subcutaneous thickens mainly through a proliferation of new adipocytes, whose mean cell volume grows only in a limited measure[157].

Therefore, following a high fat diet, in the abdominal adipose tissue the proliferation of pre-adipocytes appears very modest and poor is also their differentiation capacity, while their sensitivity to apoptotic stimuli is high[158]. Just apoptosis may be the cause of an early exhaustion of the stem cell pool, that, according to some, would explain the scarcity of preadipocytes. These progenitor cells, on the contrary, are considerably more numerous in the gluteal-femoral subcutaneous, where also they show a greater capacity for proliferation and differentiation and a lower susceptibility to apoptosis[159].

About the factors that determine these differences there are data with contrasting meaning.

Some experiments demonstrate that, contrary to expectations, in vitro, adipocytes taken from the abdominal subcutaneous show a greater replicative activity than fat cells from the gluteal-femoral region[160].

The more dynamic hyperplastic response in vivo expressed by the latter would be due, therefore, not to the intrinsic properties of preadipocytes, but to a series of micro-environmental factors, relating to the innervation, vascularization and the composition of the structure stromal vascular.

Other studies, however, reveal that preadipocytes from various districts show significant differences in gene expression, regarding, in particular, the so-called developmental genes, characterized by a common polynucleotide sequence (HOX homeodomain); the various members of the HOX family are activated at different times and in different tissues during embryogenesis and, in some cases, remain functional in adults[161,162].

The diverse patterns of gene expression that characterize the adipocytes in distinct regions of the body are acquired prenatally by tissue stemcells and are kept unchanged through repeated cycles of replication and, even, during the in vitro cultivation; this tends to demonstrate that the fat deposits in the various locations are derived from intrinsically different precursors[163] and that the morphological and functional diversities between visceral and abdominal subcutaneous fat and between the latter and the gluteal-trochanteric fat are programmed via epigenetics[164,165].

It is reasonable to suppose that the preferential accumulation of fat in one or another district depends on the local balance between the amount of lipid material which is deposited and the amount which, instead, in the same span of time, is released through lipolysis. Both processes are fine-tuned by neuroendocrine mechanisms.

The triglyceride storage into adipocytes follows, in large part, to the uptake, by the lipoprotein lipase, of the fatty acid carried by plasma lipoproteins[166]. The LPL expression in male is more intense within the adipocytes of the upper body[167,168], while in women it is superior within the cells of the gluteal-femoral region169,170, which also show a greater ability to operate the direct uptake of the free fatty acids carried in blood flow by albumins during postprandial phases[171].

As mentioned above, women have a higher body fat percentage; one would be led to believe that this is realized as a result of a more modest lipolytic activity than men's. On the contrary, in resting state, at equal energy expenditure rate, lipolysis is, overall, considerably more intense in females (about 40%)[172].

The resulting high output of circulating free fatty acids does not involve deleterious metabolic effects, also because women, in times of high energy demand, such as during exercise, preferentially oxidize lipids, while men tend to use more carbohydrates[173,174].

The conspicuous female lipolytic activity is, however, limited only to the upper body subcutaneous; in both sexes, this is the main source of circulating free fatty acids[175], but the triglyceride hydrolysis therein caused by the administration of norepinephrine is much greater in women[176].

Both males and females show a very high norepinephrine-induced triglyceride hydrolysis in visceral adipose tissue; as this is, in large part, a tributary of the portal circulation, lipolysis cause an increases of liver fat deposits and of lipoprotein synthesis, rather than of circulating free fatty acids.

Far less is, instead, the lipolytic action exerted by catecholamines on female subcutaneous of lower limbs[177]. The fairer sex people, in fact, express in the fat cells of gluteal-femoral hypodermis, a 40 times higher antilipolytic $\alpha 2$-adrenergic sensitivity than in abdominal subcutaneous, where, instead, prevails the presence of the β-adrenergic receptors, which stimulate lipolysis; consequently, the sympathetic system exerts, on the lower limbs, an effect at least in part contrary to the release of free fatty acids[178].

These observations have been confirmed by in vivo researches demonstrating that catecholamine administration, as well as prolonged fasting or strenuous exercises, induce a more modest lipolysis in gluteal-femoral subcutaneous than in upper body fat[179,180]; this occurs both in women both in men, but in the former the difference is much more relevant[181].

In determining a dissimilar loco-regional lipolysis, in addition to the differential expression of the two main classes of catecholamine receptors, also comes into play the diverse sensitivity to the antilipolytic action of insulin, which, especially in obese patients, is much smaller in visceral tissue.

Another element that contributes to making higher the triglyceride hydrolysis in splanchnic fat is represented by the increased expression, in this tissue, of the hormone-sensitive lipase (key enzyme in lipolysis[174]) and of perilipin A (a protein located on the surface of the lipid droplets[182]). The fatty acids that are obtained from the cleavage of triglycerides are intended to be used as energy material in the intervals between meals and in the starvation.

In normal weight individuals, the adipose tissue located above the waistline, characterized by a quick turnover of the lipid material, is in charge of a daily short-term management of fatty acids from food, that are sequestered in the post prandial phase and immediately released during the post-absorptive time[183]. Conversely, the accumulation in the gluteal-femoral area assumes, especially in women, the significance of a long-term storage.

In the obese people, while the lipolysis by the gluteal-femoral subcutaneous does not greatly exceed that observed in lean individuals, the upper body fat releases a free fatty acid flow proportional to its mass. The excessive amounts of FFA poured in the blood may contribute to the negative metabolic consequences of obesity, leading to ectopic accumulation in the liver and in peripheral sites and, consequently, to lipotoxicity phenomena.

Visceral adipose tissue, a tributary of the portal circulation, causes, thus, lipotoxic damage in the liver (non alcoholic fatty liver disease, in acronym NAFLD); it provokes in this way, an increase in lipoprotein synthesis by hepatocytes, but contributes only 13% to the influx of free fatty acids into the large circulation which, for the most part, comes, as has been said, from the upper body subcutaneous.

The panniculus of the gluteal-trochanteric region, not very sensitive to the "ordinary" lipolytic stimuli, is a "storeroom" that, if needed, can boost its size through adipocyte hyperplasia; it can, therefore, rake in the lipid material in excess, avoiding that fat goes to form ectopic deposits in other tissues. In this way, the lower limbs subcutaneous carries out a crucial protective function.

Evidence suggests that also the qualitative and quantitative differences in the adipokine secretion may help explain the diversities regarding the metabolic consequences of the lipid accumulation in the upper body vs. the lower body[184,185].

Generally the synthesis of leptin, adiponectin and IL-10 prevails in gluteal-femoral subcutaneous, especially in females, while in the visceral fat the expression of inflammatory cytokines is higher and contribute to determine the local tissue remodelling, and, on a systemic level, to induce the insulin resistance[16,186].

Metabolic activity	
basal lipolysis	UBS & VF > LBS
activation of lipolysis by catecholamines	UBS & VF > LBS
inhibition of lipolysis by insulin	LBS > UBS > VF
presence of lipolytic β2-adrenergic receptors	UBS & VF > LBS
presence of anti-lipolytic α2-adrenergic receptors	LBS > VF & UBS
activity of lipoprotein lipase in the male	UBS & VF > LBS
activity of lipoprotein lipase in the female	LBS > UBS> VF
release of FFAs in the portal circulation	VF
release of FFAs into the general circulation	UBS >> LBS
neoadipogenesis capacity	LBS > UBS > VF
production of leptin	LBS > UBS > VF
production of adiponectin	LBS > UBS > VF
production of IL-6	VF > UBS > LBS
production of TNF-α	VF > UBS > LBS
production of angiotensinogen	VF > UBS > LBS

Table 3 - Regional functional characteristics of adipose tissue
VF = visceral fat; UBS = upper body subcutaneous; LBS = lower body subcutaneous

Sick fat and metabolic syndrome

The increasing prevalence of obesity in industrialized countries has become a serious public health problem. In the period from 1976 to 2002, the prevalence of obesity (BMI> 30 kg/m^2) in the US population has risen from 15% to 31%[187,188], while the prevalence of overweight (BMI> 25 kg/m^2) increased from 46% to 66% (17.1% in children).

The excessive accumulation of fat, as is known, constitutes the most important factor in predisposition to diseases and to premature mortality.

Visceral adiposity

Since the time of the observations by Jean Vague[189], around 1950, we know that, from a physiopathology perspective, the quality of the adipose tissue is more important than its amount: it appears evident, in fact, that there isn't only one type of obesity, but that more variants exist, each of which has its phenotypic elements, is characterized by particular pathophysiology and is burdened with specific complications[190].

In particular, it is shown that the closer relation with the old age diseases is established not by the fat mass as a whole, but by the fatty tissue that accumulates at the central level, in the abdominal (splanchnic but also subcutaneous) district.

Central obesity (also called android because more common in males), is associated, in fact, to insulin resistance, dyslipidaemia, hypertension, inflammation: these conditions, in turn, result in an increased incidence of diabetes mellitus, of atherosclerosis (with its procession of ischemic consequences) and of some of the most common forms of cancer[191,192].

In support of these conclusions, there are the findings from a large number of epidemiological studies (to name a few, Hartz[193], NHANES III[194], Goteborg[195], Health Professional Study[196], Nurses' Health Study[197], Hoorn Study[198]) that, in any part of the world, have examined several tens of thousands of subjects, of both sexes and various ages; in all cases, a close correlation was found between the anthropometric indicators of android obesity and the incidence of diabetes and of cardiovascular disease.

The distinction between central and peripheral obesity is relatively easy and is based on the objective examination of the subject and, in particular, on the mensuration of abdomen circumference alone or on the WHR *ratio*, i.e. the ratio between the waist circumference (measured, in general, to the umbilical level) and the hips circumference (measured at the height of greater trochanters).

Abdominal circumferences > 102 cm in men and > 88 cm in women identify the abdominal obesity, although, more recently, stricter limits (94 and 80 cm respectively) have been recommended by some.

Values of WHR > 1.0 in men and > 0.90 in women (or, more stringently, > 0.95 and > 0.85, respectively) are indicative of central fat distribution, regardless of the amount of total body fat and the presence of obesity.

Lately, the use of imaging techniques such as computerized axial tomography (CT), magnetic resonance imaging (MRI), dual-energy x-ray absorptiometry (DEXA), or, (with results much less accurate) as ultrasonography, have allowed a more detailed distinction between abdominal subcutaneous adiposity and visceral obesity.

First Gerard Reaven in 1988, described, in subjects suffering from android obesity, the frequent association among seemingly unrelated alterations, such as impaired glucose tolerance, hyperinsulinemia, hypertension and hyperlipidaemia (with elevated triglycerides and cholesterol, accompanied by a reduction of HDL)[199]. According to the hypothesis formulated by Reaven and afterwards widely validated, these conditions, the common denominator of which is insulin

resistance, exert synergistic pathogenic action towards cardiovascular diseases and constitute a unified framework, which Reaven described as syndrome X and today is better known as Metabolic Syndrome.

Adipose tissue and insulin resistance

Insulin resistance can be defined as the condition in which the hormone, despite a quantitatively normal or higher than normal secretion, is unable to carry out its activity, in particular at the level of adipose tissue, muscle and liver. This results in a reduced glucose tolerance, failure in postprandial suppression of lipolysis, dyslipidaemia.

Insulin is the most powerful anabolic hormone in the body and plays a significant role in the regulation of the metabolism of all the main nutrients (glucose, fat, amino acids); it also influences the cell growth and differentiation, as well as the endothelial functions.

Insulin elicits various biological responses by binding to the alpha subunit of a specific receptor, in order to stimulate the tyrosine kinase activity of the beta subunit, which starts the next transmission sequence, through phosphorylation of various substrates; among them the four IRS (insulin receptor substrate) proteins whose distribution is tissue-specific.

The two main transduction chains thus initiated are headed respectively by PI3K (phosphatidil-inositol-3-kinase) and by the MAPK (mitogenactivated protein kinase). Especially the first signalling pathway plays a crucial role in the most important metabolic actions of insulin, including the translocation to the plasma membrane of the GLUT 4 transporter (which uptakes glucose), the synthesis of glycogen, triglycerides and proteins and the antiinflammatory and vasodilator effects[200].

Except for rare cases, in which antibodies against receptor proteins or mutations of related genes come into play, insulin resistance is due to alterations in intracellular signalling pathways subsequent to the interaction with the hormone receptor. The ensuing metabolic abnormalities result from the insulin activity defect, in the districts where the insulin resistance is most manifest, together with the harmful impact of compensatory hyperinsulinemia on tissues that retain an almost normal responsiveness.

It is now believed that the primary cause of metabolic syndrome is represented by visceral obesity[201], which is able to produce its disastrous systemic effects through three main mechanisms:

a. An altered secretion of adipokines: hypertrophic and dysfunctional adipocytes stop producing adiponectin, an hormone that normally protects the peripheral insulin sensitivity and performs anti-inflammatory and vessel protective effects. In the visceral fat, however, a rise occurs in the synthesis of resistin, angiotensino-gen II, leptin, IL-6 and TNF-α, factors that can contribute, each with own mode, to the functional changes that underlie insulin resistance and metabolic syndrome[202].
b. The induction of a pro-inflammatory systemic condition: in obese individuals, the adipose tissue, especially the splanchnic one, is widely infiltrated by a significant population of macrophages together with lymphocytes and mast cells[203]. The stimulus that causes this inflammatory reaction is not clear: it is assumed that the expansion of adipose mass is only partly offset by a parallel angiogenesis[204]. The critical factor is probably represented by adipocyte size: splanchnic fat shows little neo adipogenesis capacity. In case of excessive caloric intake, therefore, the lipid surplus is raked in through an increase of the average cell diameter, as long as this exceeds the distance within which it is possible an appropriate oxygen diffusion. Adipocyte hypertrophy thus cause hypoxic tissue conditions[205,206], perhaps even aggravated by the micro-vessels crush and by leukocyte adhesion to endothelium. This results in the secretion by adipocytes of vasoactive factors, such as HIF-1α (*hypoxia inducible factor 1 alpha*[207]), ACE *(angiotensin converting enzyme*[208]) and leptin, as well as of angiogenic cytokines, especially VEGF (v*ascular endothelial growth factor*) and MCP-1 (*monocyte chemoattractant protein-1*), also able to attract phagocytes. The decisive role played by HIF-1α is shown by the fact that transgenic mice lacking this factor, when become obese, display, in the fatty tissue, a significantly reduced inflammatory reaction[209]. Hypoxia and excessive lipid load, provoking endoplasmic reticulum stress[210], activate NFkB factor (intracellular mediator of inflammatory phenomena)[211] and can come to cause the death by apoptosis of some adipocytes[212]. All these events help to attract into the adipose tissue a large number of blood monocytes, which are trans-formed into macrophages and take frequently a circle disposition around apoptosis undergoing fat cells[213] (*crown-like structures*, see Fig. 5). The macrophages that infiltrate visceral fat express markers indicative of their M1 polarization, which involves a distinctly inflammatory functional attitude[214]. The short chain saturated fatty acids released by adipocytes concur to determine the inflammatory reaction, since they are able to excite the toll-like receptors (cellular sensors normally deputies to recognize, in a non-specific way, the presence of pathogens)[215,216].

Figure 5

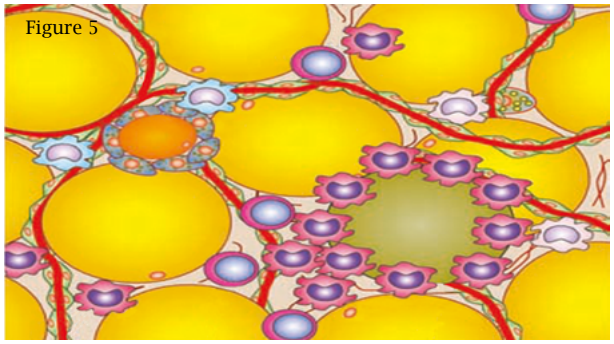

Figure 5 - Macrophages arranged in a crown-like disposition around an apoptotic adipocyte (Nishimura S. Discov Med. 2009;8:55)

A further stimulation of these receptors is due to lipopoly-saccharide produced by intestinal microbial flora, often impaired in obese individuals. The toll-like receptors activation is followed, both in macrophages both in adipocytes, by the prompting of intracellular transmission pathways which amplify the inflammatory reactions[217]. The hypoxia and the cytokines secreted by macrophages and by the hypertrophied adipocytes cause a deep restructuring of the intercellular matrix of adipose tissue (matrix remodelling) which also involves a wide-spread fibrosis[24,25,218]. The presence of tissue collagen I and collagen VI, as well as the expression of their mRNA, appear to grow exponentially[219], because macrophages become capable of inducing the adipose stem cells to transform into myofibroblasts[220]. This improved collagen deposition is started by means of the secretion of cytokines, such as TGF-β and osteopontin, capable of exercising an intense stimulatory activity of fibrillogenesis[221]. The inflammatory reaction of the hypertrophic fatty tissue determines strong repercussions on a general level, because the infiltrating macrophages and, to a lesser extent, the same dysfunctional adipocytes, pour into blood flow a large amounts of inflammatory cytokines, such as IL-6 and TNF-α[222], which not only induce a state of chronic systemic inflammation, but also alter the metabolism of the energy substrates in liver and muscle, helping to cause the insulin resistance[223].

c. The lipotoxicity: hypertrophic adipocytes, typical of the visceral fat of obese people, are failing in their fundamental role, since they lose the ability to properly store lipids. They behave, indeed, like too filled receptacles, the contents of which overflows at any attempt to further filling[224]. In other words, the triglyceride nutritional overload (which, for the modest capacity of neoadipogenesis expressed by visceral adipose tissue, cannot be allocated to new-born cells) is embedded only in an extremely provisional way into abdominal fat cells, which soon regurgitate fatty acids in the blood, owing to a significant lipolytic activity, due both to the great sensitivity of the abdominal adipocytes to the adrenergic stimulation of the lipases, both to the hypoxia, which inhibits the insulin antilipolytic effects[206]. The high release of FFA and glycerol into the portal vein[225] causes a progressive ectopic accumulation of lipid material in the liver parenchyma[226] (NAFLD), resulting in a severe anatomical and functional damage. The subcutaneous tissue of the upper body, which, in obese individuals, undergoes a remodelling processes in a similar manner to visceral fat, can pour into the systemic circulation a comparable hyper flow of FFA, causing the formation of ectopic fatty deposits in the fibres of the skeletal and cardiac muscle and in pancreatic islets. The lipids, accumulating in these sides, become strongly harmful to the cells, due to the direct cytotoxicity, as well as to alterations in gene expression and to apoptosis processes[227,228]. This leads to functional abnormalities of target tissues: defect in glucose uptake by muscle, dilated cardiomyopathy, decreased production of insulin[229]. In this way, it is started the disastrous chain of events that plunges toward the pluripathologic constellation of the metabolic syndrome[230]. The liver, submerged by a portal hyper flow of fatty acids, pours into the bloodstream an excessive amount of low-density lipoproteins. In addition, the hepatocytes become less responsive to the suppressive effect that insulin normally exercise towards gluconeogenesis, the increase of which determines hyperglycaemia, also induced by the decreased sugar uptake in the muscle fibres. To compensate for the hyperglycaemia and for the low insulin sensitivity of liver, fat and muscle, the pancreas beta-cells are forced to increase the synthesis; thus a transient hyperinsulinemic phase begins, also helped by the reduced hepatic clearance of the hormone. With the passing of time, the pancreatic islets, exhausted for the over activity, inhibited by inflammatory cytokines and damaged by the ectopic lipid depots, cease gradually to work. In the arterial tree, the inflammatory stimulus increases the expression of adhesion molecules (ICAM-1, VCAM-1, LFA-1, E-selectin); these cause the margining and the sub-endothelial migration of monocytes, which, capturing oxidized lipoproteins, are transformed into foam cells that form the atherosclerotic plaque. Central adiposity leads, therefore, the activation, in many district, of biochemical and cellular mechanisms of inflammatory type,

which, paradoxically, remain subclinical at the site of origin, and determine the most harmful metabolic effects at a distance, on the whole body, contributing to the development of insulin resistance and cardiovascular disease[231,232]. Some authors have come to define the obese adipose tissue as an "inflammatory organ"[233], for which obesity corresponds to a chronic inflammatory condition[234].

Fatty tissue and ageing

Adipose tissue shrinks important and intricate relations with the ageing-related phenomena[235,236].

On the one hand, ageing has a considerable influence on the amount and the distribution of fat. Over years, in fact, even without changes in body weight, there is a gradual rise in the relative percent of body fat compared to the lean components. At the same time, visceral fat depots expand, at the expense of the subcutaneous panniculus. Ectopic depots also grow, particularly in cardiac and skeletal muscles, pancreas and bone marrow. These changes are associated with an increased risk of morbidity and mortality[237].

On the other hand, researches conducted in recent years have clearly demonstrated that the size of the lipid stores, their site, the amount of caloric intake and the endocrine factors that regulate the metabolism of adipose tissue and, more generally, the management of the energy resources have a strong influence on longevity.

It is a common clinical observation, accompanied by a large extent of epidemiological data, that obesity and the overweight reduce life expectancy[238].

Conversely, it has been proved that calorie restriction (CR) and the consequent reduction of triglycerides reserves are able to determine a lifespan increase in a high number of animal species, ranging from yeast to worms and from insects to mammals[239,240]. The biochemical mechanisms through which this effect is expressed is not been completely elucidated: it seems, however, that the beneficial consequence of CR are not limited to the lowered production of metabolic waste, consequent to the reduced availability of energy substrates. It is assumed that a profound change occurs in gene expression patterns, resulting in the activation of cell protective genes.

The diminution of fat mass is associated with an increased longevity, even when obtained by means of different types of genetic manipulation, as well as it happens in Drosophila, following the overexpression of the transcription factor dFOXO[241].

In mammals, the protein SIRT1 (homolog of SIR2, known for increasing the lifespan in yeast) reduces lipid accumulation in adipocytes, suppressing the action of receptor PPARγ; the activation of SIRT1 and of proteins belonging to the same class (sirtuins) is one of the ways by which calorie restriction realizes its effects on longevity and is now identified as a target of possible anti-aging therapies[242].

In many biological forms, from the simplest ones till to mammals, a wide variety of genetic alterations that affect the insulin and insulin-like signalling pathways[243], including those mediated by GH and IGF-1, determine an elongation of lifespan.

So it is for example:

- in Caenorabditis elegans, following the mutation of the insulin-like receptor DAF-2[244];
- in Drosophila, in which has been suppressed the Chico protein, insulin receptor substrate[245];
- in mice strains in which was repressed the activity of the GH (by inhibition of its production or by suppression of the specific receptor)[246,247], or in which has been obtained the knock-out of the insulin receptor exclusively in adipose tissue[248] or the knock-out of the IGF-1 receptor in the brain[249].

In particular, transgenic mice in which has been achieved a deletion of the insulin receptor limited to adipose tissue (FIRKO mice) have a reduced fat mass and experience a rise in average and maximum lifespan by about 20%[250].

Of great interest is the increase in longevity and stress resistance that was observed in mice genetically lacking the protein p66Shc[251].

According to a recent interpretation, p66Shc would act as an amplifier of the insulin activity in the adipocytes, in which maximizes the synthesis and the storage of triglycerides[252]. Animals without the protein have a lower lipid accumulation and a reduced fasting resistance; on the other hand, they are less prone to diet-induced obesity and live longer than controls[253]. Again in mice, the surgical ablation of visceral fat (but not of the subcutaneous) elevates the longevity[254].

Adipose tissue appears capable of interfering on phenomena that affect the duration of the existence, even apart from effects directly related to the management of energy substrates.

This is realized, for example, through the aforementioned promotion, by the hypertrophied visceral tissue of the obese individuals, of chronic inflammatory conditions and oxidative stress[255] at the systemic level. Inflammatory cytokines poured in the blood, exercise, indeed, actions at a distance on a number of targets, triggering, into the cells, the transcription factor NFkB, and causing the leukocyte activation and the expression of the endothelial adhesion proteins.

These events help to generate the slow tissue damaging events determined by all the immune-inflammatory phenomena: these, according to the

"inflammaging" theory[256,257], represent detrimental conditions that generate cumulative harmful parenchymal micro-injuries, which, during the years, combine to cause the gradual senile decay.

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Classification of fat pad of the third medium of the face

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ABSTRACT

Studies focused on the ultrastructure of facial compartments and in particular on the adipose component have been scarce. The aim of this work is to increase information about facial fat tissue anatomy and morphology and its behaviour through ultrastructural analysis of biopsies harvested from different facial compartments. Six women ranging from 34 to 63 years underwent blepharoplasty, face lift, nose and bimaxillary surgery. Biopsies were resin-embedded and observed using transmission and scanning electron microscopy. The data collected demonstrated that the adipose tissue present in each compartment is characterised by different morphologies of adipose components and that adipocytes are organized in the tissue according to different architectures. In particular, the Bichat's fat pad differed from the other fat pads because its morphology was similar to visceral adipose tissue.

Labial and nasal deposits were characterised by small clusters of adipocyte with specific polarisation. The malar fat pad presented the morphology of structural adipose tissue. Moreover the organisation of adipocytes in facial fat deposits appeared to depend on the embryologic origin of each fat pad, and consequently, the role played by each pad is correlated with the embryologic origin of adipose tissue and not only with their function, as described at large in recent literature. In fact, the study extends at the ultrastructural level the author's observation on structural and functional features in the different adipose compartments of the face.

Keywords
Facial adipose tissue, aging, facial fat pad, Trasmission Electron Microscopy, Scanning Electron Microscopy

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Introduction

Recent advances in facial soft-tissue anatomy permit modification of current aesthetic procedures, including surgical techniques[1], volumetric rehabilitation[2] or botulinum toxin associated techniques[3], in order to plan adequate treatment/therapeutic options/techniques. Detailed knowledge of the topographical anatomy and morphology of facial white adipose tissue (fcWAT) compartments may clarify the facial anatomy, in order to improve medical and surgical plans. Many authors suggest that fcWAT was highly compartmentalised and strongly integrated with superficial muscle aponeurotic system (SMAS), and that they are implicated in the mechanisms of aging processes[4-5].

However, their role, and in particular the role played by adipose tissue in facial aging, is not fully understood, and not completely explained[6-8].

In the literature there are few papers in which the structure and role of facial fat pads are described; most papers focus on the role of SMAS[8]. The aim of this work is to increase information on facial fat tissue morphology and its behaviour through ultrastructural analysis of biopsies harvested from different facial compartments. In the present work we analysed biopsies obtained from different and specific facial fat compartments as previously described in the literature by other authors[9-11].

Specimens harvested from the periorbital, nasal, buccal and malar compartments have been analysed using transmission and scanning electron microscopy in order to describe their morphological and ultrastructural aspects and to classify the adipose tissue following previous classification found in the literature.

Materials and methods

Patients

Six women ranging between 34 and 63 years, enrolled from 2014 to 2015, who underwent reconstructive or aesthetic surgery. In the first case, surgery was performed to correct functional nasal defects or to reconstruct an injured portion of the face. In the second case, surgery was employed to correct aesthetic defects. All patients were treated under general anaesthetic and received different surgical operations: blepharoplasty, face lift, nose surgery and bimaxillary surgery. The operations performed on each patient are described in Table 1.

During a preliminary meeting with the surgeons, all the patients were informed that fat tissue would be harvested from facial compartments for scientific purposes. All the patients gave their written consent to the harvesting of adipose tissue biopsies.

Patients	Age of patient	Bimaxillary surgery	Nose surgery	Blepharo-plasty	Le Fort 1 osteotomy
#1	36	Yes	Yes	No	Yes
#2	59	No	No	Yes	No
#3	63	Yes	No	No	Yes
#4	34	No	No	Yes	Yes
#5	44	Yes	Yes	No	No
#6	56	Yes	Yes	No	No

Table 1 - Patients enrolled in the study. The table shows the patients, the age of each patient and the type of operation performed on each patient.

Fat samples

Biopsies (0.2 cm^3) were obtained from different specific facial fat compartments. The periorbital fat tissue relating to the superior, inferior and lateral orbital fat was harvested during blepharoplasty, from the supra and subciliary incision.

The malar fat tissue was harvested during face lift, from the face lift/preauricular incision.

The Bichat and labial fat pads were harvested during bimaxillary surgery, from the circumvestibular incision during the Le Fort 1 osteotomy.

The nasal fat tissue was harvested during nose surgery, through an open approach from the tip in the inter-domal and dorsal area.

During surgery, all the samples were fixed in 2% glutaraldehyde in Sorensen buffer pH 7.4 at 4° C and then analysed in the Section of Anatomy and Histology (University of Verona, Italy).

All samples were analysed using light microscopy, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).

Transmission Electron Microscopy (TEM)

Samples of fcWAT were fixed with 2% glutaraldehyde in 0.1 M Phosphate Buffer for 4h, postfixed in 1% osmium tetroxide in the same buffer for 2h, dehydrated in gradient of acetone and finally embedded in Epon–Araldite mixture (Electron Microscopy Sciences, Fort Washington, PA, USA).

The semi-thin sections were examined by light microscopy and stained with toluidine blue in order to select the region of interest for ultra-thin section.

The ultra-thin sections were cut at 70 nm thickness and placed on Cu/Rh grids with Ultracut E (Reichert, Wien, Austria), stained with lead citrate

and observed using an FEI Morgagni 268D electron microscope (FEI Company, Eindhoven, Netherlands).

Scanning Electron Microscopy (SEM)

Samples of fcWAT were fixed with 2% glutaraldehyde in 0.1 M Phosphate Buffer for 4h, postfixed in 1% osmium tetroxide in the same buffer for 1h, dehydrated in gradient of ethanol, critical point dried (CPD 030, Balzers, Vaduz, Liechtenstein), fixed to stubs with colloidal silver, sputtered with gold by an MED 010 coater (Balzers), and examined with a FEI XL30 scanning electron microscope (FEI Company, Eindhoven, Netherlands).

Results

Adipose tissue evaluation of the third medium of the face, performed by TEM/SEM, showed three different morphologic patterns of fcWAT.

Pattern 1

The first pattern of adipose tissue was observed in malar and periorbital fat pads. These fcWATs were classifiable as structural adipose tissues.

Malar fat pad

At the TEM, the malar fat pad was characterised by the predominant presence of mature unilocular adipocytes, with paucity of collagen fibres, and also by the presence of large capillaries.

A small number of multilocular adipocytes was detectable near the capillaries, and they could represent new adipose elements originating specifically from blood vessels.

These newly-formed adipocytes were in the lipid internalisation phase of the adipose cell cycle (ACC) leading to the formation of unilocular mature adipocytes (Fig. 1A-B).

By contrast, the adipocytes with a unilocular aspect, at the moment of observation, were characterised by regular shape even if, in some cases, plasmatic membranes appeared thickened (Fig. 1A-B).

At the SEM, the malar fat pad was characterised by lobules of mature adipocyte, homogenously covered by thin collagen fibres (Fig. 1 C-D).

Periorbital fat pad

At the TEM, adipocytes showed a diameter of between 80 and 100 microns.

Their plasmatic membranes were characterised by regular shape, even if, in some cases, cell membrane showed a slight increase in thickness (Fig. 2A-B).

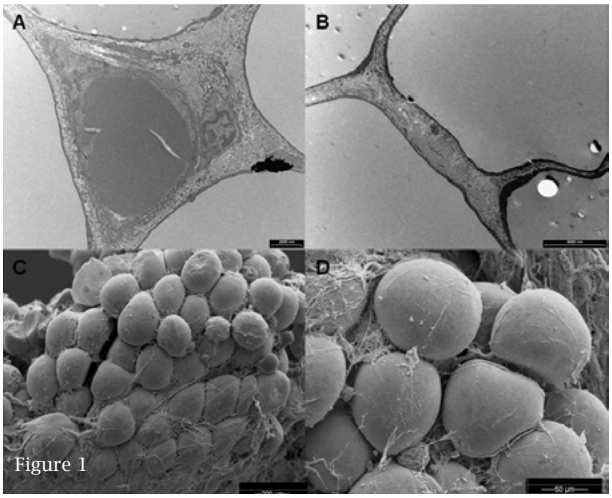

Figure 1 - Malar fat pad. The TEM shows the presence of unilocular adipocytes with scarce collagen fibres. SEM shows the organisation of adipocytes in lobules surrounded by thin collagen fibres covering the adipose cells.

This adipose tissue seemed to be richly vascularised.

At the SEM, the periorbital fat pad showed the typical morphology of structural WAT[12] characterised by the presence of thick fibres surrounding mature adipocyte, organised in clusters.

A dense network of thin fibres was localised around the lobules, forming a basket-like structure (Fig. 2C-D).

These collagen bundles were distributed in all directions to form a dense network in which lobules of mature adipocytes were embedded.

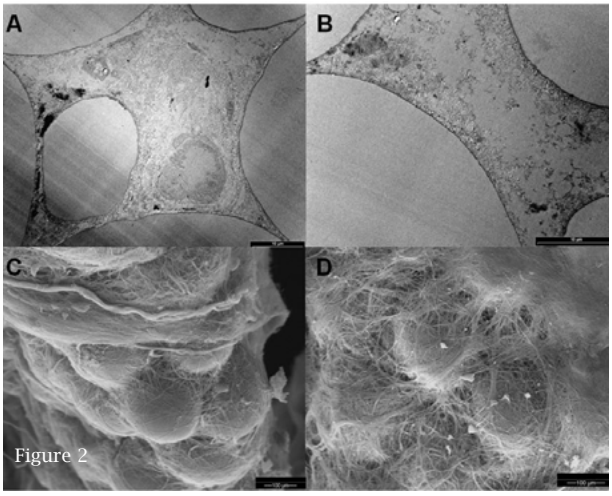

Figure 2 - **Periorbital fat pad**. The TEM shows adipocyte of about 80-100 um with a regular profile of plasmatic membrane (A-B). The SEM shows abundant thick collagen fibres that form a basket-like structure surrounding adipocyte clusters.

Pattern 2

The second pattern of adipose tissue was observed in nasal and labial fat pads. These fcWATs were classifiable as a modified form of fibrous adipose tissue.

Labial fat pad

In comparison with other fat pads the labial fat pad was characterised by the presence of few mature adipocytes. Mature cells were characterised by a unilocular aspect, with a diameter of 80 microns.

Collagen fibres were abundant and interposed between mature adipocytes. Moreover, it was possible to detect smaller multilocular adipocytes organised in clusters with longitudinal orientation (Fig. 3A-B). At the SEM, the labial fat pad was characterized by mature adipocytes embedded in a dense collagen matrix with elastic and structural fibres (Fig. 3C-D). The dimension of collagen fibres, around mature adipocytes, appeared elevated in comparison to the very thin fibres that surrounded adipocytes in periorbital fcWAT.

Isolated clusters of mature adipocytes were observed and were characterised by a unilocular aspect. Moreover, at lower magnification, it was possible to detect clusters of elongated mature adipocytes (Fig. 3C-D).

Nasal fat pad

The nasal fat pad showed a dense texture of collagen fibres surrounding adipocyte (Fig. 4A-B).

Collagen fibres were homogeneously distributed in the extracellular space and were also characterised by the presence of long-spaced collagen (Fig. 4A-B).

However, the connective tissue that formed the adipose lobules appeared well hydrated and seemed to confer a particular aspect that specifically identified the nasal fat pad.

At the SEM, the nasal fat pad was characterised by the presence of thin and dense collagen fibres around the mature adipocytes. These fibres were randomly organised around adipocytes and formed a basket-like structure around some adipocytes. The thinner collagen fibres seemed to be stuck fast to the plasmatic membrane of adipocytes (Fig. 4C-D).

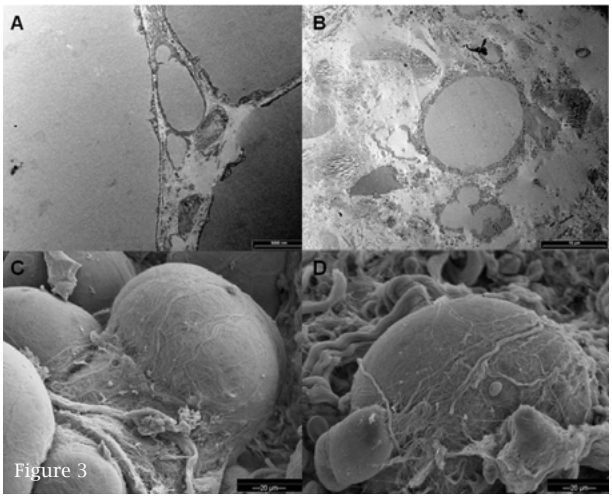

Figure 3 - Labial fat pad. The TEM shows adipocyte of about 80-100 microns with a regular profile of plasmatic membrane (A-B). The SEM shows abundant thin collagen fibre that forms a basket-like structure surrounding adipocyte clusters.

Pattern 3

The third patter of adipose tissue was detected in buccal fat pad. It was classifiable as a modified form of visceral adipose tissue[12].

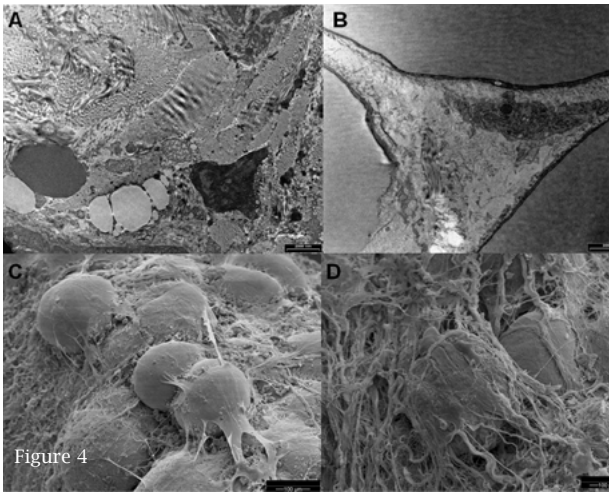

Figure 4 - Nasal fat pad. The TEM shows adipocyte of about 80 microns with a regular profile of plasmatic membrane (A-B). The SEM shows abundant thin collagen fibre that forms a basket-like structure surrounding adipocyte clusters.

Buccal fat pad

At the TEM, the adipose tissue appeared as dense tissue, but was characterised by a paucity of fibrous elements. The stromal compartment was characterised by the presence of blood microvessels.

Adipocytes appeared separated from each other by scarce electron dense material and were characterised by numerous long and thin cytoplasmic protrusions.

Cytoplasmic flaps seemed to represent a protrusion of cytoplasm of mature adipocyte, that usually surrounds the large lipid droplet.

Protrusions were extended to the nearest connective tissue for about 20-40 microns. Cytoplasmic protrusions appeared covered by

external laminae and were often characterised by branched boards.

In the cytoplasm of the protrusion, organelles were usually absent and were replaced by microfilaments, lysosomes, mitochondria and isolated and grouped unilocular lipid droplets (Fig. 5A-B).

At the SEM, buccal adipose tissue was characterised by large mature adipocytes surrounded by thick and irregular fibres (Fig. 5C-D) that do not completely cover mature cells.

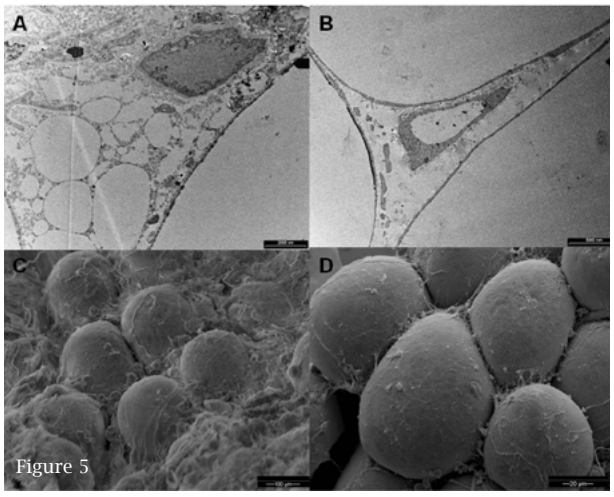

Figure 5 - Buccal fat pad. The TEM shows the scarcity of collagen fibres and the presence of large adipocytes surrounded by a collagen matrix in which small clusters of adipocytes are detectable (A-B). The SEM shows the presence of adipocyte clusters surrounded by thin collagen fibres.

Discussion

A detailed knowledge of facial anatomy is a prerequisite for rejuvenating procedures[13,14].

Many authors have accurately described the anatomy and location of facial fat pads[15-17]. Gierloff et al.[15] and Pessa et al.[18] have suggested that the aging face can be analysed as a change in the volume and position of separate compartments, both superficial and deep.

These works emphasise the importance of facial fat compartments to the facial ageing process.

The present study provides new information on the facial adipose tissue compartmentalisation hypothesis. The collected data extend, at the ultrastructural level, the observation concerning histological features among the different adipose compartments of the face.

Based on our results, the connective septa, described as separating the different pads, seem to identify ultrastructural domains as well.

Moreover, our data suggest that in each fat pad, the aging processes may be characterised by different patterns related not only to the distribution of adipose tissue and the triglyceride component,[4,5] but also to the modifications that have occurred in the intracellular scaffold, which in turn, is closely connected to microvascular distribution.

The microvessels provide nutritional and hormonal support to the adipocytes, but they are associated with the regenerative units (i.e. stem niches), and with the role of adipose tissue renewal.

Considering these features, malar, periorbital, nasal and labial depots appeared classifiable, respectively, as fibrous or structural adipose tissue, as previously described by Sbarbati et al. (2010)[12], while the buccal fat pad is characterized by several aspects of deposited adipose tissue[12].

Facial fat pads are subject to intense mechanical stimulation that is typically observed in the structural and fibrous compartments, such as the trochanteric fat pad[20]. Facial deposits are characterised by a highly complex vasculo-stromal network.

This aspect is probably due to the association of adipose deposits with visceral systems (i.e. digestive, respiratory and visual).

In this apparatus, fat deposits play a mechanical role, and for this reason they may be characterised by a different collagen fibre density and adipocyte arrangement due to their morpho-functional roles.

The results of this study highlight an important new aspect of fcWAT. The facial connective tissue seems to be characterised by an embryonic origin different from that of the adipose tissues of other body fat. While the body and limb fats have a mesodermal origin, the facial connective tissue seems to be derived from neural ectoderm.

The facial fat pads probably derive from neural ectoderm associated with the rhombomeres, from which gill arches originate. Based on this hypothesis, the relationship between innervations and facial fat depots should be studied in greater detail. This relationship could determine the interpretation of the morpho-functional compartmentalisation of facial adipose tissue. The neuro-chemistry of facial fat pads, and its role in aging processes, appears to be another very interesting field for investigation. Particular attention should be drawn to the buccal fat pad. It is located in a deeper compartment, and subject to diminution with age.

For this reason it is not surprising that the buccal fat pad is different from other pads, in the ultrastructural aspect. Its morphology shows that the buccal fat pad is dense adipose tissue, characterized by unilocular and large adipocytes interposed by multilocular and smaller ones,

originating in modified white adipose tissue[21].

This change is not frequent in subcutaneous adipose tissue, but it is typical of visceral tissue. Based on the histological data described in the literature, and on our ultrastructural data, the buccal fat pad must be considered an example of visceral adipose tissue localised in the face and, for this reason, could constitute a good model for studying the dynamic of brown adipose tissue. In particular, its differentiation into beige or white fat plays a very important role in the regulation of metabolism.

On the contrary, periorbital, malar, nasal, and labial deposits typically appear as white adipose tissues, at least at the beginning of their development. Nasal and labial deposits appear more fibrous, with rare mature adipocytes embedded in a strong collagenic network. Therefore they appear similar to the fibrous connective tissue found in other body fat deposits.

The periorbital and malar pads appear to be characterised by more solid aspect and to be arranged in accurately-structured lobules, forming large-scale clusters. The ultrastructural data collected in this study could permit accurate classification of the fcWAT. Facial adipose tissue should be divided into a medial portion of frontal-nasal ontological origin (frontal-nasal prominence or process), which appears during embryonic development under the forebrain in the anterior wall of the stomodeum, and the lateral region of rhombomeric derivation[22].

This last region should be divided into a deeper (buccal) layer that is a beige adipose tissue derived from brown adipose tissue, and a superficial (periorbital and malar) layer.

Conclusion

This paper suggests that the fcWAT is characterised by a high complexity and variety of adipose tissue typologies. The therapeutic strategies aimed at minimizing the aspects caused by aging should be focused on preserving the adipose organisation of the young face.

Disclosure of interest

All the authors declare that they have no conflict of interest in drafting this manuscript and that this study was not funded by organization or the Ministry of Health.

Ethical statement

All procedures performed in this study and involving human participants were carried out in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Histological evaluation of a biorevitalisation treatment with PDO wires

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ABSTRACT

Introduction: the hypothesis of biorevitalisation with absorbable suture in Poly-Di-Oxanon (PDO) not anchored has undoubtedly been the most widespread procedure in recent years. This study aims to assess whether what has been said can be demonstrated scientifically.

Materials and methods: 5 patients were treated with biostimulation of absorbable PDO, Basic type, from 5 different manufacturers. Skin biopsies were performed pre-treatment, at 6 months, 1 year and 18 months post-treatment. The samples were evaluated by optical microscope. The process of mechanotransduction has instead been evaluated using the voltage clamp technique.

Results: optical microscopy showed an unspecific increase in the number of collagen fibres without a significant increase in collagen type III; elastic fibres, however, showed transitional morphological changes. The greatest effect of these changes occurred especially at 6 months post-treatment; at 18 months it regressed completely to restoration of the initial state. The voltage clamp showed that mechanotransduction occurred. Thread: Not necessarily a method that results in improved aesthetic results with a positive functional biological effect. The technical object studied is one example.

Conclusions: The study demonstrated histologically that the use of bio-revitalising wires in PDO determines fibrosis and functional fabric.

Keywords
Biorevitalisation, derma, PDO wires, rejuvenation

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Introduction

The use of sutures is a very timely topic and is of significant interest in the field of aesthetic medicine. Biorevitalisation with absorbable wires that are not anchored is without doubt the procedure which has been most widespread in recent years. The number of types of wires used for biorevitalisation has progressively increased, from both a technical point of view (basic, barbed, cog, screw, mono, bi, multi-directional, etc.) as well as a structural one (polydioxanone, polylactic acid, etc.)[1,2,3,4,5,6,7,8].

As can easily be found by browsing their advertising brochures, the manufacturers claim that when the wires are inserted into the dermis, they determine:

- production of new collagen and connective tissue, with improved elasticity and turgor of the skin;
- increase in skin tone and significant antiradical effect with a substantial reduction of the oxidative stress;
- the process of mechanical transduction, the basic mechanism whereby the mechanical stress induced by the wires acts on the cells, activating a cascade of intracellular signals which promote cell growth and survival and regulate tissue morphology and architecture, influencing metabolic responses[9].

This study aims to evaluate if what has previously been said can be proved with histological evidence.

Using an optical microscope, variations in the tissues were analysed at different time intervals after the PDO biostimulating wires were inserted.

Materials and methods

1. *Patient selection*

In June 2013, 5 patients (4 females and 1 male) were treated with PDO biostimulating wires inserted. The average age of the 5 patients was 44.4 years (age range 38-50).

None of them had acute or chronic diseases in course, none was undergoing any therapies except for one subject who was undergoing EP therapy (oestrogen and progestin). Two participants were smokers.

The average BMI was 24.4 (range 23-26). None of the subjects had previously undergone aesthetic-medical treatments and/or surgical procedures in the head and neck regions. They all signed an informed consent form for biostimulation with wires.

2. *PDO wires treatment*

The anatomic regions treated were the middle and lower third of the face (from the mandibular margin to the zygomatic region). Twenty-five wires made of PDO were placed on each side of the face using the Korean technique. The wires used in the treatments were all composed of polydioxanone (PDO).

Chemically, PDO is a polymer, which originates from multiple repetitions of ether-esters. It is obtained by opening the p-dioxanone ring monomer and by successive polymerisations. The process requires heat and an organometallic catalyst such as zirconium acetylacetone or zinc L-lactate (Figure 1).

Figure 1

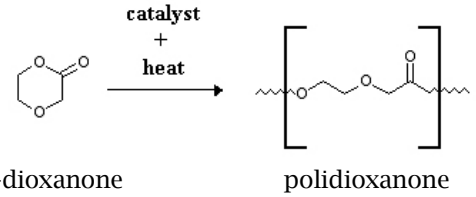

Figure 1 - Representation of the polymerisation process of the p-dioxanone monomer. In the presence of heat and a suitable catalyst the ring is opened

The PDO is characterised by a glass transition temperature in the -10 to 0° C range and a crystallinity of about 55%. In the sutures production process the PDO is usually extruded into fibres; particular attention must be given to process the polymer at the lowest possible temperature in order to avoid spontaneous depolymerisation (i.e. the return to the monomer).

The ethereal oxygen group is the support of the polymer chain, which gives it its flexibility. Five wires made of PDO, each from a different manufacturer, were used, to each of which, for commercial reasons, the name of a colour was assigned (Table 1).

PATIENT	CHARACTERISTICS
I	Wires produced by (Red) with a 31G needle diameter, needle length 30 mm, USP size 7/0, suture length 30 mm.
II	Wires produced by (Yellow) with a 31G needle diameter, needle length 30 mm, USP size 7/0, suture length 30 mm.
III	Wires produced by (Blue) with a 30G needle diameter, needle length 25 mm, USP size 6/0, suture length 30 mm.
IV	Wires produced by (White) with a 28G needle diameter, needle length 35 mm, USP size 6/0, suture length 34 mm.
V	Wires produced by (Orange) with a 31G needle diameter, needle length 30 mm, USP size 7/0, suture length 30 mm.

Table 1 - Characteristics of the PDO biostimulation wires used in the study and assigned to each patient.

The evaluations of the study were made on histological preparations of biopsies taken at different time intervals, on the dosage of the trans-cell membrane ion flow using the voltage clamp technique, and on the dosage of hydroxyproline in the urine.

3. Light microscope observations of the histological preparations

The 5 patients underwent an 18-month follow-up with histological evaluations from the treatment site, by means of pre-treatment biopsies at (T0), 6 months (T1), 12 months (T2) and 18 months (T3).

The biopsies were made with a biopsy punch 2 mm in diameter (Kai medical with CE 0197 Kai industries, Seki City, Japan). Each of the skin and subcutaneous tissue biopsies was fixed by immersion in 4% PFA with phosphate-buffered saline at a pH of 7.2-7.4 in 0.1 M solution for 24 hours, to block the biochemical reactions of the tissue. Biopsy samples were dehydrated using an ascending series of alcohols, then infiltrated with organic solvents followed by hot liquid paraffin; the paraffin, when left to cool, solidifies and provides a support for the tissue.

The preparations are then sectioned, full thickness, into 5-8 micron slices using a rotating microtome.

To obtain the anatomic preparations to stain and analyse, the paraffin is dissolved with organic solvents and the tissues are rehydrated in a series of descending concentrations of alcohols. The slides obtained were observed and photographed using Nomarski differential interference contrast microscopy. The samples were observed and photographed by three independent observers (Modena section of histochemistry laboratory TEST).

The stains adopted for the slides were haematoxylin and eosin, Weigert and Masson's trichrome:

- **The haematoxylin and eosin** permits evaluation of the collagen fibres in relation to the structural conformation of the dermis and epidermis. They were evaluated for any variations in the dermis, such as the presence of fibrosis determined by an increase in type I collagen fibres.
- **The Masson's trichrome**[10] stain technique allows a detailed study of collagen fibres. This type of histochemical staining highlights the collagen fibres in bright green (light green reagent - COLOUR INDEX NUMBER 42095) and makes them stand out from the cells that turn red in the cytoplasm and black in the nuclei, and from and keratin which turns red. The collagen was evaluated quantitatively (absence, occasional presence, abundant presence) and qualitatively according to structural organization (the various types of collagen are responsible for different structures).
- **Blue Mallory Weigert**[11] histochemical staining also shows elastic fibres in addition to collagen. Elastic fibres were evaluated quantitatively (abundant presence, occasional presence, absent) and morphologically (small punctiform fragments, discontinuous fragments, long thin continuous bands).

4. Dosage of the ion flow with voltage clamp

The technique of voltage block or voltage clamp allows the measurement of the intensity and direction of the ionic currents that flow through the cell membrane, in relation to the membrane potential and the duration for which it is applied[12,13].

The experiments are conducted in controlled intracellular and extracellular ionic conditions. The development of the current over time is related to the membrane conductance (G) as follows: Ohm: I = G (Em - Eioni). The voltage block studies how the membrane conductance varies as a result of changes in potential. In the specifics of our study the recording of an eventual ion flow would be indicative of a mechanical transduction mechanism . The voltage clamp was performed on each biopsy sample at time T1, T2 and T3, before being treated and stained. Normally the intracellular ionic values are: Na^+ 12 mM; K^+ 140 mM; Ca^{2+} <0.0001 mM; Mg^{2+} 1.6 mM; Cl 4 mM; HCO_3^- 12 mM; and A- 138 mM. Whereas in the interstitial fluid they are Na^+ 145 mM; K^+ 4 mM; Ca^{2+} 2.1 mM; Mg^{2+} 0.6 mM; Cl 117 mM; and HCO_3^- 27 mM. Each ion has an equilibrium potential: Na^+ is equal to +63 mV; K^+ is equal to -90 mV; Ca^{2+} is equal to +121 mV; Cl is equal to -85 mV.

5. Determination of urine hydroxyproline

Collagen is the principal component of the dermis. About 15% of the total collagen resides in dermal tissue. There are different types of collagen proper and proteins that have a polypeptide structure very similar to collagen. 28 types of collagen have been described in the literature. Collagen is a protein whose primary structure is made of repeating sequences of glycine, proline, hydroxyproline and hydroxylysine. Hydroxyproline is a nonessential amino acid found almost exclusively in collagen, where it represents about 14% of the total amino acid content. It is derived from the hydroxylation of proline. In collagen, hydrogen bonds between the hydroxyl groups of the hydroxyproline and hydroxylysine stabilise the structure.

These bonds form crosslinked α inter-chains (intra-tropocollagen) and interfibrillar bonds. An alteration of the concentration of hydroxyproline in collagen appears to the optical microscope as a modification in the form of the fibre (from a linear form, repeated and well-organised in irregular shapes without a repeating logical structure). Hydroxyproline is commonly found

in the urine and its concentration is an indicator of collagen metabolism. An increase in hydroxyproline is also found in the presence of skeletal diseases, in particular osteoporosis. For this reason the urinary dosage is used as a marker of increased bone absorption (i.e. senile osteoporosis, Paget's disease, cancer with bone lesions, osteomalacia, burns, hyperthyroidism, acute osteomyelitis).

This examination has allowed us to determine that when microscopically altered collagen is found, it does not depend on a pathology in progress. The urinary content of hydroxyproline was determined at 18 months post procedure (T3). Patients were put on a suitable diet for 3 days prior to the collection: no meat or meat derivatives (broths and gelatins); there were no limitations on other foods.

The urine collection was carried out over 24 hours using the traditional method: the first urination of the morning is discarded (collection starting at 7 am), then all subsequent urine samples are collected in a suitable container, including the following morning (finish the sample collection at 7 am). During the collection, exposure of the container to light and temperatures above 10° C was avoided. The reference values of hydroxyproline concentration in the urine in adults (25-65 years) of both sexes are: 6-22 Mg / 24h / m^2.

Results

The study reported the following results:

1. *Evaluation of the histological preparations using the light microscope*

The optical analyses performed on the coloured anatomic preparations stained with **haematoxylin and eosin** (Fig. 2) taken from the same individual at different times (pre-treatment, 6 months post-treatment, 12 months post-treatment, 18 months post-treatment) during this study appear light in colour: the neocollagenogenesis induced by the biostimulation treatment with wires appears during the first 12 months after treatment; however after 18 months it disappears, leaving only some sporadic neofibrils.

In view of the proverb "one swallow does not make a summer", we performed another type of staining to assess whether or not the initial findings could be confirmed (Figs. 3, 4, 5).

The histological sections were treated with blue mallory Weigert stain and observed under an optical microscope at 10x and 20x magnification.

This staining also permitted a thorough study of the elastic fibres. The histological preparations with Weigert's stain have confirmed the initial evaluation.

The collagen fibres progressively increased during the first 12 months post treatment (T2), then the neosynthesis activity of the fibroblasts starts to decline until it returns to the initial condition (T0), leaving a majority of fibrous type I collagen.

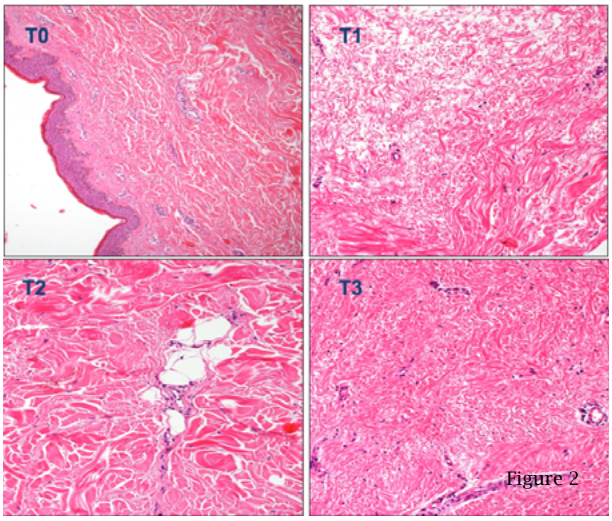

Figure 2

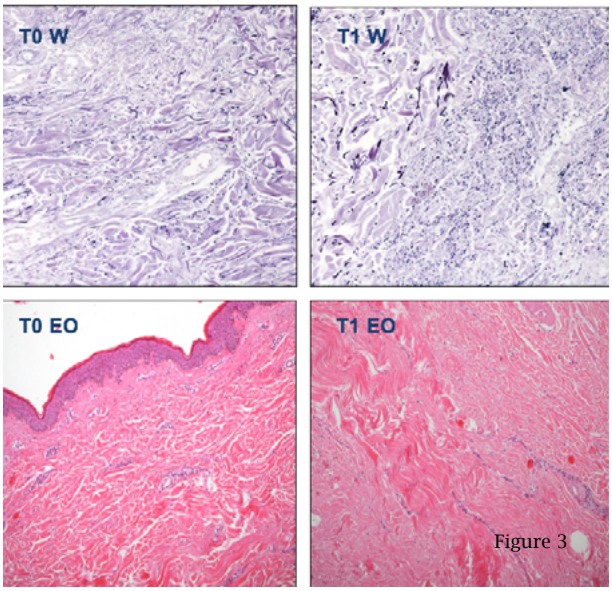

Figure 3

Figure 2 - Comparison of histological preparations, stained with haematoxylin and eosin, and evaluated with a light microscope (10x magnification). In figure T0 the tissues are evaluated in their original state, that is, prior to the biostimulation with the wires: the collagen fibres appear thin and disarranged. In figure T1, or at 6 months after the application of the wires, the collagen fibres appear enlarged mixed with thin. In figure T2, at 12 months post treatment, the histological image always shows the presence of thin and enlarged collagen fibres, but with a net prevalence of the latter. In figure T3, at 18 months, the collagen fibres have returned to their thin and disorganised form.

Figure 3 - Comparison of histological preparations, stained with mallory Weigert blue stain, pre treatment and 6 months post treatment (T0W and T1W respectively) and haematoxylin and eosin pre treatment and 6 months post treatment (T0EO and T1EO). The evaluation was made using an optical microscope at 10x magnification. Figure T1W at 6 months denotes a wavy and thickened band of collagen fibres. Around this it is possible to observe smaller fibres which are always dense and thickened (bottom left slide), and very thin, more rectilinear fibres (top right). Note the presence of elastic punctiform fibres.

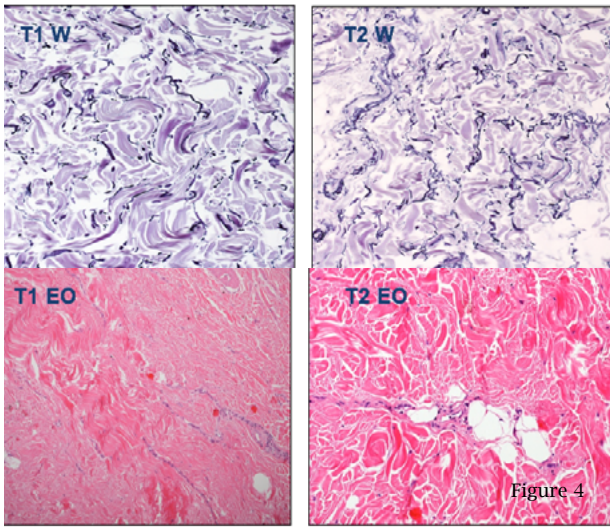

Figure 4 - Comparison of histological preparations, stained with mallory Weigert blue stain at 6 and 12 months post treatment (T1W and T2W respectively) and haematoxylin and eosin, pre treatment and 6 months and 12 months post treatment (T1EO and T2EO). The evaluation was made using an optical microscope at 20x magnification. Comparison of figure T1W and figure T2W shows that the elastic punctiform fibres have started to elongate. By contrast, comparison of T1EO and T2EO shows that the type I collagen fibres have been thickened.

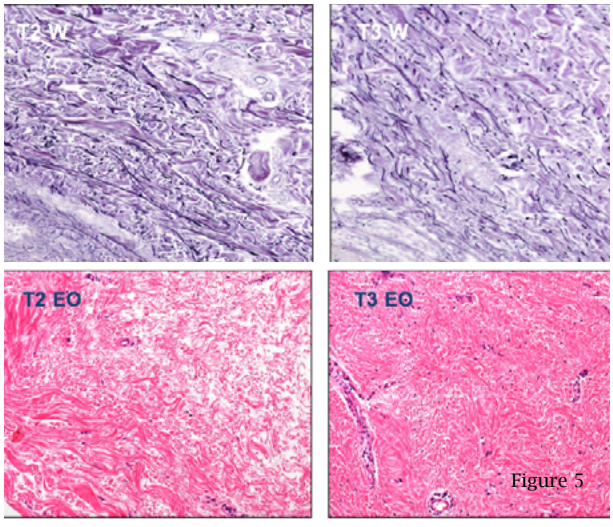

Figure 5 - Comparison of histological preparations stained with blue mallory Weigert at 12 and 18 months post treatment (T2W and T3W respectively) and haematoxylin and eosin at 12 and 18 months post treatment (T2EO and T3EO). The evaluation was made using an optical microscope at 20x magnification. Comparison of figures T2W and T3W shows that the elongated elastic fibres, distended and partially wavy, have returned to a punctiform shape. By contrast, comparison of T2EO and T3EO shows that the mixed collagen fibres have returned to their original state, fragmented, punctiform and disorganised.

The elastic fibres also increased during the first 12 months only to return to T0, their original state, without causing any major changes.

A third staining was performed using Masson's trichrome technique, which also reconfirmed the previous results (Fig. 6).

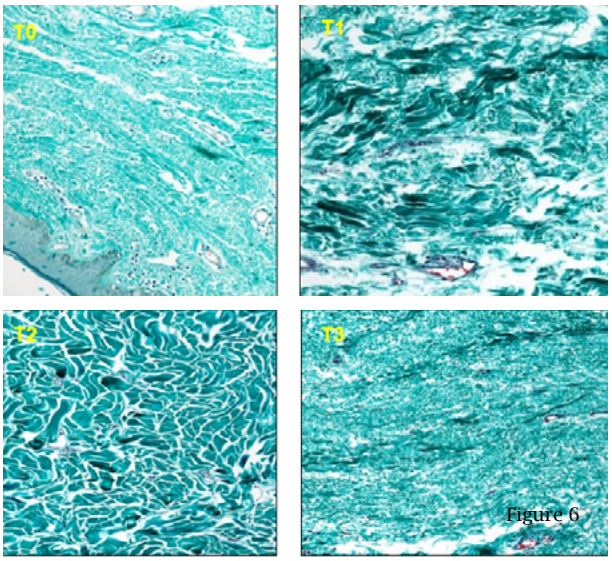

Figure 6 - Histological preparations stained with Masson Goldner stain and evaluated using an optical microscope. In figure T0 the tissue is evaluated in its original state, prior to the biostimulation treatment with the wires: the collagen fibres appear thin and disorganised (10x magnification). In figure T1, namely at 6 months after the wires were applied, the collagen fibres appear to be variable in size, thin and thickened (20x magnification). In figure T2, 12 months post treatment, the histological images show the presence of greatly enlarged collagen fibres (20x magnification). In figure T3, at 18 months, thin disarranged fibres once again become prevalent; however, some swollen collagen fibres remain (10x magnification).

2. Dosage of the ion flow with voltage clamp

Using the voltage clamp technique, no ion flow was recorded; this means there was no mechanotransduction effect.

3. Determination of urine hydroxyproline

The urinary concentration of amino acid did not increase in any of the patients, and remained within the normal range. This rules out the possibility that the changes evaluated microscopically depend on any ongoing chronic and/or acute diseases.

Discussion

As we know, the matrix of the dermis is made up primarily of type I and type III[14] collagen fibres.

These fibres, arranged parallel to the surface of the skin, give it strength and resistance.

Type I or fibrous collagen is synthesised by the fibroblasts when their CD 39 and CD 40 receptors are stimulated by an inflammatory process and TGF beta[15,16] is liberated, while **type III or reticular**

collagen is synthesised by the stimulation of the CD 44 receptors.

Type I is formed by an alpha 1 chain and two alpha 2 chains, while type III is formed by three alpha 1 chains. Type III collagen is largely represented at a young age and contains cysteine in the amino acid sequence that prevents its degradation by metalloproteinase enzymes.

Other types of collagen are also present in the integument, such as type IV collagen, a basic constituent of the basement membrane.

Biostimulation is a treatment that consists of the infiltration into the dermis of a substance that is able to favour the production of new collagen and connective tissue. The objectives of the procedure are to improve the elasticity and turgor of the skin tissue, to increase skin firmness and anti-radical action: thus it is a rejuvenation program that operates full-thickness, on different structures and using different mechanisms.

Today there are numerous techniques of biostimulation: Platelet-rich plasma (PRP), hyaluronic acid, nucleic acids, organic silicon, polylactic acid, wires, to name just a few. Even non-injected techniques can have biostimulation as their target: radiofrequency, laser, carboxy therapy and oxygen therapy. Not all techniques are equal, however: each of the above has different effects on the skin. First, it is a good idea to distinguish techniques on the basis of the biological effect.

The biostimulation inevitably produces functional variation of the cells in the tissue at which it is aimed, and this can be positive or negative. Some biostimulations produce dermal fibrosis as a result of an increase in type I collagen, with biological damage leading to the alteration of metabolic exchanges in the skin. Despite this, the aesthetic effect may be considered beneficial: the fibrosis causes the dermis to retract, producing a lifting effect in the skin.

This aesthetic improvement is accompanied by a loss of tissue function. The neosynthesis of fibrotic type I collagen, even if it produces an aesthetic improvement, always causes biological aging. To have a positive biological effect the collagen fibres synthesised must be predominantly type III.

The biostimulation treatment with PDO wires determines neocollagenogenesis of a primarily fibrotic nature. This results in improved skin appearance due to the retraction effect it produces, but it also determines functional damage with compaction and stiffening of the collagen fibres and biological damage from the alteration of metabolic exchanges. So we are faced with biostimulation whose primary results are aesthetic improvement but which cause functional damage to cellular exchange.

The follow-up performed after 18 months showed by histological examination that when the stimulus is no longer present these modifications are partially reversible, because the fibrous type I collagen component remains elevated.

Conclusions

This preliminary study has demonstrated by histological examination that biostimulation with wires made of PDO during the first 12 months post treatment determined a neocollagenesis and fibrillogenesis that was not induced by mechanical transduction. The new collagen synthesised is above all unspecific and predominantly type I.

When absorption of the threads is completed, the stimulation effect also stops, and at 18 months we witness a full recovery with a slight increase of fibrous type I collagen. The macroscopic-aesthetic improvement to the skin is associated with a negative biological effect with functional alterations.

Considering the multiplicity of biostimulation techniques and the heterogeneity of the patients, it would be appropriate to combine them properly according to appropriate indications. Even the treatment of biostimulation with PDO wires must be carried out in selected subjects with specific skin characteristics[17]. The lack of clear indications from device manufacturers is an important gap that needs to be filled.

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Mesotherapy and extracellular matrix: evidence for the treatment of skin aging

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ABSTRACT

Extracellular matrix (ECM) alteration and the consequent fibroblast dysfunctions are the main causes of aging-related changes to human skin.

This finding has provided the rationale for therapeutic interventions aimed at reversing ECM damage and rescuing fibroblast functions. Among them, the intradermal injection of biological substances with the potential to improve signs of skin aging, is a promising mini-invasive approach. This technique, called mesotherapy, uses a variable mixture of substances such as hyaluronic acid, vitamins, minerals, and amino acids which it is claimed act via different synergistic mechanisms to rejuvenate aging skin.

Although its application in aesthetic medicine is gaining in popularity, the efficacy and safety of this treatment remain uncertain, making mesotherapy liable to criticism by the generally more sceptical medical community. In this article we summarise recent information from the literature on the use of mesotherapy for the treatment of skin aging and we will discuss future innovative approaches.

Keywords
Skin aging, mesotherapy, hyaluronic acid, collagen, extracellular matrix

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Introduction

Skin aging is a complex biological process in which a combination of both intrinsic (genetics) and extrinsic (UV exposure, pollution, radiation) factors progressively leads to the loss of its integrity and functions[1]. Both are cumulative processes that share common cellular and molecular pathways leading to skin damage (Figure 1). Skin aging factors cause oxidative stress and inflammation which alter intracellular signal transduction pathways and thereby the expression of extracellular matrix genes as well as the structure and organisation of extracellular matrix (ECM) proteins (Fig. 1). Basically, intrinsic aging (chronoaging) leads to slight atrophy, loss of elasticity, and fine wrinkling.

At the histological level, the epidermis appears atrophic and flattened; in the dermis, fibroblasts, inflammatory cells, and the microvasculature are reduced. Extrinsic aging, mostly due to UV exposure (photoaging), manifests clinically with fine and coarse wrinkling, dryness, roughness, laxity, telangiectasia, and pigmentary lesions, which in some cases may represent preneoplastic and neoplastic alterations.

Photoaged skin is histopathologically characterised by acanthosis, loss of epidermal polarity, keratinocyte atypia, and irregularly dispersed melanocytes[2].

Figure 1

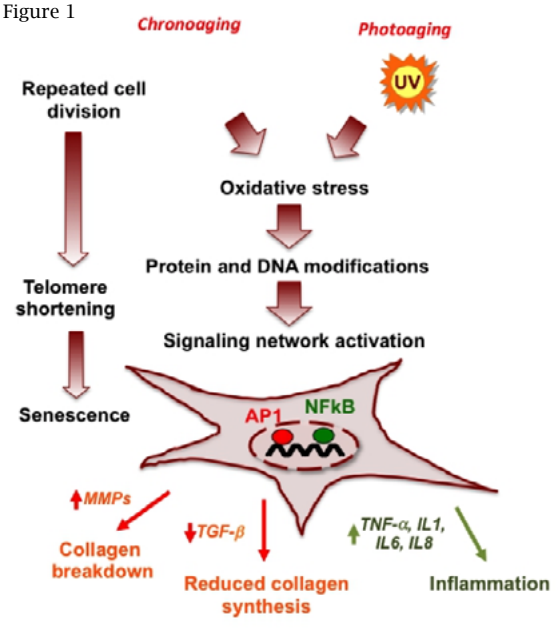

Figure 1 - Mechanism of skin chronoaging and photoaging. Genetics and metabolic processes are involved in both photoaging and chronoaging. Reactive oxygen species (ROS) induce DNA and protein modifications, altering their biological functions. Moreover, ROS activate two transcriptional factors, NF-kB and AP1. NF-kB increases the expression of inflammatory cytokines. AP1 enhances the expression of metalloproteases (MMPs) and reduces the expression of the growth factor TGF-b and type I and III collagen, altering ECM composition.

Collagen and hyaluronic acid, two key molecules in skin aging

Human skin essentially consists of two layers, the epidermis and dermis, attached via the basal lamina.

Fibroblasts represent the major skin type in the dermis, where they have a key role in producing and maintaining the ECM synthesising type I and type III collagen, elastin and hyaluronic acid (HA).

In adults, the dermis contains predominantly type I collagen (85-90%) with lesser amounts of type III collagen (10-15%). Fibroblasts possess cell surface receptors, called integrins, which link ECM proteins including collagen with intracellular cytoskeleton via focal adhesions. The ECM-fibroblast interaction generates mechanical forces that regulate cell shape and influence fibroblast functions[3].

Disruption of the ECM following reduction of collagen synthesis and increased collagen degradation (via the up-regulation of metalloproteinases) are characteristic features of chronologically aged skin and are enhanced in photodamaged skin[4-7]. All these changes negatively impact fibroblast functions reducing fibroblast-ECM binding and mechanical forces[3]. Clinically, impaired fibroblast function, coupled with reduced collagen synthesis, translates into atrophy, wrinkling, and fragility of aged skin.

It has been hypothesised that fibroblast function in naturally aged skin could be stimulated by enhancing structural support of the ECM, supporting the belief that reduced and fragmented collagen in the ECM are predominant determinants of altered functions in aged human skin fibroblasts[8].

HA represents a major component of the ECM in the epidermis. HA is a glycosaminoglycan disaccharide composed of repeating units of d-glucuronic acid and N-acetyl-d-glucosamine. Epidermal and, to a lesser extent, dermal HA displays a rapid turnover: it is synthesised by HA synthases and degraded by the enzymatic activity of hyaluronidases and reactive oxygen species (ROS)[9]. The most dramatic change observed in senescent skin is the marked disappearance of epidermal HA and the reduction of dermal HA synthesis[10]. The latter effect has been attributed to collagen fragments, which activate integrin signalling, inhibiting HA expression[11].

HA exists in high molecular weight form (HMW-HA, up to 104 kDa) and smaller forms referred as low molecular weight HA (LMW-HA). An emerging concept from *in vitro* studies and *in vivo* models of skin wound healing is that HA fragments may have different biological activities both on keratinocytes and fibroblasts[12]. HMW-HA forms are immunosuppressive, possess anti-inflammatory activity, and induce cell cycle arrest[13,14]. By contrast, LMW-HA are immunostimulatory and inflammatory.

Moreover, LMW-HA (100-400 kDa) induces keratinocyte proliferation and migration, and HA

oligosaccharides up to 4 kDa promote migration, angiogenesis and wound closure. Notably, both HMW-HA and LHW-HA seem to induce both type I and type III collagen synthesis, although controversial results have been reported[15-17]. Age-related changes in HA size have been previously described[18].

Therefore, these observations are consistent with the notion that both low levels of HA deposition and HA size modifications could contribute to skin aging.

Mesotherapy and skin aging

The main goal of rejuvenation is to increase the biosynthetic capacity of fibroblasts, inducing the reconstruction of an optimal physiologic environment and the synthesis of collagen, elastin, and HA. There exists a multitude of treatments that claim to improve the appearance of aged skin, but microinjections of therapeutic substances, such as HA, vitamins, antioxidants, and amino acids into the superficial papillary dermis of the skin, represent a promising mini-invasive approach. Although mesotherapy has been extensively used for many years to treat several localised pathologic conditions, at present scientific evidence of the therapeutic efficacy of mesotherapy formulations as a skin anti-aging treatment is lacking.

In vitro studies performed using different mesotherapy formulations on cultured human skin fibroblasts gave different results depending on the product formulation used: NCTF/NCTF-HA (Filorga) increased the expression of type I collagen, metalloprotease 1 (MMP1) inhibited metalloprotease 1 (TIMP1) but did not affect cell proliferation, while other formulations seemed to induce cell necrosis and apoptosis (Soluvit-N, MesoBK)[19].

The first clinical evaluation of mesotherapy on facial skin rejuvenation did not reveal significant effects and failed to show changes to the epidermal/dermal thickness 6 months after treatment[20].

El Domyati et al. obtained similar negative results in 6 treated patients after 3 months. Significantly, no modification was detected in the expression of type I and type III collagen[21].

In a recent clinical study, 50 participants were enrolled and divided into two groups: one group was treated with a formulation containing HA, vitamins, amino acids, minerals, coenzymes, and antioxidants; the other group received HA and the antioxidant idebenone. The authors reported significant improvements in the clinical appearance of the skin with both formulations.

A decreased expression of inflammatory cytokines (IL-6, IL-1β) and MMP1 together with increased expression of collagen I was also observed[22]. More recently, a clinical and statistically significant improvement of profilometric parameters, brightness, pigmentation, and hydration of the skin was detected in 64 women 3 months after the administration of HA, vitamins anti-oxidants (Viscoderm Skinko E)[23].

Moreover, the product showed protection against UVB damage as demonstrated by the reduction of skin erythema in the pre-treated areas. The authors attributed the protective effects of the formulation to the presence of lipoic acid whose antioxidant and protective properties are well known.

The beneficial effects of mesotherapy were also reported in patients treated with HA alone[24,25].

Quan and colleagues proposed that the injection of HA-based dermal fillers restores the structural support of the ECM and reverses the impairment of old skin fibroblasts. Thus fibroblasts displayed elongated morphology, increased proliferation and type I and III collagen synthesis[8,26].

Enhanced keratinocyte proliferation and angiogenesis was also observed[26].

In vitro studies performed on human skin fibroblasts revealed that cross-linked HA (20 mg/ml) may have an effect on ECM remodelling up-regulating the expression of MMPs, hyaluronidase, and elastase, as well as hyaluronan synthase 1 and desmoplakin[27].

Similarly, a controlled single-blind study using non-cross-linked HA–mannitol formulation (Glytone) for the hemifacial treatment of 55 women, resulted, after 3 months, in an improvement of the skin's elastic parameters and complexion radiance[28].

In a recent placebo-controlled study, HA administered to the dorsum of the hand, improved signs of skin aging as detected by high-frequency ultrasound. This technique allows the detection of the subepidermal low-echogenic band (SLEB) that is lower in photoaged skin. After 4 weeks, SLEB echogenicity significantly increased in treated hands vs placebo and this effect was maintained using monthly treatment for an additional 4 or 9 months[29].

Finally, Iannitti and colleagues recently reported that the sequential combination of non-cross-linked HA-based formulation (with vitamins, antioxidants, amino acids, and minerals) and a cross-linked HA filler provided significant improvements in skin hydration, transepithelial water loss, and wrinkle-related aesthetic appearance compared with the HA filler alone[30]. Although the HA activities on fibroblasts and keratinocytes functions have been attributed to LMW-HA (13,20), HA-based dermal fillers are composed of chains with a HMW ranging from 500,000 to 6,000,000. At present it is unknown whether the observed effects are due to the HMW-HA or to the catabolic-derived HA forms.

Future directions

Mesotherapy formulations containing HA vitamins, aminoacids and antioxidants aim principally at increasing fibroblast activities and reducing oxidative

stress, these being the main cause of aging.

The administration of Platelet-rich plasma (PRP) has attracted attention since PRP-derived growth factors are known to regulate several processes including cell proliferation, migration, collagen and ECM synthesis.

Thus, PRP has been used for the treatment of skin ulcers, several musculoskeletal disorders and recently, androgenic alopecia[31,32].

However, experimental studies confirming the effects of PRP when administered using the mesotherapy approach to the treatment of skin aging are lacking.

More recently the role of microRNA (miRNAs) as regulators of cellular aging has been increasingly studied (Fig. 2).

MiRNAs are short ribonucleic acid (RNA) molecules containing on average 22-24 nucleotides, which act as post-transcriptional regulators: miRNAs bind to complementary sequences on target messenger RNAs (mRNAs) inducing their degradation and consequently inhibiting synthesis of corresponding proteins[33] (Fig. 2).

Importantly, one miRNA targets not only one mRNA but several mRNAs, resulting in the ability to repress the expression of multiple components of one or more related pathways[33].

In this way miRNA regulates all biological functions, and their dysregulation has been linked to a wide range of human diseases, especially cancer and cardiovascular diseases[34].

Studies of miRNAs in the field of dermatology have been focused mainly on wound healing, skin differentiation and cancer[35].

However, recent findings have linked miRNA to skin chronoaging and photoaging (Fig. 3), suggesting that miRNA modulation may represent a strategy to reverse aging-associated skin alterations[36,37].

In the skin, the miR-29 family inhibits collagen, fibrillin and elastin synthesis[38], whose loss is associated with skin aging. Targeting miR-29 may increase synthesis of these proteins and provide a strategy to ameliorate and treat dermatologic signs of aging.

Similarly, modulation of miRNA regulating tyrosinase, a melanocytic membrane-bound glycoprotein that is the rate-limiting enzyme critical for melanin biosynthesis, may inhibit melanin accumulation[39].

MiRNA are not far from having a possible application in skin care, including these molecules in cosmetology and mesotherapy formulations. Several biotech companies (Mello Biotech; Sederma, Maison Chanel) are moving in this direction, developing formulations with active ingredients able to target specific miRNAs. At present the beneficial effects of these molecules, as skin antiaging products, are unknown.

Figure 2

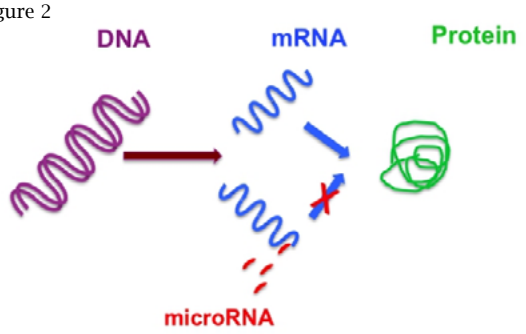

Figure 2 - MiRNA, mechanism of action. The central dogma of molecular biology begins with transcription, the process by which information in the DNA is transferred to a messenger RNA (mRNA). Then mRNA is translated, following the unique genetic code, into a functional protein. miRNA are short post-transcriptional regulators that bind to target mRNA, preventing the functional protein from being formed.

Figure 3

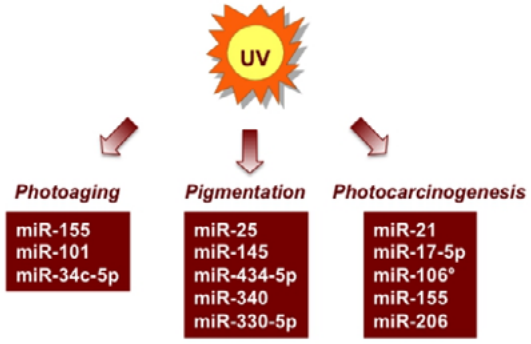

Figure 3 - MiRNA modulated following UVA and UVB irradiation.
Selected miRNA involved in photoaging, skin pigmentation and skin carcinogenesis.

Conclusions

Although mesotherapy appears to be a simple, easy and financially attractive therapeutic option in aesthetic medicine, the use of this technique is controversial. The great versatility of mesotherapy lies in the different compositions of the multiple available formulations. The synergy of different functional ingredients can treat skin in a complete way, acting on various age-related marks caused by both chrono-and photoaging, with a preventive and curative action. Few recent studies support the beneficial effect of mesotherapy as a skin anti-aging treatment and, at present, there is insufficient data to evaluate the efficacy and safety of this technique.

The mechanism of action of many of the components

presents in the mesotherapy formulations is either doubtful or unknown, and molecular and cellular studies are required to establish their in vivo effects.

Is it sufficient to use HA alone (possibly with different molecular weight) or could we achieve better results by administering HA with a cocktail containing vitamins and antioxidants as well? How many treatments are required for long-term results? Might components in the mesotherapy formulations unmask skin tumours? Continued research and well-designed controlled scientific studies are required to sustain the claims for the effectiveness of these products and to formulate guidelines and recommendations regarding their use in the field of aesthetic medicine.

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Advanced Rhinoplasty: Form and Flow

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ABSTRACT

Rhinoplastic surgery remains one of the most difficult operations performed on the face. Improving the aesthetic appearance and maintaining nasal function are inseparable goals in rhinoplastic surgery, and failure in either of these goals can be devastating for patients. Out of a variety of rhinoplastic complications, in this paper more care and attention were paid to the surgical technique for reconstruction of the dorsal aesthetic lines and nasal tip projection in patients with a prominent hump.

Based on modern cartilages conserving concept autospreader flap rotation technique should be considered when dorsal reduction is required. Familiarity with aesthetic anatomy and function is a fundamental aspect in the correct appropriate indication for the autospreader flaps technique, which is a useful tool to prevent post-operative nasal obstruction, segmental appearance, mid-facial axial asymmetry, sever supratip break and preserve ethnicity. Patients with a long nose, prominent hump, short nasal bones and low lower lateral cartilages (LCCs) are good candidates for autospreading.

Keywords
Open rhinoplasty, nasal aesthetic lines, autospreader flap, nasal aesthetic anatomy

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Introduction

The greatest challenge in performing primary aesthetic rhinoplasty is applying advanced anatomical/aesthetic/ethical/ethnic/evidence-based medicine judgments to each individual case and customising the procedure to the individual face, leading to the most natural result for the patient[1,2].

So, some principal criteria in the pre-operative assessment of patients can be made which can improve the results artistically. This must take into account the patient's anatomy, deformities, nasofacial aesthetics, ethnicity, sex, and personal desires.

The endonasal (closed) and external (open) techniques are the two main techniques in primary and secondary rhinoplasty interventions on the face and are most challenging procedures in modern aesthetic plastic surgery[3,4]. For both approaches the treatment goals are to preserve or achieve normal airflow and climatisation functions with primary aesthetic importance: aesthetically pleasing, appearing natural, retaining ethnicity.

Multiple studies have reported that the nasal obstruction is a relatively common problem in patients presenting for aesthetic rhinoplasty, with extreme prevalence of nasal deviation syndrome[5].

The functions of the nose, specifically respiration, humidification, filtration, temperature regulation, and protection, are regulated by the septum, turbinates, and nasal valves (internal and external)[5,6].

Therefore, every rhinoplastic surgeon should cultivate a full understanding of modern intranasal and external rhinoplastic anatomy[7], reach the differential diagnosis of nasal obstruction, achieve the elements of a complete nasal examination (including nasal endoscopy), be comprehensive in analysing the facial and nasal regions, and have a broad understanding of the long-term effects of healing forces on the ultimate nasal aesthetics and function[5]. Knowledge of rhinoplastic medical and surgical treatment options and side effects and anatomical correlates can assist in anticipating them intraoperatively in certain surgical manoeuvres. In prioritising nasal surgery safety issues it is crucial to have a surgical procedures protocol, and certain tools and equipment require training of staff.

Endotracheal monitored anaesthesia care is preferable and a nasopharyngeal pack can be a useful preventative measure, helping to keep the larynx clear.

The Aesthetic Anatomy of the Nose: Dorsal Aesthetic Lines

The external nose bony cartilaginous pyramid is a 3-dimentional structure composed of 3 basic regions: the upper rigid bony third, the middle semi-rigid cartilaginous third, and the lower mobile cartilaginous third. Common and uncommon nasal deformities result from loss of tripod analogy support mechanisms[4]. The nasal dorsal T-shape stabilisation keystone (K-area) is the critical area in which the quadrangular cartilage, nasal bones, perpendicular plate, and upper lateral cartilages (ULC) come together[8] - the roof of the middle nose (Fig. 1).

Figure 1

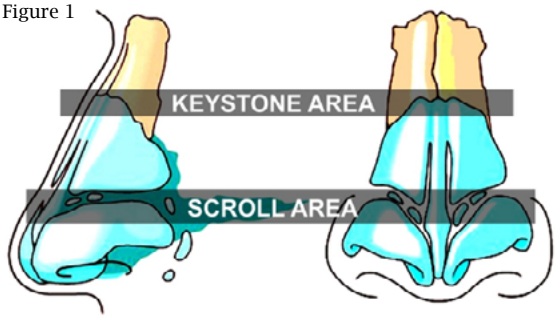

Figure 1 - The KEYSTONE AREA, where the nasal bones overlap the ULCs, and the SCROLL AREA, where the LLCs overlap the ULCs.

The soft tissue components/envelope of the nose include skin, muscles, nerves, and vascular tissues of differing densities. The tissue layers and fibrovascular membranous structures of the envelope in the inferior part of the external nose are divided into 5 layers, which are similar to the structure of the face: epidermis, dermis, superficial fascia, fibromuscular layer and perichondrium.

By density of the microangium, the order of these layers is: perichondrium/periosteum, reticular layer, fibromuscular layer, sub-papillary layer, superficial fascia and papillary layer[9]. Beneath the dermis lies the superficial fatty panniculus, and under the panniculus is a fibromuscular layer. Deep under the fibromuscular layer, a fatty layer encases another longitudinal fibrous sheet in the scroll area that connects the upper lateral cartilages (ULCs) to the LLCs (Fig. 1).

The thin, dynamic musculoaponeurotic layer of the nose is a critical structure of the nose, difficult to visualise. Preservation of these muscles is vital to nasal function and appearance[6,7,10,11].

By two divergent concave lines (nasal dorsum aesthetic lines[8]) that are unbroken extensions of the superciliary ridges, the nasal dorsum connects the radix with the lateral projections of the crura of the lower lateral cartilages (LLC) (Fig. 2).

The radix and supratip region have thicker soft tissue coverage, while the midvault area has thinner soft tissue coverage. A tip break is a place along the profile line of the nose where it comes down fairly straight all the way to the tip, without a location above the tip where the dorsal profile line takes a

little jump out beyond that straight line, angling outward to surround the tip.

Another feature of a nose that has good tip support is that the nose has a tip defining point.

A tip defining point simply means a place that the observer can easily identify as the location of the tip of the nose[8,12],. The position of the tip defining point is somewhat subjective, varying slightly according to ethnic characteristics. To achieve a balanced dorsal profile and dorsum with a supratip break it is necessary to respect differential soft tissue thickness and tip dorsal stabilisation and supporting connective ligaments, which mandates a frame with a slightly deeper nasion and tip projection beyond the dorsum[7,10-13].

Over-reduction of the dorsum causes a change in the orbital nasal relationship with a flattening of the midface, which patients feel leads to an unfavourable change in the appearance of the eyes.

Figure 2

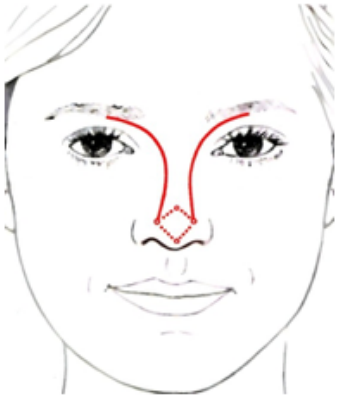

Figure 2 - The curvilinear dorsal aesthetic lines extend from the supraorbital ridges to the tip defining points. The ideal dorsal aesthetic lines run smoothly from the brow to the tip of the nose.

From an aesthetic standpoint, the area from the nasal bridge to nasal tip should be aligned and straight[14,15].

Rhinoplastic Complications

Primary rhinoplastic surgery can be fraught with many risks, where the main complications are post-operative deformities, often requiring revision rhinoplastic surgery.

Clinical manifestations of rhinoplastic complications and side effects may be classified as functional and aesthetic or a combination of both.

To solve these problems, a number of technical methods have been presented by many authors[4-16]. Out of a variety of complications described in this paper, more care and attention were paid to the surgical technique for reconstructing the nasal tip projection and dorsal aesthetic lines in patients with prominent humps. The functional insufficiency of the internal nasal valve occurs in conjunction with the inverted V deformity (disruption of the dorsal aesthetic lines) caused by collapse of the ULCs after dorsal humpectomy, which renders the caudal edge of the nasal bones visible. This combined complication can be prevented and corrected during the dorsal component hump reduction by avoiding excessive resection of the upper lateral cartilage as compared with the septum (midvault area) and by placement of spreader grafts[17].

The nasal tip presents an exceptional challenge because of its mobility and the tip-plasty is most difficult aspect of aesthetic rhinoplasty[10-15]. Sacrifice of tip support and projection by the healing forces is one of the most common surgical errors.

During dorsal hump reduction when the K-area is disrupted and not aligned with the nasal bridge, it may act as a pivot point; downward and inward rotation of the anterior septal cartilage then becomes possible, disproportionally widening the nasal base and resulting in an unnatural appearance of the dorsal aesthetic lines. This protuberant piece of cartilage can then appear as a pollybeak or supratip deformity.

A nose with pollybeak exhibits protuberance with a rounded downward pointing tip with fullness in the supratip region, very much like that of a parrot's beak. Excess scar tissue in the area of the dorsal septal cartilage or supratip region, which can cause a protuberant deformity, becomes apparent once swelling following the surgery has subsided, and is more likely to lead in patients with thick skin. To prevent this deformity, every attempt should be made to maintain adequate tip support using columellar struts or suturing techniques. Suturing the supratip subcutaneous tissues to the caudal dorsum and scroll areas eliminates dead space and formation of deep scar tissues, preserving the functional and aesthetic anatomy of the nose[10-14].

The most important parameters used to describe a nose in preparation for rhinoplasty are the following: projection, rotation, alar-columellar relationship, width and symmetry. Systematic and complete analysis of external and internal nasal anatomical regions as one unit, and knowledge of normal variation, are critical factors in creating an appropriate and realistic methodical operative (and post-operative) plan for successful rhinoplasty[5,6].

Surgical Planning and Operative Strategies

Compared to the blind operative approach to rhinoplasty, the external transcolumellar

infracartilaginous approach used today is an increasingly preferred intervention used for primary and secondary rhinoplasty in the practices of most experienced rhinoplasty surgeons[4].

Both approaches can provide the surgeon with the ability to perform successful rhinoplasty, but each has its appropriate anatomical indications and its own advantages and disadvantages.

The most significant advantage of the open rhinoplasty approach is improved surgical exposure - better visualisation for surgical manoeuvres.

Direct observation of the underlying bony cartilaginous framework dissection from the soft tissue envelope permits accurate diagnosis of nasal deformities, as well as precise manipulation of the dorsum and the nasal tip through a variety of technical tricks[14-16].

Dissection below the musculoaponeurotic layer preserves the major arterial, venous, and lymphatic channels[6,10].

During the open approach as the specific surgical manoeuvre, the deep bony cartilaginous septum and its associated components can be clearly viewed; the existing cartilaginous structures can be refined with precise suture techniques as opposed to blind techniques; better restoration of the integrity of the nasal lobule and preservation of the minor tip support mechanisms can be applied, preventing future loss of tip projection; and grafts can be fashioned and secured without fear of displacement. This degree of precision can reduce uncontrolled tissue scarring and revision rate. The three negative consequences of open approach rhinoplasty are external scarring, occasional prolonged tip oedema, and longer surgery time. Typically, transcolumellar scars heal well and are not noticeable. The occasional prolonged tip oedema always resolves without any negative consequence, using subperichondreal dissection and suturing techniques[4-15].

Autospreader Flaps Technique

Most dorsal humps can be addressed with spreader flaps after reduction of excessive critical part of the bone and cartilage dorsum at the K-area.

The Sheen's spreader graft concept remains the gold standard for internal valve reconstruction, and has been applied for surgical restoration of the disrupted nasal dorsum[16,18]. As reduction increases, so does the need for spreader grafts. The need for a spreader graft is an important consideration during all primary rhinoplasty, particularly in high-risk patients[4]. Typically, patients who have a high, narrow dorsum, a weak middle vault, short nasal bones, or a positive Cottle test pre-operatively are at risk of developing post-operative internal nasal valve dysfunction and resultant nasal airway obstruction[5,6,8,16,18].

Traditionally, spreader grafts are fashioned from cartilage taken from the septum or ear[4,5,16-18]. The disadvantages of the spreader grafts technique are expanded operating time and donor site morbidity[16,18].

The greatest shortcoming with the use of spreader grafts is the need to obtain a certain amount of cartilage graft material. Due to cartilage harvesting, unpredictable post-operative swelling is considerable after septal submucosal dissection. In all cases, if septal cartilage is removed in the treatment of a septal deformity or for grafting purposes, it is crucial to maintain a 10-15 mm L-strut of cartilage along the nasal dorsum and caudal septum. Sometimes, the width of dorsal lines is aesthetically wider after spreader grafts are applied[4,5].

Another option involves preserving the upper lateral cartilage and septum. Compared to cartilage harvesting, the autospreader flaps technique in selected cases preserves the septum, and reduces surgery time in order to maintain or restore dorsal aesthetic lines and internal valve function when performed at the time of humpectomy[16,18].

In 1995 Berkowitz and Oneal described an easily reproducible cartilage conserving technique in rhinoplasty and were among the first to utilise the ULCs as spreader grafts[16,20]. They coined the term *spreader flap*. Rohrich et al. referred to it as the *autospreader*[16]. Utilisation of all cartilage-conserving techniques (the cartilage from the reduced dorsal septum (dorsal columellar strut), lower lateral turnover, tip refinement grafts and suturing) permits successful reshaping of the middle vault and nasal tip[19]. The precise dorsal reduction allows us to use the resected cartilage fragment as a columellar strut, thereby allowing us to forego the standard septal harvest once more, reducing operative time and patient morbidity[21]. The cartilage-conserving concept can be efficient and aesthetic in well-selected patients. Anatomical differences dictate the surgical approach.

The ideal patient for this technique requires 3 mm or more of dorsal hump reduction with tip reshaping and refinement. The patient should not have any breathing problems or septal deviation that would require septal surgery. The patient has adequate tip projection based on nasal analysis but may lack tip definition because of a dorsal hump.

It is important to identify the patient with a tension tip, as he or she will certainly require maintenance or restoration of tip projection to prevent a pollybeak deformity. As mentioned above, in some cases with high dorsum there is no need to harvest septal cartilaginous fragments for grafting (spreader grafts, columellar strut, tip grafts) in order to prevent the functional and aesthetic side effects of the rhinoplasty[4,5,8,16]. If a hump is even 3 mm above the ideal dorsal line, it will usually be possible to fold the

dissected ends of the ULCs as local flaps (supplied by its attachment to the mucoperichondrium) at their interface with the septum immediately prior to performing the incremental humpectomy[18].

Procedure allows the use of this tissue which would otherwise be discarded. The excess ULCs are being appreciated just after the septum and bony hump reduction was precisely done, and autospreader flaps are bilaterally interposed between the septum and ULCs on themselves, including the portion lying under the nasal bones. Where the hump is minimal and folding over the ULCs is not possible, it may be an option to simply return the ULCs to the dorsum and suture it to the dorsal septum. With the use of asymmetric mattress sutures, the autospreader flaps are positioned horizontally, abutting the septum instead of being vertically folded and fixed to the septum. Using the ULCs without folding affords the opportunity to restore a dorsum with sufficient width[10,16]. For these technical tricks the open approach is preferred except by extensively experienced surgeons, as the closed approach is much more difficult[22,23].

The follow-up demonstrates better post-operative recovery with much less septal swelling comparing to the spreader grafts harvesting technique, and proportional projection of dorsal aesthetic lines without over-widening at the K-area space.

Preservation of the dynamic musculoaponeurotic system with its ligamentous connections permits their repair at the time of closure.

Repair of Pitanguy's midline ligament using advancement suture allows the surgeon to control tip rotation, enhance projection, and emphasise a supratip break, while reconstruction of the scroll area ligaments provides stability of the internal nasal valve[7,10,11].

Patients at risk of internal nasal valve dysfunction, such as those with a high, narrow dorsum, a weak middle vault, short nasal bones, or pre-operative internal nasal valve dysfunction, are suitable candidates for the autospreading technique[18].

Despite its significant advantages, the autospreader flap also has distinct shortcomings.

The most common problem encountered in using an autospreader flap is the technique's inability to provide adequate dorsal width compared with spreader grafts. Additionally, the use of an autospreader flap has not been described for special cases such as crooked noses, cases with minimal dorsal humps, and secondary cases.

So, limitations using this technique include those patients with a deviated dorsal septum, asymmetric dorsal aesthetic lines and ULCs of insufficient length at the caudal end of the septum. This population would more than likely benefit from traditional or expanding spreader grafts harvested from the nasal septum, perhaps combined with autospreader flaps.

The thickness of free septal grafts can be varied to control symmetries, and anatomic features must be taken into account when planning surgery on a patient with a crooked nose.

In appropriate patients with nasal axial deviation requiring septoplasty, the need for combined use of autospreader flaps and unilateral or bilateral spreader grafts technique altogether is indicated to correct asymmetric dorsal aesthetic lines.

Indications for combined use of spreader and autospreader techniques are: widening of the dorsal middle third of the nose (especially in ethnic cases); bridging and strengthening a long, narrow roof of the middle nose in patients with short nasal bones and high LLCs; straightening and stabilising a dorsally deviated septum; and creating ethnically acceptable dorsal aesthetic lines (Fig. 3). Nasal septal grafts are thicker and stronger, resisting the deforming forces of a deviated septum and thus correcting the curvature[18].

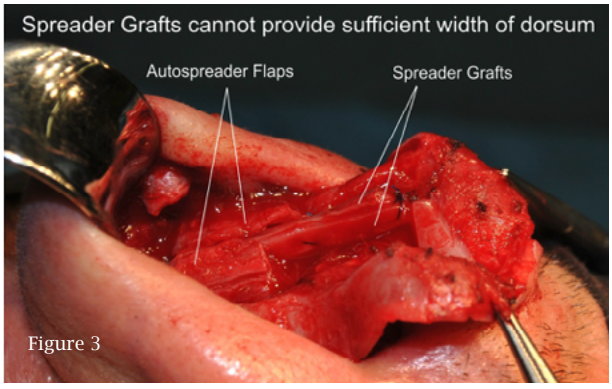

Figure 3 - Combination use of the spreading grafting.

Autospreader flaps may not provide adequate stability when there is associated collapse of the

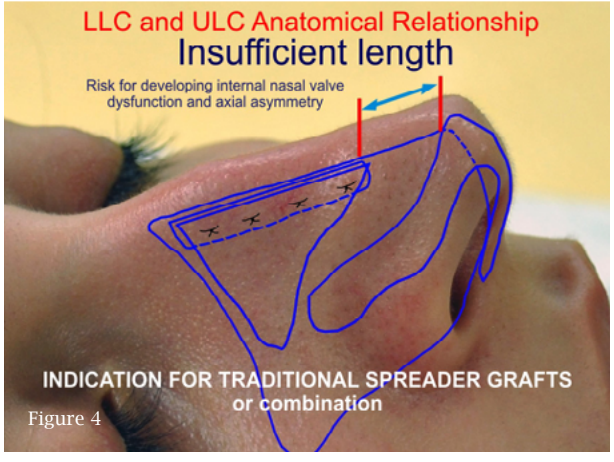

Figure 4 - Indication for spreader graft procedure.

bony sidewalls. In these instances, traditional spreader grafts that extend beyond the keystone are indicated. Another shortcoming of the autospreader flaps compared with spreader grafts is that it cannot always bring about the spreading effect in the area of the anterior septal angle. The reason for this is insufficient length of the ULCs at the caudal end of the septum and lack of sufficient cartilage material for folding (Fig. 4).

The ULCs in most cases are too short to extend down to the anterior septal angle and may not provide a free strip of cartilage tissue to be used as a spreader flap.

Especially in patients with long noses, excision of caudal septal cartilage (when significant vertical shortening is planned) has brought the anterior septal angle to the same level as the caudal edge of the ULCs, rendering the use of additional cartilage grafts. For cases in which an autospreader flaps technique cannot provide sufficient width at the anterior septal angle, this area must be supported by spreader grafts, and the effect of the autospreader flap is extended down to the entire dorsum[24].

When insufficient length of ULCs at the caudal end of the septum is diagnosed, important consideration can be taken concerning the anatomical relationship between the ULCs and LLCs.

The upper cartilaginous framework (external midvault) is comprised of the paired ULCs and dorsal cartilaginous septum. It begins at the keystone area, where the nasal bones overlap the ULCs. Normally, this is the widest part of the nasal dorsum, and resembles a T-shape in cross-section.

Restoration of the keystone area anatomical structure during the primary rhinoplasty prevents open roof and inverted V deformities.

The lower cartilaginous framework is composed of the medial, middle, and lateral crura, and begins where the LLCs overlap with the ULCs in what is called the SCROLL AREA. The LLCs are connected to each other, the ULCs, and the septum by fibrous tissue and ligaments. Disruption of these ligaments during rhinoplasty can result in diminished tip projection, requiring reconstruction to maintain or increase tip support.

The dorsal aesthetic lines originate on the supraorbital ridges and pass medially along the glabellar area to converge caudally at the medial canthal ligaments. From there, they usually begin diverging at the keystone area and ultimately conclude at the tip defining points, which become the highest point in the nasal profile. Ideally, the width of the dorsal aesthetic lines should match either the interphiltral distance or the width of the tip defining points. The width of the bony base should be approximately 80 percent of the alar base width.

The alar base width is also equal to the intercanthal distance. Thus, if the bony base width is greater than 80 percent of the intercanthal distance, osteotomies may be indicated.

Conclusion

Patients with a long nose, prominent hump, short nasal bones and low LLCs are good candidates for autospreading (Fig. 5).

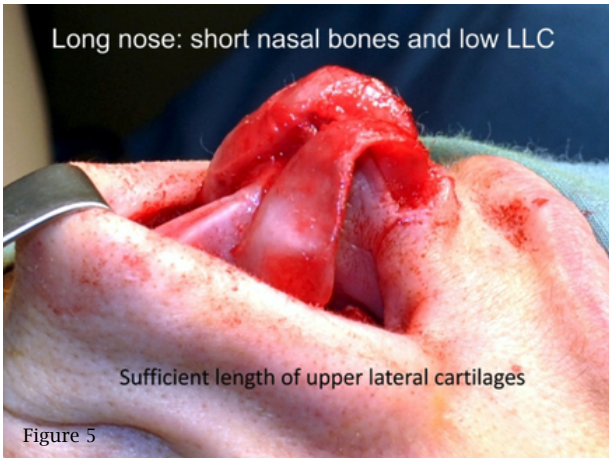

Figure 5 - Indication for autospreader grafting

The autospreader technique is simple, reproducible, and effective in shaping the dorsum while preserving the function of the internal valve in primary rhinoplasty patients if they are appropriate candidates.

Subperichondrial dissection of the nasal framework (preservation of the dynamic musculoaponeurotic system) and controlled manipulation and repair of ligaments without disturbing the overlying soft tissue allows reshaping and redraping of the nasal aesthetic lines.

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Courses and Congresses

2015

19-21 February - Malaga (Spain)
30th Spanish Congress of Aesthetic Medicine
Spanish Society of Aesthetic Medicine
Ronda General Mitre, 210, 08006 Barcelona (Spain)
President: Petra Vega
Web: www.seme.org
E-mail: secretaria@seme.org

14 March – Bucharest (Romania)
Course: Treatment of Vascular Lesions Romanian Society for Aesthetic Medicine and Dermatologic Surgery
Venue: Hotel Novotel
Calea Victoriei 37B - Sector 1
010061 BUCHAREST, ROMANIA
Organizer: Dr. Mihaela Leventer
mihaelaleventer@drleventercentre.com
www.drleventercentre.com

3-5 April – Marrakech (Morocco)
International Congress of Dermastic Moroccan Association of Surgical Dermatology – Cosmetic Aesthetic Medicine – Anti-Aging Medicine
Venue: Le Meridien Nfis
President: Ahmed Bourra
www.dermastic.asso.ma
dermastic.asso@hotmail.com

24-25 April – Brussels (Belgium)
25th Congress of the Belgian Society of Aesthetic Medicine
Venue: Radisson Blu Royal Hotel
Rue Fossé aux Loups, 47 – Brussel
Organisation
Jean Hebrant – Hugues Cartier
www.sbme-bveg.be
info@aesthetic-medicine.be

15-17 May – Rome (Italy)
36th National Congress of the Italian Society of Aesthetic Medicine
10th National Congress of the Italian Academy of Aesthetic Medicine
Venue: Congress Centre Rome Cavalieri
President of the Congress: Emanuele Bartoletti
sime@lamedicinaestetica.it
congresso@lamedicinaestetica.it
www.lamedicinaestetica.it

28-29 May – Odessa (Ukraine)
International conference
"Important issues of current plastic surgery, aesthetic medicine and dermatology"
Ukrainian Society of Aesthetic Medicine
President: Vladimir Tsepkolenko
office@virtus.ua

29-30 May – Pretoria (South Africa)
The 9th Aesthetic Medicine Congress of South Africa
Aesthetic & Anti-aging Medicine Society of South Africa
Venue: CSIR Convention Centre
President of the Congress: Riekie Smit
info@aesthmed.co.za
www.aesthmed.co.za

27-28 June – Tblisi (Georgia)
III International Congress of Aesthetic Medicine
Georgian Society of Aesthetic Medicine
Venue: "Expo Georgia" Event Hall # 3
Co-organiser:
Scientific-Professional Society of Dermatovenereologists of Georgia
Department of Dermatovenereology of TSMU
Tel. +995 (32) 2 250565 - +995 5 5 77545108
info@gsoam.ge
www.gsoam.ge

16-17 July – Montevideo (Uruguay)
Congress of the Uruguayan Aesthetics Medical Society
President: Alberto Elbaum
www.sume.com.uy

3-14 August - Buenos Aires (Argentina)
Degree course in Aesthetic and Anti-Aging Medicine
Practical module
Argentinian Society of Aesthetic Medicine
SOARME
Director: Prof. Dr. Raúl Pinto
info@soarme.com
www.soarme.com

22-23 September – Odessa (Ukraine)
Seminar and master class
"Regenerative technologies in aesthetic medicine"
Ukrainian Society of Aesthetic Medicine
President: Vladimir Tsepkolenko
office@virtus.ua

25-26 September – Paris (France)
36th National Congress of Aesthetic Medicine and Dermatologic Surgery
French Society of Aesthetic Medicine
French Association of Morpho-Aesthetic and Anti-Aging Medicine
National Institute of education in aging prevention
Venue: Palais de Congres
www.sfme.info
congress@sfme.info

1-3 October – Quito (Ecuador)
VII Ecuadorian Congress of Aesthetic Medicine
Ecuadorian Society of Aesthetic Medicine
Venue: Swissotel Quito
President of the Congress: Viveka Tinoco Kirby
seem2008cg@gmail.com
www.seem.com.ec

2-4 October - Warsaw
XV International Congress of Aesthetic and Anti-aging Medicine
X International Conference – Lasers and other sources of energy in aesthetic medicine
Polish Society of Aesthetic and Anti-Aging Medicine of Polish Medical Society
Venue: Warsaw-Hilton Poland
President: Andrzej Ignaciuk
sekretariat@ptmeiaa.p
www.ptmeiaa.pl

7 November – Lausanne (Switzerland)
XIV Congress of the Swiss Society of Aesthetic Medicine
Venue: Beau-Rivage Palace
President: Xavier Martin
www.ssme.ch
xmartin@worldcom.ch

6-7 November – Toronto (Ontario – Canada)
CAAM 12th Annual Conference
Canadian Association of Aesthetic Medicine
Venue: The Westin Prince Hotel
CAAM Office Executive Director: Susan Roberts
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12-15 November – Miami (Florida – Usa)
20th World Congress of Aesthetic Medicine
"Discoveries in Aesthetic Medicine"
American Academy of Aesthetic Medicine
Union International de Medicine Esthetique
Venue: JW Marriott Miami
President: Michel Delune
www.aaamed.org/20wcam
wcam@aaamed.org

14 November – Madrid (Spain)
VI Monographic days of SEME
Spanish Society of Aesthetic Medicine
www.seme.org

26-27 November – Alger (Algeria)
14th National Congress of Aesthetic Medicine and Surgery
Algerian Society of Aesthetic Medicine
Venue: Hotel Hilton
President: Mohamed Oughanem
oughanem_m@hotmail.com
www.same-dz.com

2016

January – Caracas (Venezuela)
Degree course in Corporal Aesthetic
16 hours of University Credits
Degree Course in Facial Aesthetic
18 hours of University Credits
Degree Course in Metabolism, Nutrition and integral management of obesity
10 hours of University Credits
Tel. 00 58 416 6219974
www.fuceme.org
fuceme@gmail.com

18-20 February – Malaga (Spain)
31st National Congress of Aesthetic Medicine
Spanish Society of Aesthetic Medicine
Ronda General Mitre, 210, 08006 Barcelona (Spain)
President: Petra Vega
Web: www.seme.org
E-mail: secretaria@seme.org

3-5 March – Mexico City (Mexico)
XI Pan American Congress of Aesthetic Medicine

XIII Mexican Congress of Aesthetic and Anti-Aging Medicine
XIII Venezuelan Congress of Aesthetic Medicine
Mexican Scientific Society of Aesthetic Medicine
Aesthetic Medicine Society of Venezuela
Venue: Pepsi Center, WTC México
Calle Dakota S/N, Nápoles, 03810
Presidents: Blanca Miller Kobisher – Victor Garcia Guevara
info@ippcvtas.com
www.congresodemedicinaestetica.com

25-27 March - Casablanca (Morocco)
International Congress of Dermastic
Moroccan Association of Surgical Dermatology – Cosmetic Aesthetic Medicine – Anti-Aging Medicine
President: Ahmed Bourra
www.dermastic.asso.ma
dermastic.asso@hotmail.com

31 March - 2 April - Buenos Aires (Argentina)
26th Argentinian Congress of Aesthetic Medicine
Argentinian Society of Aesthetic Medicine
SOARME
President: Prof. Dr. Raúl Pinto
info@soarme.com
www.soarme.com

13-15 May – Rome (Italy)
11th European Congress of Aesthetic Medicine
37th National Congress of the Italian Society of Aesthetic Medicine
11th National Congress of the Italian Academy of Aesthetic Medicine
Venue: Congress Centre Rome Cavalieri
President: Emanuele Bartoletti
sime@lamedicinaestetica.it
congresso@lamedicinaestetica.it
www.lamedicinaestetica.it

9-21 May – Pretoria (South Africa)
The 10th Aesthetic Medicine Congress of South Africa
Aesthetic & Anti-aging Medicine Society of South Africa
Venue: CSIR Convention Centre
President of the Congress: Riekie Smit
info@aesthmed.co.za
www.aesthmed.co.za

16-17 September – Paris (France)
37th National Congress of Aesthetic Medicine and Dermatologic Surgery
French Society of Aesthetic Medicine
French Association of Morpho-Aesthetic and Anti-Aging Medicine
National Institute of education in aging prevention
Venue: Palais de Congres
www.sfme.info
congress@sfme.info

2017

22-24 September - Almaty (Kazakhstan)
9th National Congress of Aesthetic Medicine and Plastic Surgery
Kazakhstan Association of Aesthetic Medicine and Plastic Surgery
President: G. Zhumatova
info@estetic.kz
www.estetic.kz

27-29 October - Istanbul (Turkey)
21th World Congress of Aesthetic Medicine
Turkish Society of Aesthetic Medicine
President: Hasan Subasi
Rumeli Caddesi Durak Apt N° 2, D.7
Nisantasi, Istanbul - Turkey
www.estetiktipdernegi.org.tr
subasihasanm@superonline.com

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