THE EUROPEAN JOURNAL OF AESTHETIC MEDICINE AND DERMATOLOGY

General notes
The European Journal of Aesthetic Medicine and Dermatology is a quarterly Journal published by Marllor Biomedical srl Editor every April, August, and December. The 2015-2016 subscription rates are: 225,00 € (institutional) and 95,00 € (individual), respectively, for the European countries, and 245,00 € (institutional) and 110,00 € (individual), respectively, for the extra-European countries, including taxes and shipping fees (for the printed version). For special issues (e.g. supplements) different rates can be applied. The publication of any article is intended free of charge and no fees are available for Authors; the only charges requested to the contributors are for articles off print when requested.*

Editorial Office
EJAMeD Marllor Biomedical srl
Via Don Minzoni, 12.
I-47842 San Giovanni in Marignano (RN), Italy.
Phone: +39 0541 410286. Fax: +39 0541 954644.
E-mail: media@ejamed.com

Subscription, distribution and back issues
Marllor Biomedical srl
Via Don Minzoni, 12.
I-47842 San Giovanni in Marignano (RN), Italy.
Fax: +39 0541 954644.
E-mail: editor.office@ejamed.com

Business/marketing matters, advertising, supplements and reprints
Marllor Biomedical srl
Via Don Minzoni, 12.
I-47842 San Giovanni in Marignano (RN), Italy.
Fax: +39 0541 954644.
E-mail: media@ejamed.com

Copyright
The entire contents of The European Journal of Aesthetic Medicine and Dermatology – EJAMED are protected by copyright. No material in this journal may be reproduced photographically, stored on compact disc, pen drive, video, electronic database, etc. without written permission from Marllor Biomedical srl.
© 2015 Marllor Biomedical srl.

Info
www.ejamed.com

*General notes and instructions for Authors are given on the last page of the Journal.
EDITORIAL BOARD

The European Journal of Aesthetic Medicine and Dermatology

EJAMED. 2015. Volume 3. ISSN 2240-5046.

Editor in Chief
Eugenio Luigi Iorio, MD, PhD
Salerno, Italy

Co-Editor
Giovanni Scapagnini, MD, PhD
Campobasso, Italy

Lawyer
Federica Lerro
Italy

Editorial Board

Roberto Amore, MD
Italy

Patrick J. Treacy, MD
Ireland

Gerhard Sattler, MD
Germany

Domenico Amuso, MD
Italy

Vincenza Leonardi, MD
Italy

Ferdinando Terranova, MD
Italy

Vitor Figueiredo, MD
Portugal

Joe Niamtu III, MD
USA

Vladimir Tsekpolenko, MD
Ukraine

Kostantinos Gritzalas, MD
Greece

Hernan Pinto, MD
Spain

Barbara W. Cyranska, MD
Poland

Liga Jacevica, MD
Latvia

Patrizia Sacchi, MD
Italy

Lorenza Cicale, MD
Italy

Senior Advisory Board

Nikolay P. Serdev, MD Phd
Bulgaria

Pierantonio Bacci, MD
Italy
5
EDITORIAL
The traditional concept of aesthetics as a medical and surgery branch aimed to improve – in cooperation with the dermatology – the “body’s appearance” through the treatment of conditions like skin spots, wrinkles, localised adiposity, cellulite and so on is rapidly changing.
IORIO EL

8
THE NODULE OF DISCORD.
The unresolved diatribe on the pathogenesis of cellulite in the light of the adipocyte pathophysiology.
TERRANOVA F, IORIO EL, AMUSO D

46
OXIDATIVE STRESS EVALUATION AND HISTOLOGICAL ANALYSIS in the assessment of cellulite: lights and shadows towards a multidisciplinary approach.
AMUSO D, IORIO EL, BONETTI I, AMORE R, TERRANOVA F, LEONARDI V

54
THE MANAGEMENT OF SKIN NECROSIS associated to intralipotherapy with an adipocytolytic solution for treatment of localized adiposity.
AMORE R, AMUSO D, TANZARELLA L, GRITZALAS K, LEONARDI V
EDITORIAL

The traditional concept of aesthetics as a medical and surgery branch aimed to improve – in cooperation with the dermatology – the “body’s appearance” through the treatment of conditions like skin spots, wrinkles, localised adiposity, cellulite and so on is rapidly changing.

First of all in the last years it has been recognised that the external wellness as it can be shown by the “body’s appearance” is the mirror of internal physical and biochemical processes that take place continuously into the cells and the surrounding extracellular matrix where are blood and lymphatic vessels and nerve ending, all potentially targets of diseases as well as of treatments.

Therefore in order to reach as suitable and permanent as possible results both the aesthetic physician and the dermatologist is now considering not only what is happening outside but also inside the body of the patient who must be involved in the preventive or therapeutic options.

Indeed the purpose to pursue is not simply to exploit all the available medical, surgical and diagnostic devices to treat imperfections but also to take care of the person as a whole, encouraging any people to adopt healthy lifestyles in harmony with the nature and possibly to make sustainable choices even in cosmetics field in order “to be beautiful on the outside and on the inside”.

At the same time, this new collective consciousness of beauty together with the incredible progress technology has made is leading to develop not only new medical and surgical devices but also specific cosmetics, drugs and supplements, among which are the newly branded cosmeceuticals and nutricosmetics. Cosmeceuticals – in some way a combination of cosmetics and pharmaceuticals – are cosmetic products with biologically active ingredients claiming to have benefit beyond the traditional moisturizers where nutricosmetics brag the same advantages but after ingestion by oral route.

In the wake of these changes patients themselves are driving the aesthetic doctor and the dermatologist to propose, for the treatment of their most visible imperfections, more and more minimally invasive and “natural” procedures in order to respect the anatomy and physiology of the body and to improve own compliance to the treatment and, therefore, own satisfaction.

On this background the classical “clinical visit” makes available to health professionals involved in the fields of aesthetic as well as dermatology a powerful opportunity for a personalized, multidisciplinary and sustainable approach, in agreement with the modern concept of preventive medicine.

On the other hand the continuous research and development of new bio-materials, diagnostic procedures, devices and techniques are rightly forcing the physicians to deal with previously unknown or not considered issues. These major challenges determine the positive effect of expanding the horizons of traditional aesthetic medicine and dermatology thus pushing doctors to gradually shift their study and intervention fields from organs and tissues to cells and molecules, in an increasingly specialized perspective. This in turn may expose doctors to become super-specialists with the concomitant risk of losing the uniqueness of the person, composed of body, mind and spirit but, on the other side, the lack of knowledge can make real the unwanted possibility of passively accept any new proposal coming from sector’s companies and, therefore, to trigger dangerous ethical drifts of legal relevance.

Down in the more technical details, what has changed in recent decades, in the field of aesthetic medicine and dermatology? And what are the prospects?

In agreement with the available scientific literature, a first series of news can be found in the knowledge of physiology and biochemistry of the traditional therapeutic target tissues/organs of aesthetics, i.e. the skin and its subcutaneous, the connective and adipose tissues, the extracellular matrix environment, the microcirculation, and so on. For instance it is now clear that adipose tissue is not simply a food surplus store but acts as a multi-hormone endocrine system, able to modulate food intake, to regulate the metabolism of caloric substrates in the other body’s districts and to produce a series of additional important systemic actions. These new find-
ings are opening new way to learn more about local adiposity and cellulite and to diagnose, prevent and treat them, as is reported in this issue. In particular increasing evidence suggests the key role of reactive processes including oxidative stress and inflammation in the pathogenesis of most aesthetic imperfections finally leading to early senescence; from this point of view it is often difficult to separate “aesthetic medicine” from “anti-aging medicine”, both being connected by the thread of “positive biology”. This latter rather than making diseases the central focus of research, seeks to explain the causes of positive phenotypes, trying to explain the biological mechanisms of health and well-being in order to prevent and/or reduce frailty and disability.

In turn such extraordinary development of biochemistry and molecular biology, associated the bio-informatics and robotics, in recent decades has allowed to identify, quantify and localize the distribution, in various living organisms, many thousands of chemical species, according the various classes or families (nucleic acids, proteins, lipids, glucides, etc.). The first example of this analytical approach was provided, probably, from genomics, which pursues, in fact, the primary objective to “map” the entire genome or to study the structure, function and evolution of nucleic acids (DNA or RNA), custodians of genetic information in all living organisms. In relatively recent times, the potential of genomics has been enhanced by new conceptually similar branches of biology to which it was given, by analogy, the suffix “-omics”. Thus, recalling the famous “central dogma of molecular biology” (DNA>RNA>Protein) or, much better, by following the flow of information in living beings, transcriptomics, proteomics, metabolomics, and lipomics, each closely associated with a well-defined set analytical methods, were born. Furthermore, we expect to get more new information from the most recent approach, such as redoxomics, exposomics and microbiomics, in combination with the emerging field of epigenetics.

The development of such suitable biomarkers has been accompanied by the manufacture of new instrumental techniques of imaging based on magnetic resonance and computed tomography with the final purpose to personalise and to monitor any therapy in the field of aesthetics as well as dermatology. Particularly relevant appears the real time display of oxygen level or other compounds, like ascorbic acid, into the tissues.

In parallel the options of treatment in aesthetics in dermatology have been surprising grew with the availability of new medical devices for gas therapy (e.g. oxygen propulsion, carboxytherapy, ozone therapy), radio-frequency, ultrasound, light, laser and so on for treatment of almost all aesthetics imperfections. Unfortunately the real efficacy of some such devices is still to establish while more consistent appear the approach of regenerative medicine.

Regenerative medicine is a branch of translational research in tissue engineering and molecular biology that deals with the “process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function”. This field holds the promise of engineering damaged tissues and organs via stimulating the body’s own repair mechanisms to functionally heal previously irreparable tissues or organs. Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and safely implant them when the body cannot heal itself. And if a regenerated organ’s cells would be derived from the patient’s own tissue or cells, this would potentially solve the problem of the shortage of organs available for donation, and the problem of organ transplant rejection.

Besides this, new challenges await health professionals engaged in the field of dermatology and aesthetics who are continuously rising new attracting and some time “hot” topics, including the impact of the aesthetic treatments on the patient’s psychology and the behaviour as well as on the society customs, in terms of mass media and law roles and rules. In this context rejuvenation, face “westernization” in oriental peoples, anatomical changes in transgender, robotics are also noticeable key words of such novel aesthetics.

In line with these principles, the European Journal of Aesthetic Medicine and Dermatology (EJAMED), a peer-reviewed quarterly scientific journal funded in 2011 by Pasquale Motolese, is aimed to translate the findings of Aesthetic Medicine, with a special focus on Dermatology, from the basic research to the clinical practice. In particular EJAMED intends stimulate the integration of all the branches of Medicine that are involved in aesthetic care, from the Biochemistry to the Surgery, in order to make available to the health profes-
sionals a validated and continuously updated tool for a multidisciplinary and personalised approach of patients. In this context the Editorial board looks forward to collaboration with all scientific societies that share our mission.

Therefore, in agreement with the above aim and mission, EJAMED offers not only to aesthetic physicians, plastic surgeons and dermatologists but also to pharmacologists, nutritionists, biologists, engineers, and chemists the unique opportunity to disseminate virtually worldwide their case reports, experimental and clinical trials, as well as innovative research and reviews to all healthy professionals.

Moreover EJAMED supports educational programs, stimulates discussion and round tables, promotes consensus conference for shared protocols, position papers, and guidelines in the common fields of Aesthetic Medicine. Furthermore EJAMED provides to the lecturers and subscribers some additional services including the news from the world, congress calendars, and so on with a direct interaction via web between the official site and social networks.

Thanks to this global approach EJAMED intends to enhance the practice of aesthetic medicine and, above all, contribute to the creation of a training and educational programs which, through a multidisciplinary and integrated vision of the Medicine (where there are internal medicine, geriatrics, dermatology, endocrinology, andrology, gynecology, nutrition, physical therapy and rehabilitation, posturology, ambulatory surgery, phlebology, cosmetology, psychology, thermal medicine, motor science, wellness, lifestyle, genetics and biochemistry) finally should create the professional medical aesthetic, which still does not yet exist in most countries.

As Editor in Chief of EJAMED that Today restarts I would like to express my sincere gratitude to the late Professor Motolesse that although having us left so early has bequeathed a huge patrimony of knowledge that we intend to protect and to develop in the next years. A heartfelt thanks goes to all the staff of EJAMED.

Eugenio Luigi Iorio
THE NODULE OF DISCORD.
The unresolved diatribe on the pathogenesis of cellulite in the light of the adipocyte pathophysiology.

Ferdinando Terranova MD (1), Eugenio Luigi Iorio MD, PhD (2), Domenico Amuso MD (3).
(1) International School of Aesthetic Medicine, Rome (Italy). (2) International Observatory of Oxidative Stress, Salerno (Italy). (3) University of Palermo, Palermo (Italy).

European Journal of Aesthetic Medicine and Dermatology

Corresponding Author
Dr. Ferdinando Terranova
fierranova52@alice.it

Conflict of Interest: None declared.

Keywords
cellulite, adipose tissue, subcutaneous, fibrosis, inflammation, metabolic syndrome.

ABSTRACT
The studies about cellulite published by high-impact-factor journals are limited in number and reach antithetical conclusions. Consequently, it is not yet possible to resolve the serious differences among the ideas that, for years, have been dragging on the nature of this disease, on its origin and even on the most basic aspects of its histopathology. It is also lacking a universally recognized name.

Over the past decades, five main hypothesis about its pathogenesis have been opposing, with varying success, indicating, as the cause of the imperfection, respectively:
1. an oedema caused by an excessive hydrophilicity of the intercellular matrix;
2. an alteration of the homeostasis at the end of the micro-circulation district; this pathogenic theory is summarized in a concise and explanatory name: edematous-fibrous-sclerotic panniculopathy;
3. the protrusion of intradermal adipocyte hernias, favoured by the peculiar anatomy of the female subcutaneous, different from the male one;
4. the unequal reaction that the fibrous interlobular septa oppose to the stretching induced by the enhanced adipose thickness;
5. the protease-dependent lysis of the fibrous septa.

Today, perhaps, all these assumptions are going to be updated in light of recent findings about the sophisticated and complex pathophysiology of adipose tissue, which acts not only as a food surplus store, but also as a controller of the energy balance of the entire organism and as multi-hormone endocrine and paracrine system, able to modulate food intake, to regulate the metabolism of caloric substrates in the other districts and to produce a series of additional important systemic actions.

The several adipocyte skills make it seem unlikely that this element remains a powerless victim of external factors, when there is a widespread anatomical and functional alteration of the tissue that houses it.

The adipose organ, as rightfully today it is named, is distributed in various regions of the body; different deposits show extremely variable dimensions, in relation to caloric balance, gender and age; they manifest very different biological activities, depending on the location and on the expansion. A very wide set of data demonstrates that the upper body adipose fat (i.e. the visceral fat and the subcutaneous fat of the abdominal wall), 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, dyslipidemia and cardio-vascular atherosclerotic illness. The subcutaneous tissue of the lower limbs is well developed in women, becoming the elective cellulite localization; even when in excess, it does not entail an increased risk of systemic complications, against which seems, in fact, to perform a protective function. Some observations lead, however, to believe that, even in this place, processes of inflammatory remodelling, may occur, capable of contributing to produce the typical alterations of cellulite.

The aim of the present review was to provide an update on the pathogenesis of the so-called “cellulite” with a special focus on the emerging role of adipose tissue.
Introduction

Every doctor is bound to experience a deep discomfort when he is forced to treat any disease on the basis of scarce and contradictory knowledge; unfortunately, a similar embarrassing situation is carried out all the times when a female patient requires to the attending physician to help her to resolve the problem of cellulite. This condition, almost exclusively feminine, compromises the silhouettes (and the good humour) of millions of women all over the world. All media that identify their target in women younger than 50 years make a lot of talk about 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 an ephemeral glory, before proving to be ineffective; this does not prevent beauty salons and beauty-farms to continue to collect huge amounts of money, passing off unlikely panaceas.

Faced with a very felt problem, the progress of medical science appears remarkably small. Questioning the Medline database, 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 that in the "cellulite problem" 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 still continue to provide valuable contributions to the advancement of knowledge about the skin pathophysiology.

Consequently, it is still not possible to resolve the serious differences of ideas that, for years, have been dragging on the nature of this disease, its origin, and even on its most elementary histopathologic aspects. It is also lacking a universally recognized name: most of the authors that touch the subject are used to begin the discussion with an introduction that explains that the word “cellulitis”, came into international use, is inappropriate and inadequate, as suggestive of an inflammatory nature of the affection. However it was not found a better name. Indeed, the term "cellulitis" is used, in English medical science to indicate a disease altogether different: the gangrenous-suppurative infection of the subcutaneous fat (12). In the chapters of panniculitis (13), of liposclerosis (14), and of lipodystrophy (15) there are included morbid forms clearly not comparable to the common cellulite. Finally, the remaining designations, from time to time employed, (e. g. lipoedema, adiposis oedematosa, dermopanniculiosis deformans, edematous-fibrous-sclerotic panniculopathy, status protrusus cutis, etc.) summarize morphological and pathogenic conceptions not shared by everyone.

There can be several causes of the continuing knowledge inadequacy: 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 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 organization difficult and controversial.

Fortunately, in recent years, this disastrous gap in the understanding of one of the most common female blemishes was partially filled in, so we could say, indirect way: the ever rising prevalence of the obesity and the related diseases has produced an incentive for a growing interest of researchers around the world to study the pathophysiology of the adipose tissue. Therefore, thousands of articles have been published which, although 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 the 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 good note of those tissue changes which, not being described in any of these publications, likely cannot be part of the histopathology of a so commonly seen condition as cellulite, you may be able, with difficulty, to obtain the necessary information to unravel the jumbled tangle of theories on the pathogenesis of this blemish.

This article will review the pathophysiology of cellulite on the basis of the available scientific literature starting from the name itself of such clinical condition and then facing the issue according to the most validated hypotheses with a wide focus on the possible role of adipose tissue.
The adipose tissue

Cellulite is a condition that, in women, electively affects subcutaneous fat, with a trend to prefer some anatomic areas. 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.

1. Morphological aspects

Adipose tissue is a special type of connective tissue, whose constitutive elements are 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. 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 shows 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, i.e. the white and the brown adipose tissue.

2. The white adipose tissue

Unilocular adipose tissue is often referred to as “common” or “yellow” or “white” (see also below), 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 mono-glycerides, 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 “uni-locular” 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 (Figure 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).

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.

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).

3. The 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. Indeed 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 F18-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) that 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 Authors 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) (Figure 2).

Among 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 PGE2 (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).

Figure 2.
Histogenesis of white, brown and “beige-brite” adipocytes.
4. 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. On the contrary, today we know that, even after the development age, fat tissue holds stem 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 local 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 remodelling (52), accompanied by the formation of new blood vessels (53, 54). Nuclear receptors PPARγ (targets of the oral antidiabetic drugs tiazolidinediones) 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 lipoprotein-lipase (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 acyl-CoA 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: \( \beta_1 \), \( \beta_2 \), \( \beta_3 \) and \( \alpha_2 \). The three \( \beta \) subtypes, and particularly the \( \beta_2 \) one, transmit a lipolytic stimulus, while \( \alpha_2 \) units send off a contrary signal; the adipocyte is the only cell that hosts simultaneously, on its membrane, agonist and antagonist adrenoceptors. The amount of triglycerides hydrolyzed depends on the local balance between these opposite inputs (64). The \( \beta_2 \) 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). The \( \alpha_2 \) 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) (Figure 3).

Under normal conditions, the \( \beta_2 \) 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 \( \alpha_2 \) adrenergic receptor. The balance between lipolytic and antilipolytic catecholamines stimulation depends, in a determined district, from the numerical ratio between \( \beta_2 \) and \( \alpha_2 \) 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 adeniltato-cyclase enzyme and, therefore, of the cAMP production. Caffeine and theophylline are adenosine antagonists (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).

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 di-glycerides (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 ob-

---

**Figure 3.**
Mechanisms of regulation of adipocyte lipolysis.
tained. At this point, the PKA phosphorylates also the HLS: 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 mono-glycerides (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.

Table 1. Adrenergic receptor agonists and antagonists and post-receptor modulators capable of interfering on lipolysis.

<table>
<thead>
<tr>
<th>MODULATOR</th>
<th>MAIN MOLECULAR EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>Non-selective beta and alpha-adrenergic agonist</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>Non-selective beta-adrenergic agonist</td>
</tr>
<tr>
<td>Propanolol</td>
<td>Beta-adrenergic antagonist</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Selective alpha-2 agonant</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>Selective alpha-2 antagonist</td>
</tr>
<tr>
<td>Forskolin</td>
<td>Direct adenylate cyclase activator</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>Selective inhibitor of phosphodiesterase</td>
</tr>
<tr>
<td>Dibutyryl-AMP</td>
<td>Stimulator of protein kinase A</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Adenylate cyclase inhibitor</td>
</tr>
<tr>
<td>N6-(L-2-Fenilisopropil)-Adenosine</td>
<td>Adenosine receptor agonant</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Adenosine antagonist</td>
</tr>
</tbody>
</table>

Figure 4. Various lipases work in sequence to hydrolyze the three molecules of fatty acids from triglycerides.
• **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. Free fatty acids (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, they 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, this 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 characterized 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 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 store, 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 and 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 means of influence on the functions of circulatory system, immune system (75), kidney, gonads and scaffold bone, with important impacts even on cell proliferation, inflammatory processes (76, 77), reproduction and aging pathophysiology (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), TNF-α (88, 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), adipin (100), vaspin (visceral adipose tissue-derived serine protease inhibitor) (101), chemerin (102), prostaglandins (103, 104), lipoprotein lipase (105), angiotensinogen and angiotensin II (106), FGF (fibroblast growth factor) (107), TGF-β (transforming growth factor-beta) (108), PAI-1 (plaminogen activator inhibitor-1) (109), VEGF (vascular endothelial growth factor) (110, 111), MMP (matrix metalloproteinases) (112, 113) (Table 2).

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, glucocorti-
coids, 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 enhancing 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

<table>
<thead>
<tr>
<th>ADIPOKINE(S)</th>
<th>SITE OF ACTION</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>Hypothalamus</td>
<td>Represses hunger, increases energy metabolism</td>
</tr>
<tr>
<td></td>
<td>Immune system</td>
<td>Keeps immune system up-regulated</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular system</td>
<td>Exhibits anti-inflammatory effect</td>
</tr>
<tr>
<td></td>
<td>Endocrine system</td>
<td>Regulates puberty and reproduction</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>Improves insulin sensitivity</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Immune system</td>
<td>Decreases the release of inflammatory molecules</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>Increases fatty acid oxidation, glucose uptake, and lactate production</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Reduces the levels of molecules involved in gluconeogenesis, increases free fatty acid metabolism</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular system</td>
<td>Exhibits anti-atherosclerotic effect</td>
</tr>
<tr>
<td>Resistin</td>
<td>Immune system</td>
<td>Stimulates inflammation</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular system</td>
<td>Impairs vascular relaxation</td>
</tr>
<tr>
<td>Retinol-binding protein 4</td>
<td>Plasma</td>
<td>Transports vitamin A</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>Impairs insulin signalling</td>
</tr>
<tr>
<td>Tumor necrosis factor alpha (TNF-α)</td>
<td>Skeletal muscle</td>
<td>Impairs insulin signalling</td>
</tr>
<tr>
<td>Viscatin</td>
<td>Skeletal muscle</td>
<td>Binds to insulin receptors and mimics insulin</td>
</tr>
<tr>
<td></td>
<td>Immune system</td>
<td>Causes release of TNF-α and interleukins (inflammatory signals)</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>Skeletal muscle</td>
<td>Impairs insulin signalling</td>
</tr>
<tr>
<td>Angiotensinogen and angiotensin II</td>
<td>Vascular system</td>
<td>Induces smooth muscle cell contraction and raises blood pressure</td>
</tr>
<tr>
<td></td>
<td>Adipose tissue</td>
<td>Exhibits pro-inflammatory effect</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>Skeletal muscle</td>
<td>Promotes insulin resistance</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Promotes insulin resistance</td>
</tr>
</tbody>
</table>
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 shows effects similar to those of insulin; it seems to activate the insulin receptor but by binding to a different site to that of such hormone. 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 diabetogenic effects. In rodents, the circulating levels of resistin 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 studies suggested that the plasma levels of resistin (120) and/or individual variations in its amino acid composition (121) can affect the onset 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 CRP, 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).

5. 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 peri-renal 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, epididymal, 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 and 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 characterized by a reduced amount of visceral fat and by a 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, 135). These two opposing models of fat distribution occur from puberty, making clear the role that, in their genesis, sex hormones play (136, 137). 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 (138). In menopausal women, characterized 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 (139). 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 (140, 141).

The androgens, however, determine, on the adipose tissue distribution, different effects, depending on gender (142). In men, testosterone declines with age and this decay is accompanied by an increase of body fat, which is mainly localized in the abdomen (143, 144). In elderly males the testosterone replacement therapy causes a decrease in visceral fat deposits and an increase in lean muscle mass (145). Conversely, in females, testosterone administration induces a significant increase in splanchnic fat (146) that is also found in women with polycystic ovary syndrome (PCOS), characterized by an overproduction of androgens (147, 148). Finally, animal studies show that a prenatal testosterone administration to female rats causes, in adulthood, a male pattern of fatty deposits distribution (149).

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 oestrogen receptors: both nuclear receptors (ERα, ERβ and its variants) and membrane G protein-coupled receptors (150). 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 (151). Conversely, the abdominal fat contains more ERα proteins than the gluteal-femoral one (152). Consequently, the ratio between waist and hips circumferences (WHR) appears correlated to the ERα/ERβ ratio, pointing out that oestrogen receptors play a role in modulating the regional fat distribution. Overall, oestrogen activity is more intense 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 testosterone) into estrone and estradiol, respectively (153).

Estrogens stimulate the proliferation of preadipocytes; this mitogen effect has been demonstrated both in visceral and in subcutaneous fat, but it appears much more pronounced in the latter, especially in females (154). It is also possible that sex steroids influence adipose tissue biology primarily by means of effects on the central nervous system, rather than through a 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 (155).

Gender differences in fat distribution involve not only the size but also the number of cells. The visceral adipocytes are greater in men than in women (156); 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 tends 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 (157). 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 excess 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 (158). Therefore, following a high fat diet, in the abdominal adipose tissue the proliferation of pre-adipocytes appears very modest and poor as well as their differentiation capacity, while their sensitivity to apoptotic stimuli is high (159). Just apoptosis may be the cause of an early exhaustion of the stem cell pool, that, according to some Authors, would explain the scarcity of pre-adipocytes. 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 (160).

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 (161). 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 number of micro-environmental factors, that are related to the innervation, the vascularization and the blood vessels structure. 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 (162, 163). The diverse patterns of gene expression that characterize the adipocytes in distinct regions of the body are acquired prenatally by tissue stem-cells 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 (164) 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 (165, 166).

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 (Table 3).

The triglyceride storage into adipocytes follows, in large part, to the uptake, by the lipoprotein lipase, of the fatty acid carried by plasma lipoproteins (167). The LPL expression in male is more intense within the adipocytes of the upper body (168, 169), while in women it is superior within the cells of the gluteal-femoral region (170, 171), 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 (172).

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%) (173). 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 (174, 175). 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 (176), but the triglyceride hydrolysis therein caused by the administration of norepinephrine is much greater in women (177). 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

<table>
<thead>
<tr>
<th>METABOLIC ACTIVITY</th>
<th>ANATOMIC AREA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal lipolysis</td>
<td>UBS &amp; VF &gt; LBS</td>
</tr>
<tr>
<td>Activation of lipolysis by catecholamines</td>
<td>UBS &amp; VF &gt; LBS</td>
</tr>
<tr>
<td>Inhibition of lipolysis by insulin</td>
<td>LBS &gt; UBS &gt; VF</td>
</tr>
<tr>
<td>Presence of lipolytic β2-adrenergic receptors</td>
<td>UBS &amp; VF &gt; LBS</td>
</tr>
<tr>
<td>Presence of anti-lipolytic α2-adrenergic receptors</td>
<td>LBS &gt; VF &amp; UBS</td>
</tr>
<tr>
<td>Activity of lipoprotein lipase in the male</td>
<td>UBS &amp; VF &gt; LBS</td>
</tr>
<tr>
<td>Activity of lipoprotein lipase in the female</td>
<td>LBS &gt; UBS &gt; VF</td>
</tr>
<tr>
<td>Release of ffas in the portal circulation</td>
<td>VF</td>
</tr>
<tr>
<td>Release of ffas into the general circulation</td>
<td>UBS &gt;&gt; LBS</td>
</tr>
<tr>
<td>Neoadipogenesis capacity</td>
<td>LBS &gt; UBS &gt; VF</td>
</tr>
<tr>
<td>Production of leptin</td>
<td>LBS &gt; UBS &gt; VF</td>
</tr>
<tr>
<td>Production of adiponectin</td>
<td>LBS &gt; UBS &gt; VF</td>
</tr>
<tr>
<td>Production of IL-6</td>
<td>VF &gt; UBS &gt; LBS</td>
</tr>
<tr>
<td>Production of TNF-α</td>
<td>VF &gt; UBS &gt; LBS</td>
</tr>
<tr>
<td>Production of angiotensinogen</td>
<td>VF &gt; UBS &gt; LBS</td>
</tr>
</tbody>
</table>
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 to explain the diversities regarding the metabolic consequences of the lipid accumulation in the upper body vs. the lower body (186, 187). 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 remodeling, and, on a systemic level, to induce the insulin resistance (16, 188).

**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/m²) in the US population has risen from 15% to 31% (189, 190), while the prevalence of overweight (BMI > 25 kg/m²) 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.

### I. Visceral adiposity

Since the time of the observations by Jean Vague (191), 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 (192). 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 and 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 (193, 194).

In support of these conclusions, there are the findings from a large number of epidemiological studies (to name a few, Hartz (195), NHANES III (196), Goteborg (197), Health Professional Study (198), Nurses’ Health Study (199), Hoorn Study (200) 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 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) (201). 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.

2. 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, and 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, fats, 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 (mitogen-activated 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 anti-inflammatory and vasodilator effects (202).

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 (203), which is able to produce its disastrous systemic effects through three main mechanisms, i.e. an altered secretion of adipokines, the induction of a pro-inflammatory systemic condition, and the lipotoxicity.

About the first proposed mechanism, 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, angiotensinogen 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 (204).

Regarding the second mechanism, i. e. 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 (205). 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 (206). 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 (207, 208), 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 the hypoxia inducible factor 1 alpha (HIF-1α) (209), the angiotensin converting enzyme (ACE) (210) and leptin, as well as of angiogenic cytokines, especially the vascular endothelial growth factor (VEGF) and the monocyte chemoattractant protein-1 (MCP-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 (211). Hypoxia and excessive lipid load, provoking endoplasmic reticulum stress (212), activate NFκB factor (intracellular mediator of inflammatory phenomena) (213) and can come to cause the death by apoptosis of some adipocytes (214). All these events help to attract into the adipose tissue a large number of blood monocytes, which are transformed into macrophages and take frequently a circle disposition around apoptosis undergoing fat cells (215) (crown-like structures) (Figure 5). The macrophages that infiltrate visceral fat express markers indicative of their M1 polarization, which involves a distinctly inflammatory functional attitude (216). 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) (217, 218). A further stimulation of these receptors is due to lipopolysaccharide 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 (219). 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 widespread fibrosis (24, 25, 220). The presence of tissue collagen I and collagen VI, as well as the expression of their mRNA, appear to grow exponentially (221), because macrophages become capable of inducing the adipose stem cells to transform into myofibroblasts (222). 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 (223). 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 (224), 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 (225).

Finally, about the third mechanism, i. e. 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 (226). 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 antilipo-lytic effects (208. The high release of FFA and glycerol

Figure 5. Cellulite: macrophages surrounding an adipocyte in apoptosis.
3. Fatty tissue and aging

Adipose tissue shrinks important and intricate relations with the aging-related phenomena (237, 238). 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 (239). On the other hand, research 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 (240). 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 (241, 242). 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 (243). 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 by 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 (244).

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 (245), including those mediated by GH and IGF-1, determine an elongation of lifespan. So it is for example:

---

European Journal of Aesthetic Medicine and Dermatology

---
The first attempt to define cellulite and the very factors leading to the widespread unsightly named cellulite. As the site of the alterations, yet not uniquely defined, adipose tissue assumes a further significant value, for professionals working in the field of cosmetic medicine, 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% (252). Of great interest is the increase in longevity and stress resistance that was observed in mice genetically lacking the protein p66Shc (253). 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 (254). 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 (255). Again in mice, the surgical ablation of visceral fat (but not of the subcutaneous) elevates the longevity (256).

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 (257) 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 (258, 259), represent detrimental conditions that generate cumulative harmful parenchymal micro-injuries, which, during the years, combine to cause the gradual senile decay.

Nature and causes of cellulite
For professionals working in the field of cosmetic medicine, adipose tissue assumes a further significant value, as the site of the alterations, yet not uniquely defined, leading to the widespread unsightly named cellulite.

The first attempt to define cellulite and the very plethora of the term are due to the French doctors Alquier and Pavot who, in 1922, described a mesenchymal tissue dystrophy, devoid of inflammatory aspects, characterized by a stagnation of interstitial fluids. The two authors considered this affection as a primary reaction of the connective tissue, in response to damaging agents of various nature (traumatic, toxic, infective, endocrine).

Over the past decades, five main hypothesis about cellulite pathogenesis have been opposing, with varying success, indicating, as the origin of the imperfection, respectively: 1) an edema caused by an excessive hydrophilic activity of the intercellular matrix, where an inordinate amount of proteoglycans accumulate; 2) an alteration of the homeostasis at the end of the micro-circulation district; this pathogenic theory is summarized in a concise and explanatory name: edematous-fibrous-sclerotic panniculopathy (EFSP); 3) the protrusion of intradermal adipocyte hernias, favoured by the adiposity and by the peculiar structure of female subcutaneous, different from male anatomy; 4) the unequal reaction that the various fibrous interlobular strands oppose to the stretching induced by the enhanced adipose thickness; 5) the protease-dependent lysis of the fibrous septa.

1. Excessive hydrophilic activity of the intercellular matrix
In 1964, Bassas-Grau described, in the connective matrix of subcutaneous tissue in patients suffering from cellulite, phenomena of hyper-polymerization of acid mucopolysaccharides, to which he claimed responsibility for an abnormal increase in tissue hydrophilicity. The persistence of this anomaly would lead to the formation of a chronic oedema, which would undergo a process of organizing, evolving in fibrous-sclerosis (260). The observation, although not confirmed by other authors (261, 262), has exerted a lasting influence on the therapeutic approaches, justifying the administration, topical or by mesotherapy, of hyaluronidase and other drugs accredited of lytic activity against proteoglycans.

In times closer to us, Lotti et al., using ruthenium fixation techniques in electronic microscopy, examined the dermis of the skin overlying the areas of adipose tissue affected by cellulite, where, besides some signs of fibroblast activation, abnormality of the micro-vascular wall and sub-epidermal depletion of collagen and elastic fibres, it was found an increased presence of glycosaminoglycans (263). According to the hypothesis of the Authors, reiterated in a more recent revision (264), this histochemical change, corresponding to a primitive connective abnormality, may result, by attracting fluids...
into the interstitial substance, in a showy synthesis of new fibres in the subcutaneous.

2. Microcirculatory alteration

The theory that explains the cellulite as an outcome of a primitive dysfunction of the tissue microcirculatory network is, at present, the most followed, at least in Italy and, to a lesser extent, in other European countries. European researchers have, after all, made a decisive contribution to its development.

Binazzi, in the second half of the 70s, has set a milestone in the interpretation of the cellular histopathology (265), with a series of observations that have enabled him to develop a pathogenic hypothesis even today current and to summarize it in a synthetic and explanatory name: Edematous-Fibrous-Sclerotic Panniculopathy (EFSP) (266).

The initial stage, in the opinion of Binazzi, is often associated with an excessive fat deposits and it is clinically characterized by irregularities of the skin surface, in the regions of buttocks, thighs, abdomen and shoulders: the so-called "mattress-like" skin. Histological examination showed only an extreme variability of the shape and size of adipocytes (aniso-poikilocytosis), along with a gel-like oedema of the dermis, dilatation of lymphatic vessels and patches of follicular hyperkeratosis (orange peel skin).

The next phase is characterized by the possibility to appreciate, by palpation, sliding and painful nodular lesions, with diameters ranging from 1 to 5-6 mm; tissue sections, according with Binazzi, let see a profound subversion of the subcutaneous tissue, where fibrous strands, tending to become sclerotic, surround fat cell clusters, forming nodular structures; thrombo-hemorrhagic vessel alterations are seen too.

Ryan (267), Merlen (268, 269, 270) and, above all, Curri (271, 272), have interpreted these regressive processes in the context of a pathogenic conception that identifies the damage primary cause in an impaired microcirculatory homeostasis.

Curri was inspired, for its consideration, by a careful anatomical and physiological study of the terminal circulatory branches: the main functional element of the peripheral vessel network is, in its view, the microvasculature-tissue unit (or histangium) (273), formed by the distal vessel district (afferent arterioles, meta-arterioles, precapillary sphincters, arteriovenous anastomosis, capillaries, efferent venules, initial lymph vessels) and by the connective tissue matrix surrounding the vessels. The latter, besides acting as a mechanical support to the thin capillary wall (mucopolysaccharide sheath) (274), performs tasks of dynamic filter in the metabolic exchange between the blood and the parenchyma (275).

Motor of the blood microcirculatory flow is the vasomotion, a rhythmic, contractile activity of arteriolar muscle cells (276, 277, 278), whose frequency varies between 3 and 20 cycles per minute, according to local conditions (interstitial pressure, PO2, etc.); it causes, at the capillary level, undulatory changes in blood flow (flowmotion) (279, 280).

Recent acquisitions have clarified that the endothelium is the hinge of the microcirculatory homeostasis; it not simply fulfils a purely mechanical task of intraluminal coating, but modulates blood-tissue exchanges and (through a complex activity of biosynthesis that assimilates it to a glandular scattered system), oversees the equilibrium between pro- and anti-coagulant, fibrinolytic and anti-fibrinolytic, vasodilator and vasoconstrictor phenomena, in order to adapt, in real time, the operation of the local microcirculation to the tissue changing needs.

A lot of data have shown, in recent years, the important role that the endothelial dysfunction can play in the pathogenesis of several diseases (281, 282, 283).

Many indications suggest that the female oestrogen hormones influence decisively the endothelial functions, which assumes distinctive aspects in women of childbearing age (284, 285).

Curri has elucidated the morphological characteristics of the subcutaneous microvasculature-tissue unit (286), characterized by:
- capillary network with very tight mesh, in intimate closeness with the adipocytes, reducing to a minimum the "diffusion space";
- absence of arteriovenous anastomosis, which leads us to believe that the continuity of the adipocyte perfusion constitutes a non-expendable need;
- branches that connect arterioles and venules of adipose tissue to the vessel network of dermis and muscle: they realize microvasculature-tissue cylindrical units, arranged, perpendicular to the skin surface and super-extended from the latter to the underneath layers of hypodermis (287). This justifies the frequent association of subcutaneous microcirculatory debts with blood flow alterations propagated to the adjacent skin and muscles, where cause clinical symptoms (hyperkeratosis, cramps) (288) and instrumental (289, 290) signs.

The presence of estrogen receptors in endothelial cells and smooth muscle (291, 292) makes reason of the functional peculiarity of the female microcirculation (293, 294), with particular regard for vascular tone (295, 296) and permeability (297).

On the basis of these assumptions and of a careful analysis of histological and clinical-instrumental data, Curri constructed his hypothesis on the origin of the EFSP. The causal factor is identified in a chronic "microcirculatory maldistribution", in turn, related to a prim-
itive defect of the arteriolar flow modulation or to an inappropriate vasomotion. This condition, sometimes framed as part of the so-called hypotonic phlebopathy (preclinical phase of venous insufficiency), is followed by:

- slowing of blood circulation;
- erythrocyte sludge;
- impaired capillary hydrostatic balance;
- reduced parietal and tissue oxygenation;
- endothelial damage (endothelial swelling, micro aneurysms, haemorrhages);
- abnormal permeability of capillaries and venules;
- increased hydrostatic pressure of the interstitial fluid and of its protein content;
- recurrent episodes of inter-adipocyte oedema.

Adipocytes suffer from damages that are expressed initially with unequal cell sizes and abnormal shapes (aniso-poikilocytosis), later with plasma membrane ruptures and leakage of lipid material. The subsequent pathological event, that characterizes in a specific way, the evolution of the histological and clinical aspects, is represented by fibrosis. The very thin web of delicate filaments that serves as scaffold for the adipose lobule, wrapping each single cell (22, 25), progressively thickens. Strands of young connective tissue subdivide the lobules, surrounding clusters of degenerate fat cells (298, 299, 300); the nodules thus formed tend, then, to be lumped together by the further attachment of collagen material, tending to a sclerotic evolution, until palpable macro-nodules are shaped, that cause the skin surface irregularity (Figure 6).

Curri traced back the fibrosis pathogenesis to the so-called “fibrous organisation” of the chronic oedema and of its rich protein content. This interpretation was shared, most recently, by Brazilian authors who have, however, attributed the cause of the stagnation of interstitial proteins to a primary disorder of the lymphatic system (301, 302).

The deposition of collagen in the interstitial matrix which is assumed to be realized in the subcutaneous affected by cellulite, seems to have some common aspects with the much more heavy fibrous-sclerosis which occurs in chronic lymphatic stasis (303) and in lipodermatosclerosis (304). We now know that every fibrosis is a highly regulated phenomenon, which involves a series of cytokines, growth factors and hormonal substances (305); the hypoxia and the consequent oxidative stress can be considered among the first links in the pathogenic chains (306).

In the development of the EFSP, Curri identifies four developmental stages (307) (Table 4):

---

Table 4. The four evolutionary stages of Edematous-Fibrous-Sclerotic Panniculopathy (EFSP) according to Curri’s opinions.

<table>
<thead>
<tr>
<th>CLINICAL PRESENTATION</th>
<th>PATHOGENESIS</th>
<th>HYSTOLOGIC AND HISTOCHEMICAL ASPECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st STAGE</td>
<td>Micro-circulatory misdistribution, vasomotion defects</td>
<td>Adipose tissue oedema and aniso-poikilocytosis, membrane breaking</td>
</tr>
<tr>
<td>Pale and pasty skin</td>
<td>Stasis, erythrocyte sludge, micro-vessel ectasia, abnormal permeability, zonal hypovolemia and hypoxia</td>
<td>Regressive adipocyte manifestations, massive micro-vessel ectasia, new fibril synthesis</td>
</tr>
<tr>
<td>2nd STAGE</td>
<td>Reduced capillary flow, increased areas of relative hypoxia</td>
<td>New fibril synthesis, encapsulation of clusters of degenerate adipocytes into small nodules</td>
</tr>
<tr>
<td>Hypothermia, skin elasticity failure, paraesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin orange peel (patches of hyperkeratosis), thin granules at palpation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd STAGE</td>
<td>Hypovolemia, telangiectasias, little varicose vein</td>
<td>Connective sclerotic strands surrounding macro-nodules, local dystrophic phenomena of dermis and epidermis</td>
</tr>
<tr>
<td>Mattress-like skin, painful nodules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th STAGE</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="http://www.ejamed.com">www.ejamed.com</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---
In the first stage owing to an excessive accumulation of triglycerides, the adipocytes increase their volume, although in an irregular way. Signs of a defective micro-circulatory regulation mark their appearance: areas of vasodilation alternate with areas of capillary hypovolemia. The arterioles show reduced pulsations and vasomotion loss. Progressively a condition of venous and lymphatic stasis is established. The increased permeability of the capillaries generate a tissue oedema which determines cell deformation, loss of intercellular connections, expansion of the interstitial gaps and, finally, the breaking of the cytoplasmic membranes. The oxygen shortage and the insufficient drainage of interstitial fluids lead to the dissociation of the reticular, collagen and elastic fibres. Physical examination shows an increased skin softness with reduction of dermal-epidermal elasticity. Areas of pallor and superficial hypothermia are also seen.

In the second stage hypoxia and interstitial oedema promote the densification of the fibrous stroma surrounding the fat lobules, while the epidermis gets thinner. Simultaneously, the progression of hypodermal microangiopathy can be detected; the small vessels show extensive alterations, with reduced contraction rhythm and appearance of ectasias and micro-aneurysms.

In the third stage the microcirculatory alterations also affect the wall of greater arterioles. The blood stasis in capillaries and little veins becomes more apparent and significant haemorrhages occur. The interstitial connective matrix further thickens, until the collagen fibres form a compact texture that stifles vascular and cellular elements, hampering the vital functions. The new fibril synthesis tends to encapsulate small groups of adipocytes, with formation of micro-nodules. The border between the dermis and the subcutaneous is progressively eroded. The epidermis participates in the disorder with a focal hyper-keratosis. Clinically the third stage is characterized by the appearance of a skin surface roughness, known by the term of "orange peel"; tissue palpation allows to detected the presence of thin granules and a widespread tenderness (cellulalgia or pain syndrome of cellulite areas).

In the fourth stage arteriolar sclerosis, dilations of small veins, hypovolemia and capillary rarefaction create a serious deficit of the microcirculatory flow. The subcutaneous thickens, for the appearance of the typical lesion of the disease: the macro-nodule. The hypodermal normal partitioning into lobules is completely overthrown, replaced by irregular adipocyte clusters, and encapsulated by thick connective strands.

The histopathologic picture described by Merlen, Curri and Binazzi was recently confirmed in its essence, by a study of Japanese researchers who examined, with light and electron microscopy, biopsy specimens taken from trochanteric subcutaneous of seven women with cellulite, aged between 37 and 46 years (308). The Authors noted that, while, in normal subjects, are found large adipocyte lobules, divided by connective fibrous strands, in patients with cellulite the lobules are fragmented into smaller cell clusters, by coarse newly formed septa, containing collagen and elastic fibres. This fibrosis is accompanied by signs of micro-vascular disorder (with vacuolar degeneration of endothelial cells and capillary thrombosis) and of neoadipogenesis, upheld by the presence, on the edge of nodules, of adipocytes with small lipid droplets. Regressive phenomena, as focal collagen sclerosis and atrophy of skin annexes, are also detected in the dermis. From a clinical viewpoint, the skin surface plan appears uneven, since introflections and globular prominences alternate giving rise to a mattress-like aspect. The epidermis looks hypotrophic and skin palpation reveals an intense tissue tenderness.

The alterations that the subcutaneous undergoes in EFSP advanced stages can be followed, in their evolution, by means of high-resolution ultrasonography, using probes with emission frequency around 20 MHz. A significant contribution to the ultrasound study of cellulite has been provided by Giannini, whose observations appear in agreement with the hystogenetic hypothesis of EFSP (309, 310). It should be noted, however, that other authors draw different conclusions from ultrasound examinations (311, 312, 313).

In the footsteps of Curri, over 20 years ago, Bartoletti et al., as part of the scientific activity of the Italian Society of Aesthetic Medicine and of the International School of Aesthetic Medicine, have made an important contribution to the definition of cellulite dystrophy.

A first contribution was clinical in nature: Bartoletti has completely revised the semeiological classification (314, 315), has codified the diagnostic pathway (316) (identifying the key moments in general examination, phlebological study, postural assessment and ultrasound examination (317, 318) and has developed treatment protocols that are currently applied by many doctors (319, 320). Another important contribution of Bartoletti and his school was nosological in nature. Curri, accrediting to an adipose tissue perfusion debit the skill to increase the lipid deposits (321), joined the EFSP pathogenesis to a district adiposity. Bartoletti has made a clearer distinction between the cellulite and the localized female gluteo-femoral adiposity which he has re-
located it in a context of morphological and functional normality, while not denying the possibility of intermediate forms (322). In this approach has received support from the identification of the estrogen receptors on adipocyte membrane; this discovery as well as the finding of a from site to site different rate between the activities of the β2 and α2 adrenergic receptor, allow, as has been said, to interpret the storage of fat in the gluteal-femoral region as a physiological consequence of the modulation of lipase (323) and lipoprotein lipase (324) actions that sexual hormones and catecholamines exert.

In years closer to us other AA provided data that substantiate the microangiopathic origin of cellulite. Rossi and Vergnanini reviewing the literature that accredits this theory, cited laser-doppler flowmetry data showing in the adipose tissue of the affected regions, a 35% reduction in flow, compared to undamaged areas (325). Inspired by completely different assumptions, Emanuele et al. have come to envisage an etiological role played by factors which affect the microcirculatory function. With the intention of checking whether a specific genetic predisposition to cellulite exists, the authors analysed the DNA of 200 female subjects suffering by the blemish, without being overweight (BMI > 25 kg/m²), and of 200 controls. The investigation was intended to evaluate the distribution of 25 polymorphisms, regarding 15 different genes, operating in different functional areas, as they regulate, respectively, lipid metabolism, inflammatory processes, extracellular matrix synthesis, estrogen peripheral action, endothelial activity. The only detected statistically significant correlations with the clinical picture were relative to two genes, both implicated in the pathophysiology of either the adipocytes and the endothelium: the ACE (angiotensin I converting enzyme) and HIF-1α (hypoxia-inducible factor 1 alpha) genes (326, 327, 328). In both loci, allelic variants associated with cellulite were likely to cause a tendency to reduce blood flow in the vessel. The same authors have documented a decreased expression of adiponectin in adipocytes taken from adipose tissue affected by manifestations of cellulite (329). Another study has confirmed the probable pathogenic role of ACE gene polymorphisms, whose association with cellulite was still tighter in women, who, at the same time, were devoted to tobacco use (330).

### 3. Intradermal adipocyte hernias

Numerous authors, from Cambar (331), Braun-Falco (332), Ribuffo (333) and Calvieri (334), have denied that, in areas where clinical signs of cellulite are seen, light and electron microscopy can demonstrate histological changes other than those commonly observed in macroscopically "normal" sites of fat accumulation. These observations are the basis of a very widespread (especially in English-speaking countries) interpretation, which, in sharp contrast with the microangiopathic hypothesis, leads cellulite to a simple expression of lipid accumulation (335). If cellulite is nothing more than the expression of a localized overabundance of adipose tissue, what is, then, the cause that limits, almost exclusively, the appearance of this imperfection to the female subcutaneous and to particular districts and how it is possible explain so different dystrophy manifestations in individuals with overlapping BMI? In 1978, two German doctors, Nürnberg and Müller, reporting the results of histological examinations carried out on a large survey (150 samples from cadavers and 30 from living), denied that they had found phenomena of oedema or fibrosis in the areas affected by cellulite (336). They attributed, however, the appearance of skin irregularities, in the typical feminine areas, to the combination of two causal factors:

- an excessive local lipid deposit ("there is no cellulite without adiposity");
- the peculiar architecture that, in women, characterizes the subcutaneous of these locations. The male hypodermis is crossed by collagen fibrous bundle with oblique course, which intersect, delimiting relatively small adipocyte lobules, with polygonal shape. Conversely, in females the fibrous septa are oriented perpendicularly to the skin surface. These strands, running from the muscle fascia to the dermis, separate bulky lobules, with rectangular section; their apices press against the reticular dermis and there protrude, in the form of "fat buds", little adipose hernias named "papillae adipose" (Figure 7).

Women who develop a thickening of the subcutaneous adipose tissue have, in the typical areas of gynoid adiposity, lobules much increased in high, which at the edges are held by fibrous strands, but, centrally, tend to protrude into the dermis, with hypertrophic fat humps. In the "cellulite areas" the dermal-hypodermal border assumes, therefore, an undulate "hills-like" profile which, in conditions of perfect normality, can make evident with the "pinch-test": the manoeuvre, putting in traction the inextensible collagen septa, highlights the surface undulations even in relatively lean subjects causing a dimpled skin appearance (mattress phenomenon). The non-aesthetic alteration of relief and depressions becomes, gradually, spontaneously manifest: at first appears evident only during standing, later also in decubitus.

Conversely, in the male subcutaneous, adipocyte lobules, separated by intersecting connective tissue septa (oriented, as has been said, according to an angle of about 45° with respect to the perpendicular to the skin) do not tend to protrude toward the dermis, neither in
case of lipid over accumulation. Consequently, in men, even in obese ones, the pinch-test does not determine surface "mattress-like" irregularities.

It is interesting to note that, in hypo gonadal males, the adipose tissue takes on a female morphology, demonstrating that, with regard to the collagen scaffold, the causal factor of gender-related differences is represented by the androgenic hormones activity.

The importance that today the English-speaking authors attribute to the anatomical female hypodermis predisposition is such that many of them, surpassing the original conception of Nürnberger and Müller, devalue the pathogenic role of obesity and consider the cellulite like a normal phenotype of the adipose tissue of women in post-adolescence (337): a kind of secondary sexual character, from which no member of the fairer sex is completely free, even the top-models, the Olympic athletes and the anorexic girls (338). Mutatis mutandis, the concept of a cellulite "normality" seems in tune with the perception that, about this aspect, had our ancestors, who, even exalted it as a true beauty standard, immortalizing it in famous paintings, in which they celebrated the triumph of feminine attractiveness.

Some of the best reviews designed to recapitulate the acquisitions on the clinical and histopathological aspects of cellulite, agree in assigning to the intradermal adipocyte humps the role of pathognomonic lesion (339, 340, 341, 341).

Rosenbaum et al. have found in the subcutaneous tissue, in addition to gender-related differences, even an inter-individual variability. Women with cellulite, according to the AA., have, at the dermal-hypodermal border, a more irregular and discontinuous dividing connective plan, that predispose to fat buds protrusion (343), which come to configure real hernias: the tips of the adipocyte lobules, in fact, come to pierce into the thickness of the reticular dermis.

In a paper published in 2008, a group of German researchers has developed a calculation algorithm in order to derive, from ultrasound scans of the subcutaneous tissue, a numerical coefficient able to define the degree of irregularity of the line which delimits the hypodermis from the dermis. This parameter showed a good correlation with a clinical "cellulite score", that the authors have developed, analysing, with special software, high resolution images of the skin (344).

Querleux and coll. have submitted more than sixty subject to MRI (magnetic resonance imaging), employing both imaging techniques, both spectroscopy (345, 346). The former documented the corrugated profile that the adipose hernias give to the dermal-hypodermal border and confirmed the diverse orientation of the connective strands in different gender individuals (although the distinction was perceived as less clear than proposed by Nürnberger and Müller). Females showed, in fact, an almost vertical course in a significantly higher percentage of the interlobular septa. This trend was accentuated in women with cellulite, which also manifested a greater thickness of subcutaneous tissue (especially of the deep fatty plane, below the fascia of Camper).

The most surprising observation stemmed from the three-dimensional reconstruction of the hypodermal fibrous scaffold, whose overall thickness, according to the Authors, was not increased in subjects with cellulite, but greatly reduced compared with controls, with weakening of both the septa, both the fibrous plan at the dermal-hypodermal boundary. At the same time, the dermis appeared thinned and "indented" by a greater number of fat protrusions.

An equally important relief, from the pathogenic point of view, has been derived from MR spectrometric data, which, according to the extensors of the article, excluded that in the cellulite is realized any form of oedema, at least within the adipose lobules (the MR resolution power was not sufficient to permit an assessment of the water content in the context of the interlobular fibrous septa) (347).

The MRI technique has been applied to the study of
the morphology of subcutaneous adipose tissue also in another paper (348) which, although penalized by the small size of the sample, has allowed interesting observations. First, the authors return to attest that the two genders have a different conformation of lower limb subcutaneous. Women showed thinner septa, oriented perpendicular to the skin surface, with adipose lobules of greater size, and also reveal a thinner dermis. The anthropometric aspect that seemed more closely related to the onset of female cellulite was the thickness of the subcutaneous tissue that, in the group of patients with BMI below 30, appeared much higher in women affected by the blemish, than in non-affected women and in males. Among individuals with BMI > 30, fat thickness was similar in both genders, but in females, the larger values correlated with a higher cellulite incidence. Other features related to the presence of such lipodystrophy were the thinning of the dermis and the rise in the adipose lobules volume. In the MRI images obtained by the females with BMI > 30 affected by cellulite, the indentations of the dermal-hypodermal border were particularly evident; the AA attributed to these the genesis of skin surface irregularities. The most important observation, however, was related to the further confirmation, that these authors provide, about a lesser thickness of the subcutaneous fibrous scaffold, with more scarce and subtle interlobular septa, in women carrying gynoid dystrophy, compared to the exempt ones. These data on the one hand seem to show that cellulite does not involve a sclerotic-fibrous involution of female subcutaneous, on the other hand lead us to believe that other factors, besides the mere overweight, intervene in the cellulite pathogenesis; the authors identified a possible causal element in a constitutional subtlety and laxity of the collagen inter-lobular beams (348). A confirmation of this hypothesis can be drawn from a recent study, in which the viscous-elastic properties has been valued in the undamaged and in the skin affected by cellulite (349); the latter was found more subject to undergo deformations after mechanical stress, revealing a lower tissue compactness. This increase in skin laxity seems, in some ways, to assimilate cellulite to skin aging, which is also characterized by a degradation of the fibrous dermal-hypodermal matrix (350, 351).

4. Unequal reactions to stretching of the inter-lobular septa

The Belgian group of Pierard, basing on the examination of 39 autopsy samples, while confirming the presence of fat hernias in women, noted that these formations are evident across the feminine subcutaneous, also outside the areas commonly affected by cellulite, and are visible even in individuals exempt from this condition. In addition, the fat protrusions into the dermis are small in size, incapable, as such, to produce, on the skin surface, the coarse alternation of convexity and depressions typical of cellulite (352).

Such a disorder, according to the Authors, would indeed due to the progress of adiposity, that determines the increase in height of the adipocyte lobules, roughly cylindrical in shape, arranged with the largest axis perpendicular to the skin plane. The adiposity puts in tension the septa that laterally delimit the lobules: some of these, due to the well-known ability of fibroblasts and myofibroblasts to activate as a result of mechanical stresses, undergo to phenomena of reactive thickening, fibrous-sclerosis and retraction. Other collagen bundles suffer, however, from partial or total tearing, in ways that closely resemble the genesis of stretch marks (353). Consequently, on the surface of the skin, soft bulging areas, due to adipose tissue protrusions, secondary to the rupture of the interlobular septa, alternate with sunken areas, firmer on palpation, in which the dense hypertrophic scar-like fibrous beams, bring near the dermis to the muscle fascia (354) (Figure 10).

High-resolution MRI investigations, made by Brazilian authors (355), have documented, in correspondence of the depressed areas found on the buttocks skin in...
patients with cellulitis, the presence of thickened and retracted collagen bundles, with linear course, perpendicular to the skin surface, which extend from muscle fascia to the reticular dermis, causing the dimples of the latter; furthermore, MR imaging did not show the ring-like arrangement around the nodules of the fibrous strands that, according Binazzi and Curri, would be characteristic of the EFSP.

The same Brazilian authors, in a subsequent work (356), have submitted to MRJ investigation the bulging areas which alternate with the depressed ones in the skin affected by cellulite; the images did not show different conformations of the connective tissue compared to the corresponding undamaged areas of normal subjects. The fibrous septa seemed not different by number, thickness, orientation and ramifications and also the lobular structure of the tissue appeared preserved and similar to the tissue structure of exempt subjects of the same age.

The stretching of the fibrous bundles caused by adipose lobule hypertrophy may extend to the papillary dermis collagen, in which in vivo confocal microscopy observations have revealed ripping images, similar to those found in the stretch-marks (338).

Once that it has occurred, the alteration which the stretched interlobular branches undergoes is, in large measure, irreversible, as shown by Smalls et al., who conducted a study in order to verify the effects of a weight loss on the manifestations of cellulite (357). The authors noted that the clinical picture underwent an improvement only in subjects who, starting from a very high BMI, obtained a considerable slimming. In all other situations, cellulite was not resolved, but rather seemed to worsen.

The observation appears to confirm the hypothesis of a pathogenesis mediated by the rupture, triggered by stretching, of the interlobular septa; these, once have been "ripped", cannot longer be repaired, even in cases where a weight loss reduces the tension to which had been subjected. Therefore, weight loss is a good starting point to combat, or, better, to prevent, the cellulite in obese women, but it is not enough to improve the uneven skin surface: neither the association between diet and exercise exhibits scientific evidence of effectiveness.

5. Proteolysis of the inter-lobular septa

A further development of the theories of Pierard done thanks the work of Mareus, which suggested that the protruding of adipose humps towards the dermis could be facilitated by the degradation of the dermis collagen component, subsequent to the action of some proteases (358).

Recently, this concept has been taken up and expanded by Pugliese: this author has proposed a new pathogenic hypothesis (359), framing the cellulite within a group of disorders of the female connective tissue, including the stretch marks, the laxity of the pelvic floor, the varicose veins dilation, the temporomandibular joint sickness, the knee anterior cruciate ligament weakness, the pregnant tooth missing.

All these events, in the view of the Author, are due to a functionality excess of a class of proteolytic enzymes, the Matrix Metalloproteinases (MMPs), among which many collagenase and elastase are included, normally engaged with the turnover of the peptide components of the intercellular substance of connective tissues. It was shown that the adipocytes are able to secrete these enzymes which come into play in the phases of tissue scaffold remodelling that accompany every increase or reduction of the fat mass (360).

In particular, it is well-known that both the neo-adipogenesis induced by an excessive caloric intake and the remodelling process characteristic of abdominal adipose tissue in obese subjects involve an increased expression of the metalloproteinases (361) and a reduced activity of their tissue inhibitors (362, 363).

In the opinion of Pugliese, the synthesis and activation of MMPs would be increased by an estrogen-induced stimulation, similar to what occurs in the uterus, where the cyclic over-expression of these protease induced by female hormones, plays an important role in determining the monthly menstrual endometrium cleavage (364). At the level of the gluteal-femoral subcutaneous, the hyperactive proteases may cut the collagen fibres of the fibrous scaffold. Thus, in the view of Pugliese, the clinical aspects of cellulite are produced by the rupture of the interlobular fibrous septa (in agreement with Pierard); the phenomenon would be yet ascribed not only to the excessive mechanical tension, but also, and especially, to a partial digestion, actuated by endogenous collagenase, functionally over-stimulated by the estrogens (337, 365).

The enzymatic laceration of the collagen fibres could be also favoured by the their destabilization, caused by an altered distribution of the interstitial fluids in the matrix: Challahan et al., have recently used the in vivo confocal microscopy to examine the dermis of the female thighs and buttocks (the subcutaneous cannot currently be explored by this technique), identifying "dark spaces filled with fluid" not attributable to vessel branches (338).

The authors hypothesized that these areas correspond to the accumulation of GAGs (confirming the observations of Lotti et al. (263) about an increase in the presence of these substances in the dermis of the areas affected by cellulite). The subsequent pathogenic evolution, would, to some extent, match the suggestion by Maibach et al. (366), according to which, in particular conditions, abnormal localized deposits of GAGs in the
context of the hydrophilic matrix, trapping the water, "sequester" it, and prevent its binding to collagen. Thus, the GAGs excess gets the paradoxical effect of producing, in the dermis and hypodermis, a dehydration of collagen fibres bundles, making them more rigid, fragile and susceptible to enzymatic attack and to tearing.

The theory that attributes the cellulite main causes to a disorder of the intercellular matrix has suggested therapeutic approaches that exploit the capabilities of restoring the fibrous network homeostasis attributed to vitamin A; however, the topical application of emulsions containing retinol got an albeit modest benefit (367).

6. The fibrous-sclerosis under investigation

From what has been just referred, it appears clear that there is a large mass of observations that, excluding the presence of a massive thickening of the collagen bundles in the tissue affected by gynoid lipodistrophy, seem to completely contradict the description of the histopathology of cellulite advanced stages as it results according to the theory of EFSP.

A further consideration which contributes to cast doubts on the development of an extensive process of fibrous-sclerosis in the gluteal-femoral panniculus of a large part of the female population comes from the fact that such a generalized alteration has not been identified and described in any of the numerous works that, in recent years, were devoted to the pathophysiology and morphology of subcutaneous fat in its various localizations (298, 368, 369, 370); clearly, these research had not, among their objectives, any investigation into the pathogenesis and the characteristics of the cellulite-induced tissue damage, but given the prevalence of this blemish, if it really would produce, in the hypodermis of a lot of women, an extended sclerotic-nodular alteration, this aspect would have been definitely documented.

It is no doubt, however, that both in the cellulite, both in the alterations displayed by the splanchnic fat in obese people, comes in the play the relationship between the adipocytes and the extracellular matrix that surrounds them, a relationship whose contours now appear delineated in forms much more intricate than had previously been suspected.

As it is known, the adipose organ is an highly dynamic structure: the changes in the energy balance following every imbalance between calorie input and output translate into even considerable variations in fat mass, involving the size and also the number of adipocytes. It is necessary that to these variations correspond suitable modifications of the extracellular matrix, which must continually adapt to the new requirements both the supporting fibrous scaffold both the microvascular network which branches between the fat cells.

The adipose tissue extracellular matrix takes, thus, an unsuspected functional importance that recently made it the object of extensive researches (371): its composition has been studied (24, 362, 372) and researches have been conducted about the role that the adipocytes and/or their precursor cells play in the matrix synthesis (373), as well as in the tissue remodelling that accompanies every change in fat cell number and size (362, 374). Other researches have put in evidence the great influence that the vascular and stromal support structure and its individual cellular and extracellular components in turn exert on the metabolic activities of the adipocytes (375-377). It is legitimate to hope that these researches will soon bring new light on cellulite pathogenesis and histopathology.
Conclusions. New acquisitions, new questions and new perspectives

The debate on cellulite, as we see, is yet open and divergent ideas, efficiently summarized in some recent, valuable reviews (341, 342, 378, 379), still continue to face. The existence of opinion differences on scientific matters, it is not, in itself, an unusual circumstance. In this case, however, the fact that the dispute concerns also (and especially) the distinguishing histopathological characters of the tissue alteration, yet described in completely discordant ways, arouses a certain perplexity: it seems surprising that there are still so many margins of disagreement in the definition of morphologic aspects concerning a so easily accessible tissue on biopsy.

Sadly, it must be held that the scientific content of the discussion is rather feeble, contaminated by a multitude of publications eminently direct to commercial purposes, and penalized by the small number of actual researches, by the limited case studies and, in general, by the modesty of employed resources.

It is possible to bring all the different interpretations to a single shared vision in its etiologic and pathological aspects? Or, perhaps, Bacci is in the right when, in one of its publications about this subject (380, 381), in the title itself (The Cellulites) overshadows the suspicion that the current confusion arises from the fact that, under one nosological “hat”, different diseases have been incorrectly included, which share only some clinical similarities?

The impasse that still drags on for the failure to solve fundamental questions (such as those concerning the pathological basis of the lesion), may, perhaps, be, sooner or later, overcome with the help of the progresses that other branches of medicine, which have nothing to do with the study of cellulite, are making in understanding the pathophysiology of adipocytes, and endothelial cells, the two cell types more involved in the development of this disorder.

In particular, today’s acquisitions bring out a limit that unites the main theories about the origin of cellulite. In the conception of Curri, as well as in that of Nürnbergber and Müller, and in the more recent other ones, the adipocytes appear to be the specific targets of an alteration in whose onset they play no significant role: they seems, in fact, not intervene at all, remaining passive “victims” of a micro-circulatory dysfunction or of a primitive defect in the connective matrix, or participate only with purely physical mode, generating a mechanical tension, through their hypertrophy.

These formulations are affected by an idea, now outdated, which sees the adipocyte as a cellular element with scant capacity, only involved in tasks of storage of surplus calorie substrates; to a so trivial cell type it is not logical to ascribe great pathogenic "responsibility", so that the causal factors of cellulite have been sought elsewhere.

The importance that the excess fat, especially visceral, seems to take in the pathogenesis of insulin resistance and diabetes (382, 383), as well as of atherosclerosis (384) and cardiovascular diseases (385), explains the huge amount of studies that, in recent years, have been dedicated to the adipocyte biology.

Today we know that the body fat tissue carries out sophisticated and composite tasks (386), acting as:

• a control device of systemic energy balance, capable to modulate food intake and substrate metabolism in other tissues;
• a glandular system, assigned to multiple hormone and para-hormone secretion (387, 388, 389, 390), able not only to make a bio-conversion of circulating steroid hormones, but also to de novo synthesize a lot of protein regulatory factors (adipokines).

Moreover, in adipose tissue, as it has been said, undifferentiated mesenchymal elements are found, capable, in case of need, to transform non only into new adipocytes, but also in other cell types.

The extraordinary performances today recognized in adipose tissue cannot be devoid of significances regarding the cellulite pathophysiology. For example, the hypothesis that the subcutaneous passively suffer the consequences of a dysfunctional microcirculatory system, has, in all likelihood, to be revised in the light of the findings that delineate, between vessels and adipocytes, a network of much more dynamic relationships, placing in both directions (391, 393, 393): we have learned, in fact, that, according to the needs, the adipose tissue is capable of modulate, directly or indirectly, the blood flow passing through it (394, 395). Furthermore, the adipocytes secrete neo-angiogenic factors (396, 397, 398) and many other substances that regulate the activities of the endothelial cells and of the smooth muscle fibres of the arteriolar wall.

The importance that the adipocytes, almost certainly, assume for the onset of cellulite, is confirmed by recent researches demonstrating that women suffering from this disorder, display, in the fat cells of the affected areas, less mRNA expression of adiponectin, an adipokine that performs multiple tasks in the local and systemic metabolic regulation; an adiponectin deficiency appears also involved in the pathogenesis of the adipocyte dys-
function typical of obesity (329).

It has been said, in the above paragraphs, that, in obese subjects, visceral adipocytes, becoming, gradually, more and more bulky, undergo functional and morphological disorders. Indeed, a series of observations tend to show that hypertrophic visceral adipocytes suffer from a condition of relative hypoxia: the lack of oxygen is the cause of the secretion of cytokines, able to produce a chronic systemic inflammatory state and to reduce insulin sensitivity in several tissues (399, 400, 401). At the level of visceral fat, the inflammatory condition hesitates in a large macrophage infiltration (402, 403, 404) and in a thickening of the fibrous network surrounding the adipocytes (405). It is possible to hypothesize that inflammatory processes, similar, to some extent, to those seen in the central fat, could be realized even in the adipose depots affected by cellulite where, in a sliding manner, they could play a role in the pathogenesis of the histologic damages, being responsible, for example, of the endothelial alteration (406), of the increased deposition of collagen fibres and of the enzymatic proteolysis of the interlobular septa. Cellulite, so, could, perhaps, be worthy of its name, regaining, rightly, the connotations of an, at least in part, inflammatory disease.

An attempt to validate a hypothesis of this kind meets a first difficulty in the existence of substantial morphological and functional differences between the subcutaneous tissue, elective site of cellulite, and the visceral adipose tissue in which the processes of inflammatory remodelling have been documented. It is hard, therefore, to understand to what extent the observations made on the abdominal fat can be used to interpret the alterations affecting the peripheral subcutaneous; much of the difficulty stems from the fact that the subcutaneous tissue, being less important than visceral fat regarding the pathogenesis of the metabolic syndrome, has been object of a far less attention by the scientific research, for which the available elements of knowledge are much more scant.

Nevertheless, in recent years, some observations have documented that, in particular circumstances, even the subcutaneous tissue may be affected by histopathological alterations similar to those that involve the central adipose tissue, characterized by an increase in the average size of adipocytes, by a diffuse infiltration of macrophages and by a thickening of the texture of interstitial collagen fibres (407, 408). This unfortunately is not enough; to make even more and more problematic the interpretation of the data, there are, in fact, the researches that demonstrate the existence of further significant differences between the anatomical and functional characteristics of the subcutaneous adipose tissue in its various districts (409).

We have seen, in the preceding paragraphs, that the abdominal subcutaneous possesses intermediate characteristics between the gluteal-femoral and the visceral fat, so much so that, together with the latter, undergoes macrophage infiltration (410) and fibrosis (411, 412) induced by obesity; indeed, it may present a fibrotic aspect even in non-obese subjects carriers of a family history of diabetes (413). In the scientific literature, the observations concerning the inflammatory remodelling of subcutaneous fat are almost exclusively related to the subcutaneous of the abdomen wall; this is by far the most studied, also owing to eminently practical reasons, consisting in the easy possibility to achieve histological samples from this site, taking off them on the occasion of laparotomy surgery.

Lower limbs fat has been investigated much less depth. Generally, however, it is believed that the gluteal-femoral subcutaneous is exempt from inflammatory alterations; it presents, in fact, a greater neoadipogenesis capacity, so that, in the face of an increased calorie intake, it puts in place hyperplastic phenomena, instead of cellular hypertrophy (414). In this way, the average size of lower limbs adipocytes does not reach the high values seen in the splanchnic district (157), which are the first cause of the adipocyte hypoxic stress which, in turn, causes the inflammatory alterations and the tissue fibrosis.

Recent data, however, lead to think that the physiological and pathophysiological disparities between the gluteal-femoral and the upper body subcutaneous fat are less clear than previously believed.

A work of Evans et al. (2011) surprisingly revealed that the expression of different inflammatory markers is higher in gluteal-femoral subcutaneous than the abdominal wall fat, both in lean both in obese women (415). This is in line with the data reported by Tchoukalova (2010) (161), who found that in normal weight subjects of both genders, the abdominal fat and the gluteal-femoral deposits contain an equal number of macrophages. Huang et al found that, in women with PCOS, the subcutaneous of the gluteal region has a strong inflammatory remodelling of macrophages that express the C11 marker, index of a M1 (i.e. inflammatory) polarization; very often these macrophages are found in a circle disposition, around adipocytes in apoptosis, thus replicating the crown-like structures typical of the hypertrophic fat tissue of obese people (416).

You et al. have found that, contrary to expectations, in basic conditions, the abdominal fat shows a higher gene expression of adiponectin and a lower IL-6 release compared to the gluteal adipose tissue (188). Similar ob-
servations have been made, in obese women, by Mal-
ěšová, who did not detect significant differences between
the abdominal and the gluteal-femoral subcutaneous, as
regards the expression of inflammatory genes, in basal
conditions as well as after diet therapy treatment (417).
In transgenic mice in which it had been deleted the
PRDM16 gene (implicated in the differentiation of the
brown-like adipose cells known as brite or beige adipo-
cyes), the subcutaneous fat assumes characteristics simi-
lar to those seen in visceral fat of obese individuals, with
increased secretion of pro-inflammatory cytokines and
accumulation of interstitial macrophages (418).
This data set helps us to deem not entirely far-fetched
a pathogenic hypothesis based on a chronic-inflamma-

tory interpretation of cellulite, according to which it
may be assumed that this alteration can occur as a result
of a process of drifting in a like-visceral direction of the
morphological and functional properties of female glu-
teal-femoral adipose tissue.
Thus, one can imagine that, even in individuals with
normal BMI, critical episodes, characterized by periods,
albeit brief, of a calorie intake increase too fast and in-
tense to allow to carry out of a sufficient hyperplastic
response, could compel the gluteal-femoral adipocyte to
annex the lipid material through a growth of cell size,
capable of triggering hypoxic conditions; in the deter-
mining an oxygen deficiency could help, as suggested by
the work of Emanuele et al., some genetic predisposi-
tions that compromise an appropriate adaptation of the
microcirculatory function (326).
Hypoxia would realize, in subcutaneous tissue, a
complex process of tissue remodelling, characterized (as
it happens in visceral fat), by the infiltration of mac-
rophages and by a slight new collagen apposition around
adipocyte clusters. This subtle fibrosis would be config-
ured as a very different alteration from the massive fi-
brous-sclerosis described as a constituent element of the

histopathology of EFSP according to the conception of Curri; from this it also differs as regards the pathogenesis,
which does not appear simply reduced to an organisation
process of a proteinaceous oedema, gushed from a
primitive micro-angiopathy, but would involve the acti-
vation of a much more complex network of inter-cellu-
lar signalling, in which the fat cells and their dysfunction
would play a central role.
According to this interpretation, women affected by
cellulite would present, in lower body fat, inflammatory
phenomena similar to those typical of visceral adipose
tissue in obese subjects. It is likely that this condition
could involve the loss, at least in part, of the protective
effectiveness against cardiovascular disease commonly
expressed by lower limbs fat. If this is true, the mani-
festations of cellulite, which become evident since the
post adolescent age, would combine, in later life and in
overweight women, with an increased incidence of obe-
sity-related diseases.
There are still not studies that can testify to the ac-
curacy of such a theory, for example, demonstrating,
with histochemical methods, in women affected by the
blemishing, an increased loco-regional expression of in-
flammatory factors correlated with the severity of the
clinical and histopathological presentation; however ele-
ments do not exist, that can deny it, with security. In
this lack of data, the preliminary results seem suggestive
of a work not yet published (419), from which it is here
possible to extrapolate an image (Figure 12). This is the
photomicrograph of an histological sample obtained
from a biopsy specimen taken from the trochanteric re-

Figure 12.
Cellulite: macrophages surrounding an adipocyte in apoptosis
(courtesy of Dr. Domenico Amuso).
References


145. Allan CA, Strauss BJ, Burger HG, et Al. Testosterone therapy prevents

THE NODULE OF DISCORD • 39

www.ejamed.com


186. Fuente-Martín E, Argente-Arizón P, Ros P, et Al. Sex differences in adipose tissue: It is not only a question of quantity and distribution.


355. Huang G, Greenspan DS. ECM roles in the function of meta-
356. Terranova F, Berardesca E, Maibach H. Cellulite: nature and aetio-
357. Small LK, Hicks M, Passeretti D, et al. Effect of weight loss on cel-
358. Dealessi WD, Marcus MD. Cellulite. Etiology and purported treat-
trix metalloproteinases 2 and 9: involvement in adipose differentiation.
edulin resistance is correlated to adipose tissue vascular endothelial
matrix remodeling in adipose tissue pathophysiology: relevance in the
363. Charrey C, Mari B, Monthoul MD, et al. Matrix metalloprotei-
nes are differentially expressed in adipose tissue during obesity and modu-
late adipocyte differentiation. J Biol Chem. 2003. 278(14): 11888-
1196.
364. Perry TE Jr, Oster K. The matrix metalloproteinase system: changes,
regulation, and impact throughout the ovarian and uterine re-
221-2.
366. Wailer JM, Mailbath HI. Age and skin structure and function, a quan-
titative approach (II): protein, glycosaminoglycan, water, and lipid con-
367. Parvisi T, Borelli C, Korting HC. Cellulite – the greatest skin problem
861-70.
368. Avram MM, Avram AS, James WD. Subcutaneous fat in normal and
663-70.
369. Avram AS, Avram MM, James WD. Subcutaneous fat in normal and
diseased states: 2. Anatomy and physiology of white and brown adipose
371. Yee B. Biology of the extracellular matrix: an overview. J Glauc-
372. Divoux A, Clement K. Architecture and the extracellular matrix: the
373. Nakajima I, Yamaguchi T, Ozutsumi K, et al. Adipose tissue extra-
cellular matrix: newly organized by adipocytes during differentiation.
374. Lee BC, Lee J. Cellular and molecular players in adipose tissue in-
flammation in the development of obesity-induced insulin resistance.
375. Huang G, Greenspan DS. ECM roles in the function of meta-
376. Craft CS, Petka TA, Schappe T, et al. The extracellular matrix protein MAGP1 supports thermogenesis and protects against obesity
1920-32.
377. Williams AS, Kang L, Wasserman DH. The extracellular matrix and
378. Terranova F, Brandoessa E, Mailbath H. Cellulite: nature and aeo-
379. Dealessi WD, Cellulite pathophysiology in Goodman MP, Bacci PA,
Hansel D, Angelina F Editors - Cellulite Pathophysiology and Treat-
mant. 2006. Taylor & Francis NY.


OXIDATIVE STRESS EVALUATION AND HISTOLOGICAL ANALYSIS in the assessment of cellulite: lights and shadows towards a multidisciplinary approach

Domenico Amuso MD (1), Eugenio Luigi Iorio MD, PhD (2), Luca Bonetti MD (3), Roberto Amore MD (1), Ferdinando Terranova MD (4), Vincenzo Leonardi MD (1)

(1) University of Palermo, Palermo (Italy). (2) International Observatory of Oxidative Stress, Salerno (Italy). (3) Policlinico di Modena, Modena (Italy). (4) International School of Aesthetic Medicine, Rome (Italy)

ABSTRACT

Introduction
Cellulite is a localised disorder affecting mainly the subcutaneous tissue of gluteal-femoral trochanteric areas and classically manifesting with a padded or orange peel-like appearance, finally responsible of a body shape alteration. Although its pathogenesis remains unclear a possible role of an unbalance between free radicals and antioxidants (i.e. oxidative stress) cannot be excluded. Unfortunately no agreement can be found between pathologists about its histological features and this may affect the possibility to treat such disorder that should be approached by means of a multidisciplinary strategy.

Aim
The aim of this study was to evaluate risk factors, clinical features, instrumental and laboratory findings (especially oxidative stress) in a group of patients suffering from cellulite and to evaluate the possible relationships between such findings and the histologic picture of cutaneous/subcutaneous gluteal-femoral trochanteric affected areas, as obtained through serial biopsies.

Materials and Methods
This was an observational open clinical trial on 60 voluntary apparently healthy women suffering from cellulite. All recruited women underwent classical clinical examination followed by instrumental evaluation (i.e. thermography, optical videocapillaroscopy, subcutaneous tissues ultrasound, colour flow Doppler, and skin performances), laboratory analysis (routine tests plus oxidative stress evaluation on blood samples by means of d-ROMs test and coenzyme Q10 dosing), accompanied by serial biopsies of the affected areas.

Results
Clinical examination and instrumental assessment confirmed the diagnosis of cellulite in its different stages. Serial biopsies showed that cellulite impairs not only the subcutaneous tissue but also the epidermis and the derma thus accounting for 5 different histological patterns that can be present in the same subjects at the same time. The only laboratory abnormal parameter out of the range was the oxidative stress, as measured by d-ROMs test (477.38±69.26 A.U.; normal range 250-300 A.U.). Interestingly according the linear regression analysis d-ROMs test values showed a positive relationship with age and BMI and a negative relationship with CoQ10 plasma levels, skin hydration and elasticity, all statistically significant (p>0.05).

Discussion
This study demonstrated that cellulite is a disease affecting not only the subcutaneous fat but also the skin with different degree of damage that were related to the clinical classification of the diseases according to Curry. In agreement with previous trials oxidative stress was increased especially in older peoples with high BMI and associated to skin abnormalities thus suggesting that an unbalance between free radicals and antioxidants can be not only a pathogenic factor but also a bridge between the local and systemic alterations found in the cellulite.

Conclusions
Cellulite is a local disease with a systemic impact that can be mediated by oxidative stress. Such finding should open new specific ways for its treatment (e.g. antioxidant cosmeceuticals/nutraceuticals). Further studies must define in the future the relationships between clinical and histological features of this disease.

Keywords
Cellulite, adipose tissue, subcutaneous tissue, oxidative stress, histology.
Introduction
Cellulite is a localised disorder affecting mainly but not exclusively the subcutaneous tissue of gluteal-femoral trochanteric areas, lower limbs and abdomen, and classically manifesting with a padded or orange peel-like appearance, finally responsible of a body shape alteration (1).

The aetiology as well as pathogenesis of cellulite remain still unclear although its first almost universally occurrence in post-pubertal women may suggest an involvement of gender-related factors (2, 3). However an impairment of oxidative balance cannot be excluded (4). Unfortunately, at present, there is no agreement between the pathologists about the histologic features of such disorder and different patterns have been described including, oedema, fibrosis, nodular liposclerosis, etc. (5).

On the other hand, considering that not all authors agree in defining cellulite as a “disease”, the possibility to control its course with the medical and/or surgical treatments is often reduced, with all the inevitable consequences for the quality of life of patients (6).

On this background a multidisciplinary diagnostic approach may be useful in order to better understand the pathophysiology of cellulite and therefore to open new horizons in its treatment (4).

Materials and Methods
In the period between 2010, February, and 2011, April, we recruited sequentially 60 voluntary apparently healthy women, in their fertile or menopause period of life, and suffering from cellulite – as diagnosed and staged according to Curri, i.e. I edema, II reticular fibrosis, III micronoduli, IV macronoduli (7) – which onset was dated at least one year before as main inclusion criteria. Women showing one or more the below conditions were excluded by the trial: age <18 years, pregnancy, lactation, severe chronic/degenerative disorders, current topical or systemic pharmacological treatments or supplements, and previous inclusion before one month in other similar trials. All the women who fit the inclusion criteria were enrolled and gave their informed consensus to the investigations.

The classical clinical visit allowed to evaluate, in particular, the pain feeling and the classical skin features of cellulite. The first one was assessed by using a quantitative visual analogical scale giving a score ranging from 1 (none) to 5 (severe) while the second one was measured according to a specific arbitrary scale where 0 indicated no abnormalities, 1, a limited presence of craters or uneven protrusions of skin surface (madras skin or mattres phenomenon) and 2, a generalized skin roughness (orange peel skin).

The physical examination was integrated by i) the antropometric evaluation (including Body Mass Index or BMI assessment, plicometry, and main circumferences measurement); ii) the bioimpedance analysis (through the High Capacity Body Composition Analyser device model BC-420MA, Tanita Corporation, Tokyo, Japan); and iii) the postural assessment (through the device LUXTM, Chinesport s.p.a, Udine, Italy), in order to identify any possible asymmetries of the trunk and the spine, and eventual varism, valgism, and cavus/flat-foot.

Hence photos (CANON EOS 350 digital camera) on interest’s areas (gluteal, femoral, trochanteric, lower limbs and so on) as specific instrumental analyses and biochemical assessment as below specified were carried out.

Thermographic examination was carried out before the video-capillaroscopy on the front of the thigh on the vertical and 15 cm from the right leg sapheno-femoral junction by using the Thermo Cell Test DGT5 high resolution system (IPS patent, Thermo Cell, Milan, Italy) with professional microencapsulated 8-colours liquid crystal (ELC) plates. The assessment was done in stand-up position after 15 minutes stay at constant room temperature (18 to 25 °C) in order to balance external temperature with body temperature. The thermographic plate is able to highlight “spot” patterns that correspond to the four stages of cellulite described by Curri (7, 8) as identified on the basis of presence or absence of images to stains, which are the expression of the local distribution of temperature. Practically no spots (e.g. a uniform image) is associated with the absence of cellulite; the presence of not clear/nuanced spots suggests a edematous cellulitis (stage I-II according to Curri); patchy spots even more evident (like leopard skin) correspond to micro-nodules and, therefore, denote a cellulite in a more advanced stage (stage III of Curri); finally, the presence of holes blacks is indicator of macronodules (stage IV of Curri). Such findings were collected and interpreted according to the formula of Curri (7,8). Results were evaluated on a scale ranging from 0 to 25. Values from 0 to 3 are normal (T0), from 4 to 7 indicate initial microcirculation alterations (T1), values from 8 to 13 indicate venous–capillary stasis (T2), values from 14 to 19 mean cold areas with hypothermal “black holes” (T3), and values from 20 to 25 indicate clear fibrosis and liposclerosis (T4).

Optical videocapillaroscopy was made using an optical probe video capillaroscopy device (NEW VIDEO-CAP 3.0, DS MEDICA SRL MILANO) in the same conditions for thermography, by using a 200x magni-
fication lens in order to analyse at the best blood flow both erythrocyte aggregation and capillary vessels and venulae where blood circulation is slowed. Pooled data were analysed according to their static or dynamic nature. Static or morphological parameters included either descriptive (e.g. visibility of sub-papillary venous plexus, morphology, orientation, handle tortuosity, neangiogenesis) or measurable parameters (length and diameter of handle, ectasie, megacapillars, vascular areas, microhaemorrhage). Dynamic or functional parameters included the features of the flow (9). In our evaluation we considered:

- the subpapillary venous plexus (SPVP), according to score ranging from 0 to 4 i.e. 0, not detectable SPVP; 1, barely visible SPV; 2, detectable only in some sections of the evaluated area, without proximal extension; and 3, detectable in the whole evaluated area, with proximal extension;
- avascular areas (i.e. apparently without capillary vessels areas more than 500 micron square), according to a score ranging from 0 to 2 i.e. 0, not detectable avascular areas, 1, 1 to 2 small avascular areas, and 2, more wide avascular areas.

Ecography of subcutaneous tissue was carried out with a portable ultrasound device (Genera Electric LOGIC E, UK) with a linear probe at 12 MHz never used before this study in patients suffering from cellulite. The reference point for the test was the front area of the thigh on the vertical line at 15 cm from the saphenofemoral junction. The echography test was carried out after the termographic and the videocapillaroscopy tests so as not to distort the obtained data. The purpose of ultrasound scanning has been to evaluate the thickness of subcutaneous adipose, by studying its general architecture (that is profoundly altered in cellulite, with branches fragmented and irregular) and searching for striking images of nodules, i.e. a hypochogenic areas with multiple hyperechogenic striae.

Colour flow Doppler imaging was performed on loer limbs by using a high resolution color flow Doppler imaging device equipped with a 7.5 Hz linear probe (Siemens Acuson Antares, Munich, Germany) in order to detect eventually concomitant venous insufficiency.

Non invasive skin parameters (hydration, elasticity, and pH) were carried out with the analytical instrumentation Soft Plus (Callegari, Parma Italy) on the area that underwent to treatment (see below) at room temperature (range 15° to 30 °C). The normal range of such parameters depends on the gender and age.

Basic biochemical assessment included: hemochrome with leukocytes count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), transaminases, gamma-glutamyl transpeptidase, alkaline phosphatase, creatinine, glycemia, nitrogen urea, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, bilirubin, urinary analysis, HCV, HbsAg, Ca 125. A specific hormone profile was carried out by means of i) the determination during follicular and luteinic phase or in menopause of serum/plasma estradiol, progesterone, FSH, and LH; ii) the measurement of morning levels of FT3, FT4, and TSH; and iii) the quantification of prolactin on three blood samples (time 0’, 15’, and 30’) also in menopausal women. Furthermore blood plasma levels of leptin, adiponectin and resistin were measured. Finally all recruited women undergo oxidative stress evaluation by means of i) the assessment of total oxidant capacity through d-ROMs test (Diacon International s.r.l. Grosseto), which normal range is 250 to 300 A. U., on a fasting sample of whole capillary blood (10), and ii) the blood serum levels of Coenzyme Q10 (normal range 0.6–1.6 µg/mL). All the analyses were performed in a certified private laboratory by using commercially available test kits (Laboratorio TEST, Modena, Italy).

At the time of clinical visit 2-mm diameter specimens of soft tissues from areas of interest (using as control specimens from trunk areas) were obtained with punch biopsy after local anestheisa with mepivacaine hydrochloride 10 mg/mL s. c. Slices obtained with paraffin inclusion method underwent to specific coloration methods (Hematoxiline-eosine, Verhoef, Weigert, Blu Mallory) as a function of the aim of study (11). Slides were observed and photographed with photomicroscope Zeiss Axiophot (Zeiss, Obercochen, Germany) equipped with contrast of differential interference according to Nomarsky. All samples were analysed (direct observation and photos) blindly by two independent investigators. Generally the correctness of clinical evaluations and instrumental finding interpretations was warranted by independent measurements of clinicians according to the best clinical practice guidelines.

Results were expressed as means ± SD and data were processed for statistical analysis thorough a dedicated software. Comparisons were performed by means of t Test and p values lower than 0.05 were considered statistically significant.

Results

Sixty women fit the inclusion criteria and were enrolled. They were 39.90 ± 11.08 years old (range 22 to 61 years), showing a mean weight and a height of 68.37 ± 6.75 kg and 162.88 ± 5.41 cm, respectively, thus accounting for a 25.13 ± 2.06 mean BMI. Eleven of them (18.33%) were in physiological menopause and not suffer from particular disturbances at the time of visit and
52/60 (86.77%) became almost one time pregnant in her past. All declared to be sexually active. Forty-three of them (71.67%) were physically active too. Nine (15%) were cigarette smokers.

In order to classify the severity of cellulite, according the chosen scale for skin (see above) none showed the score 0, 6/60 (10%) showed the score 1 and the remaining 54/60 (90%) was at the highest degree of cellulite, i.e. 2. and stretch mark in 39/60 (65%). A painful reaction to pinching was observed in 15/60 (25%) cases while 45/60 (75) reported a spontaneous pain of various degrees. Hydration, elasticity and pH were in the physiological range. However searching for co-existent vascular abnormalities the clinical examination revealed:

- feeling of heaviness in the legs 19/60 (32.67%);
- paresthesia and/or heartburn 1/60 (1.67%);
- night cramps 12/60 (20%);
- ankle oedema: 9/60 (15%);
- telangiectasias: 37/60 (61.67%);
- varicose veins: 6/60 (10%).

No abnormalities were shown after postural and podometric evaluation. Thermographic assessment as evaluated on a scale ranging from 0 to 25 (see above) showed no women neither in T0 nor in T2 stage, 8 in T3 and 52 in T3.

Optical videocapillaroscopy showed

- no women in stage 0, 12 in stage 1, 28 in stage 2, and 20 in stage 3, regarding the subpapillary venous plexous (SPVP);
- none in stage 0, 10 in stage 1 and 50 in stage 2, regarding avascular areas.

Ultrasound examination of subcutaneous layer showed the presence of images suggesting the presence of noduli having different dimensions in all the recruited women (60/60, 100%).

Colour flow Doppler imaging showed patterns of venous insufficiency in 6 patients (10%), in 4 cases at level of safen and in 2 cases at level of other veins, bilaterally, although with different level of impairment.

The laboratory data were all in the normal range (data not shown), including plasma coenzyme Q levels (1.23±0.23 μg/mL), except for total oxidant capacity that was over the upper limit, i.e. 477.38±69.26 A.U.

In order to make as possible quantitative the histological evaluation we identified 5 different pattern of increasing tissue damage by computing the microscopic features of epidermis, derma and hypoderma, as summarized in Table 1.

---

### Table 1. Histologic patterns of cellulite.

<table>
<thead>
<tr>
<th>PATTERN</th>
<th>EPIDERMIS</th>
<th>DERMA</th>
<th>HYPODERMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No abnormalities</td>
<td>No abnormalities</td>
<td>No abnormalities</td>
</tr>
<tr>
<td>2</td>
<td>No abnormalities</td>
<td>Mild oedema in the reticular dermis</td>
<td>Anisopoikilocytosis; oedema in the adipose layer close to the dermis</td>
</tr>
<tr>
<td>3</td>
<td>Islets of hypertrophic keratinocytes alternated do a flattened the stratum corneum; decreased deepness of dermal spines; abnormal cell replication at baseline</td>
<td>Impaired quality of hyaluronic acid; subversion of the regular structural architecture of the collagen and elastic fibers; the former have a disordered orientation; the second are shorter and in some places appear fragmented; alteration of skin appendages; alterations of the microcirculation with collapse of arterioles and venules, dilated lymphatics; oedema in the reticular dermis, thickening of connective fibers in some areas</td>
<td>Anisopoikilocytosis; fibrotic thickening of collagen that delimits the lobes; concentric organization of fat cells that participate in the formation of nodules; alteration of the microcirculation due to both arterioles and venules that appear collapsed and/or unstructured, as well as to dilated lymphatic vessels that are absent or dilated in the peripheral area of nodules</td>
</tr>
<tr>
<td>4</td>
<td>As in 3</td>
<td>As in 3</td>
<td>Further thickening of the connective beams that surround the small nodules; areas of fatty tissue where the connective beams appear under lysis; presence of micro-nodules that melt between them; microcirculation completely altered with veins and arterioles with no content in blood; no evidence of lymphatic vessels</td>
</tr>
<tr>
<td>5</td>
<td>As in 3</td>
<td>Hernias of adipose tissue into the dermal layers of skin</td>
<td>Complete subversion of the adipose tissue organisation; considerable presence of macronodules, within which there are fat cells with giant calcified concretions in them; wide rarefaction and complete disorganization of microcirculation</td>
</tr>
</tbody>
</table>
Paradigmatic examples of such pattern are shown in Figure 1. In an attempt to correlate such patterns with the original classification of Curri, our patterns 1 and 2 may correspond to stage I of Curri, while 3 corresponds to II and III stage and 4 and 5 to the IV stage. In the herein study, the pattern 3 was found in 12/60 patients (20%), the pattern 4 in 29/60 patients (48.33%) and the pattern 5 in 19/60 (31.67%).

In particular the features of adipose tissue were shown to be different compared to those of abdominal and breast subcutaneous (figure 2). Because the only abnormal laboratory parameter

---

**Figure 1.** Histological patterns of cellulite.

**Figure 2.** Histological features of adipose tissue in the cellulite.
**Left:** abdominal fat. **Middle:** breast fat. **Right:** trochanteric fat. Staining with Hematoxiline-eosine, Verhef, Weigert, Blu Mallory.
was the total oxidant capacity, any possible relationship between this variable and all other evaluable parameters was assessed. The linear regression analysis showed a direct, positive and statistically significant relationship (p<0.05) between total oxidant by a hand and age (r=0.34) and BMI (r=0.25) by another hand (Figure 3 and 4). On the contrary a direct, negative and statistically significant correlation (p<0.05) was shown between total oxidant capacity and skin hydration (r=-0.37) and elasticity (r=-0.35), respectively (Figure 5 and 6). Interestingly total oxidant capacity was significantly (p<0.05) and negatively (r=-0.18) correlated also to
plasma levels of coenzyme Q10, a powerful antioxidant (Figure 7). No significant relationships were found between total oxidant capacity and other evaluable clinical and instrumental parameters, including pain, noduli, thermography, and video-capillaroscopy findings as well as histological findings (data not shown).

**Discussion**

In this preliminary study firstly we analysed the anamnestic, clinical, instrumental, bioptic and laboratory data of a sample of women suffering from cellulite and secondly proposed an empirical approach to standardize in some way its histological findings. Patients showed the classical clinical and instrumental signs of cellulite in all its various degrees according to the classification of Curri (8). However such signs were not associated to any abnormal values of classical metabolic and inflammatory parameters. Surprisingly adipokines levels were also in their normal range, although cellulite is often described as a disease involving inflammation as well as adipocyte endocrine metabolism (12). The only significant parameter that increased was the total oxidant capacity, a surrogate biomarker of increased production of oxygen reactive species (13). Accordingly the detected high total oxidant capacity was negatively related to plasma levels of Coenzyme Q10, normally involved in the control of blood free radicals. In other words a condition of oxidative stress, i.e. an imbalance between the production and the elimination of free radicals by antioxidant systems was associated to cellulite (13). Moreover it is well known that skin performance can be affected by oxidative stress and in this study total oxidant capacity was higher as hydration and elasticity were lower. As expected oxidative stress was positively related to age and to BMI thus confirming that cellulite is related to age and body mass through blood free radical level. Therefore the proposed hypothesis of cellulite as the expression of a local/systemic disease able to influence the whole body functions/performances apparently confirmed a possible role only for oxidative stress, as previously described (4, 13). This does not mean that metabolic, hormonal or reactive abnormalities may have occurred in the tissues affected by cellulite but without reaching a enough level to have systemic impact (12). In this regards histochemical studies are in progress in order to analyse the expression of cellular and tissue biomarkers potentially correlated to the pathophysiology of cellulite (e.g. transcriptional factors, adipokines, nitric oxide synthase and so on).

On the other hand an accurate analysis of histological slices from repeated biopsies revealed that cellulite appears to be not only a disease of fat tissue but it involves all the skin layers including epidermis, dermis and hypoderm and therefore, keratinocytes, extracellular matrix, collagen, reticular and elastic fibres, adipocytes, blood vessels and so on. Unfortunately scientific literature is still lacking of homogeneous criteria non only to allow doctors to make a histological diagnosis of cellulite but also to provide a validate tool to quantify the histological findings (e.g. a grading) both being indispensable and to allow a histological staging of the diseases. Moreover, according
to our experience deriving from the collection of specimens from different affected areas, cellulite appears also as a multifocal disease where each focus seems to undergo a independent evolution. In other words one case labelled as “phase II cellulite according to Curri” can show in different tested areas different degrees of tissues impairment. This makes perhaps impossible to understand fully the underlying biology and pathophysiology of such disease and to treat it in a satisfying way (14).

**Conclusion**

The lacking of a control group was the main limitation of this study. However, keeping this limit in mind, we proposed a model to evaluate globally the patients suffering from cellulite and to standardise the histological findings of cellulite by describing the main abnormalities of dermis, dermis and hypoderma in affected areas in comparison with unaffected areas used as controls. We derived 5 main patterns which severity increases from 1 to 5. Further studies are in progress to make more “quantitative” the description of each pattern but it is evident that cellulite is a disease of all skin layers including hypoderma. Finally the herein found relationships between cellulite and oxidative stress may open new therapeutic opportunities through an oral antioxidant supplementation (4) and/or topical formulations (15).

### References

THE MANAGEMENT OF SKIN NECROSIS associated to intralipotherapy with an adipocytolytic solution for treatment of localized adiposity

Roberto Amore, MD (1), Domenico Amuso, MD (1), Loredana Tanzarella, MD (2), Kostas Gritzalis, MD, PhD (2), and Vincenza Leonardi, MD, PhD (1).
(1) University of Palermo, Palermo (Italy). (2) Freelance.

ABSTRACT

Introduction
Nowadays the localised adiposity – a common cause of concern or discomfort – can be treated successfully through non-invasive approaches like intralipotherapy i.e. the subcutaneous injection of adipocytolytic agents. However the possible incidence of unwanted side effects like skin necrosis should be carefully taken in account in order to develop safe and effective preventive or therapeutic strategies.

Aim
The aim of this study was to evaluate retrospectively the incidence, the risk factors, the possible mechanism and the clinical features of skin necrosis after intralipotherapy and to evaluate the effectiveness a dedicate protocol for an optimal treatment of such unwanted side effect.

Materials and Methods
The digital data shits obtained from a group of doctors who practiced one or more sessions of intralipotherapy (with a sodium deoxycholate-based formula, AqualixTM) in a defined wide interval of time were analysed. When a clinical case of skin necrosis really related to such treatment was identified the patients undergo a specific protocol of treatment tailored on the basis of their clinical history, physical examination, and laboratory tests.

Results
The data – collected from January 2010 to December 2014 (accounting for a period of 5 years) – revealed 158,000 intralipotherapy sessions performed by 3,160 medical doctors. Skin necrosis occurred only in 12 cases (equivalent to 0.0076%) and was treated successfully with the proposed treatment protocol; the healing was by secondary intention with irregular wider scars when the therapy was delayed. Risk factors for skin necrosis were technical errors, especially those leading to an abnormal accumulation of the device in the treatment area, the older age (>60 years) and the lower limbs as anatomic region of interest.

Discussion
Skin necrosis was very rare and showed no relation to the quantity of injected deoxycholate per session. Rather it was possibly related to a non-homogeneous spreading of the detergent that became more concentrated in some areas thus inducing an unspecific cytolysis around the localised adiposity under treatment. No general unwanted side effects were detected. These findings are in agreement with the observation that adipocytolitic agents have never shown systemic toxicity due to overdosing. In any case an early treatment is able to warrant an optimal healing process.

Conclusions
Local adiposity can be safely and effectively and non-invasively treated through intralipotherapy that was shown associated to a very very low incidence of skin necrosis. A right technique (e.g. a “fragmented” distribution of the adipocytolytic formula, with a careful dosing of the injected volume per unit area) can prevent this unwanted side effect. This latter, when it appears, can be safely tackled and solved with a specific protocol.

Keywords
Adipocytolysis, intralipotherapy, localized adiposity, skin necrosis, sodium deoxycholate.
Introduction

Although it is a non-symptomatic “benign lesion”, the so-called “localized adiposity” – i.e. a delimited accumulation of non-inflamed fat tissue in the subcutaneous layer of one or more areas of human body (e.g. the submental or the abdominal region) – is often a cause of concern or distress for the patient who can ask specifically the clinician to undergo non-invasive treatments. On this background intralipotherapy has been proposed in order to destroy selectively fat cells (“adipocytolysis”) by using the anionic detergent deoxycholic acid. This naturally occurring bile acid having emulsifying and fat solubilising properties, after injected into subcutaneous fat was shown to really disrupt cell membrane of adipocytes thus inducing the required adipocytolysis (1-4). However the progressive spreading of this technique worldwide may increase the probability to detect unwanted side effects like skin necrosis due to the relative unspecific (detergent) action of deoxycholic acid.

Aims

The aims of this study were i) to assess the incidence of skin necrosis on a wide cases-series of intralipotherapy sessions, ii) to analyse its relative risk, possible mechanism, and clinical features, and iii) to evaluate the suitability of a specific protocol to treat such unwanted side effect.

Materials and Method

This study analyses the partial results of a wider survey of our independent working group that was asked to monitor the safety profile of deoxycholate-based intralipotherapy and to provide some specific practical indications to physicians, who were using such approach for localized adiposity, in the case of undesired side effects like skin necrosis.

Specifically, the herein reported experience refers to the use of a CE certified Class III medical device (approved in 2009) for the reduction of localized fat in combination with ultrasound therapy (AqualyxTM, Marllo International, San Giovanni in Marignano, Italy), as previously described (5). Such medical device is a subcutaneous injectable buffered solution containing a biocompatible, slow-release, short half-life and biodegradable detergent (i.e. the sodium salt of 3-alpha, 12-alfa-dihydroxy-5-beta-24-oic cholic acid or deoxycholic acid) in a sugar matrix (i.e. a polymer of 3,6-anhydro-L-galactose with D-galactose) (5). Therefore all doctors participating to the survey who detected in their practice a case of skin necrosis, informed us properly and, after a deep analysis of each clinical situation (through two specific application forms, as below reported), received by us the instructions to follow a specific protocol of treatment based on history, physical examination and specific practical indications.

The application form for history data collection included:
- general data of the patient: age, sex, health risk factors (e.g. cigarette smoking, alcohol drinking), physical and psychological condition, concomitant medical/surgical therapies, and so on through semi-quantitative scales;
- specific data on the area of treatment: anatomic region of localised adiposity to treat and co-existence of other blemishes (e.g. cellulite, vascular malformations, scars, irregularities, asymmetries) in the same area;
- technical data about intralipotherapy: total amount of injected solution per area and per session, number of sessions, time interval between the sessions, and concomitance with other therapies;
- occurrence of any particular events during the infiltrative session: i.e. pain intensity, appearances of characteristic cutaneous manifestations, and so on through semi-quantitative scales;
- features of skin necrosis: clinical sign, time of first appearance and duration, and any eventual accompanying systemic manifestations;
- clinical follow-up, either in case or not of treatment with the suggested protocol (see below): recovery time for skin integrity and complications.

The application form for physical examination data collection included details about:
- the anatomic region of skin necrosis and its general features (e.g. colour, amplitude, deepness and so on), any signs of pain, paresthesia/dysesthesia, and superimposed infections, the region involved, the characteristics of the surrounding skin and subcutaneous regions, and so on;
- the features of eventual other regions treated: integrity level of the skin, presence of skin lesions, pain, paresthesia/dysesthesia, and so on;
- the general conditions: atypical vital signs (e.g. body temperature, heart rate, blood pressure), and eventual systemic changes (rash, rheumatic pain, generalized skin manifestations or of other body systems).

The doctors were asked also to report and to send us, in addition to the above application forms, some laboratory data indicating a systemic inflammation (e.g. white blood cell count, ESR, and CRP) together with a photographic reportage at the time of first visit.

The doctor who reported us the case, after showing the photos and laboratory analyses and discussing with our team, was asked to apply to his patient the following specific protocol of treatment:
- accurate inspection of the affected area in order to
identify any sign of skin lesions including boils/blisters and underlying bruises;
- local anaesthesia (according to the country-specific guidelines) and linear incision of the wound site, along the longest axis;
- debridement of the necrotic adipose tissue, and squeezing of the wounded area in order to drain any eventual liquid collections;
- packing of the surgery wound with povidone iodine, soaked gauze and bandages;
- systemic protection from infections through antibiotics (e.g., fluoroquinolones or cephalosporines);
- medication and change of the packed dressing every 3-4 days until ecchymosis regression and apparent tissue recovery from skin damage;
- in the total absence of skin suffering signs, debridement of wound margins and single stitches application at several different planes.

Finally, we asked the doctors participating to our survey to monitor the treatment either clinically or by serial photographic assessment at variable intervals, from 3 to 7 days, until the full recovery, and to send us a detailed report in order to allow us to analyse the findings.

Results

From January 2010 to December 2014 (accounting for 5 years of follow-up) our study group monitored 3,160 European doctors who were specifically trained to practice intralipotherapy in combination with ultrasound therapy as previously described (5, 6). They performed an average of 158,000 estimated sessions of treatment either in male and females.

The skin necrosis occurred in 12 out of 158,000 injections accounting for an incidence of 0.0076%. Its greatest area was 10 cm². The lesion was single in 10 of 12 cases (83.3%) and multiple in the remaining ones. Ecchymosis and skin blisters appeared in 11 of 12 cases (91.6%) within 48 h and 96 hours, respectively, and were accompanied by signs of inflammation, including redness, pain, and heat feeling. No signs of infection were ever reported. Loss of skin integrity during a period ranging from 4 days to 14 days (in cases where the problem was reported late and the incision, as indicated in the protocol management proposed by the study group, was not performed immediately). The other treated areas showed no pathological changes. No signs of systemic involvement or generalized manifestations were ever reported. Plasma C-Reactive Protein was only transiently and not significantly increased in few patients. The general health of the subject was not impaired.

The Figure 1 shows the evolution of a paradigmatic skin necrosis case of our series treated according to the suggested protocol.

The herein proposed treatment protocol was implemented from the onset of skin necrosis in 3 cases (group A). In 4 cases, the disclosure of the adverse event occurred late, when the integrity of the skin had already been compromised (group B). In the remaining 5 cases doctors were made aware of the problem late in the case (Group C).

The comparison among groups revealed that:
- the group A demonstrated a more rapid resolution of the lesion (9-14 weeks) with respect to B, which in turn was more rapid than C (over than 18 weeks);
- the group A resolved with a linear scar similar to a surgical wound (see Figure 1D) unlike the other two groups in which the inevitable healing by secondary intention caused irregular wider scars.

The treatment was well tolerated and no unwanted side effects were observed.

Risk factors associated with skin necrosis were:
- some technical errors during injection (91.7%): such
errors, including an excessive dosage of active principle per unit area, possibly led to a release too much superficial and/or to a unequal distribution of sodium deoxycholate that became more concentrate in an area compared to another affected fat area, thus increasing the probability of tissue damage e.g. skin necrosis;

• an interval after the previous session less than 4 weeks (in cases where no problems were reported in the first session) (85.7%);
• the lower limbs as treatment area (83.3%);
• the patient’s age over 60 years (75%);
• a session of treatment subsequent to the first one (58.3%);

There was no relation between the onset of skin necrosis and the following factors: smoking, concomitant pathologies (which do not contraindicate the treatment), the coexistence of other blemishes at the wound site, the total amount of formula used per session, some particular events occurring during the infiltration (especially severe pain, typical skin signs, and so on).

Discussion

The subcutaneous injection of adipocytolytic formulas is a generally suitable and effective strategy for the non-invasive treatment of localized adiposity, a relatively common distressing clinical condition. This approach was firstly described in 2001 by using an injectable combination of phosphatidylcholine and deoxycholic acid (LipostabylTM) (1, 2). A number of medical devices were then developed in order to treat different anatomic areas affected by local fat accumulation (3, 4). When it was discovered that the adipocytolytic effect of such formulas depended by deoxycholic acid (6-8) a new generation of medical devices containing only this natural occurring bile acid was proposed. These formulas seems more effective than previous ones although less manageable (9, 10). Indeed their lytic effects are direct but non-selective for adipocytes and therefore, depending on the procedure and the dose, they can affect also the surrounding tissues thus increasing the risk of unwanted skin structural/functional changes (11-13). However, it is important to emphasize that it has been shown both in vitro and in vivo that deoxhycolic acid decreases its cytolytic effect as the total amount of tissue protein increases. Indeed the deterging effect of deoxycholate appears preferentially directed towards the adipocytes than muscle cells these latter being more resistant due to their significantly higher protein content (14). On the other hand adipocytolitic agents have never demonstrated systemic toxicity due to overdosing.

In this scenario the incidence of skin necrosis as undesired side effect of intralipotherapy is still understood as well as its possible mechanism and treatment. Therefore in order to fill at least partially this gap we revised the data obtained by monitoring for 5 years a wide sample of intralipotherapy sessions performed with a commercially available adipocytolytic medical device associated to ultrasound therapy, as previously described (5, 6).

According to our data the incidence of skin necrosis was very low affecting only the 0.0076% of all sessions. Interestingly this effect – unlike reported by previous studies – was mostly related to technical errors responsible of a non homogeneous spreading of the active principle in the subcutaneous finally leading to a relative accumulation of deoxycholate in an area beyond the safety limit rather than to the total amount of the salt injected per session (9). Such finding is supported by three fundamental considerations:

• skin necrosis occurred even when total quantity of administered active principle per session was minimal (1/2 vial for example, as shown in Figure 2);
• larger amounts of active principle per session but distributed according to the normal dosage calculations for area (and therefore treatment of several zones) did not favour the onset of skin necrosis;
• larger amounts of active principle for session was not associated to systemic toxicity.

Moreover the risk of skin necrosis was higher in older peoples (>60 years-old) and in lower limbs and increases after first session especially when the interval between the treatments was lower than 4 weeks.

Figure 2.
Initial skin necrosis after injection of ½ vial (4 mL) in an area of approximately 4 cm² for the treatment of an underlying lipoma in the thigh in a 52 year old male subject (4 days after treatment). The excessive quantity used for the small area resulted in skin necrosis. The total amount used for the session was ½ vial.
The pathogenic processes possibly responsible of the skin necrosis were:
• a vascular damage as documented by the extensive ecchymosis;
• a necrosis of the superficial fat tissue, as seen after the surgical approach.

Both mechanisms were able to induce ischemia in the skin, as documented by the boils, with a subsequent loss of integrity/continuity and healing by second intention. However even rarely when a necrosis occurs the herein reported treatment protocol showed safe, well tolerated and effective especially when performed early.

Conclusion
Skin necrosis is a not a common event that can occur following an intralipotherapeutic treatment with adipocytolytic agents. Like any other aesthetic medical treatment the mechanisms, risk factors and treatments must be recognized. Adipocytolytic substances differ considerably from one to another and therefore cannot be compared in terms of dosage, efficacy and safety. Such substances are recognized as having aspecific cell lysis as their mechanism of action, although the adipocyte is by far the most vulnerable cell. On this background the present study – despite some evident methodological limitations (e.g. the lack of some epidemiological data and a more deep statistical analysis due to incomplete data reporting from doctors) – provided the first and widest follow-up analysis of intralipotherapy at our memory. The medical device herein evaluated was associated to a very very low incidence of skin necrosis that was successfully treated with a specific protocol. Moreover the here proposed therapy proven effective to favour the healing process and resulting scarring. Finally the study by identifying some risk factors of skin necrosis provided a useful preventive tool in order to avoid such although rare unwanted effect. In fact taken together our findings indicate that adipocytolytic formulas must be released directly into the fatty tissue as homogeneously as possible and at different levels. In order to reach this goal and to prevent the onset of unwanted side effects a right injection technique (“fragmented distribution”) as well as a right dosage per unit area (a measure of the active principle concentration) are fundamental, as summarized in the Table 1. In particular, we strongly advise against using adipocytolytic agents with the mesotherapy technique because of the high risk of irregularities of the skin (pseudocellulite effect) and nodules (15).

Table 1. Guidelines to prevent skin necrosis associated to intralipotherapy.

<table>
<thead>
<tr>
<th></th>
<th>Guidelines to prevent skin necrosis associated to intralipotherapy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Perform the technique properly, distributing the solution in the most homogeneous way possible.</td>
</tr>
<tr>
<td>2</td>
<td>Do not release the formula superficially.</td>
</tr>
<tr>
<td>3</td>
<td>Do not release the formula as boluses.</td>
</tr>
<tr>
<td>4</td>
<td>Maintain the dosage indicated for the zone, extension, and thickness.</td>
</tr>
<tr>
<td>5</td>
<td>Reduce the dosages in subjects over 60.</td>
</tr>
<tr>
<td>6</td>
<td>Respect the indicated minimal intervals between one session and another.</td>
</tr>
<tr>
<td>7</td>
<td>In case of necrosis: don’t delay the protocol for the necrosis proposed in this study; for more information and technical/scientific support contact your reference instructor or the producer.</td>
</tr>
</tbody>
</table>
References

The European Journal of Aesthetic Medicine and Dermatology is aimed at dermatologists, cosmetic surgeon, plastic surgeons, anti-ageing and aesthetic physicians and all those interested on the rapidly expanding field of aesthetic medical practice.

Features include:
- Cosmetic surgery, including facial rejuvenation, hair removal and skin resurfacing
- Adipose tissue treatments including stem cell, meso therapy and intralipotherapy
- Molecular biology applied to aesthetic procedures
- Use for lasers and light sources for cosmetic treatment
- Use of mechanical and physical technologies on aesthetic fields
- Applications of peeling agents, fillers, injectables devices and other buccoskeletal modalities
- Topical treatments
- Practical Procedures
- Safety issues

Subscription Order Form

<table>
<thead>
<tr>
<th>EJAMeD Print + Online</th>
<th>EJAMeD Print Only</th>
<th>EJAMeD Online Only</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EUROPE</strong></td>
<td><strong>EUROPE</strong></td>
<td><strong>EUROPE</strong></td>
</tr>
<tr>
<td>□ Individual € 105,00</td>
<td>□ Individual € 95,00</td>
<td>□ Individual € 85,00</td>
</tr>
<tr>
<td>□ Institutional € 285,00</td>
<td>□ Institutional € 225,00</td>
<td>□ Institutional € 85,00</td>
</tr>
<tr>
<td><strong>EXTRA</strong></td>
<td><strong>EXTRA</strong></td>
<td><strong>EXTRA</strong></td>
</tr>
<tr>
<td>the european countries</td>
<td>the european countries</td>
<td>the european countries</td>
</tr>
<tr>
<td>□ Individual € 125,00</td>
<td>□ Individual € 110,00</td>
<td>□ Individual € 85,00</td>
</tr>
<tr>
<td>□ Institutional € 315,00</td>
<td>□ Institutional € 245,00</td>
<td>□ Institutional € 85,00</td>
</tr>
</tbody>
</table>

Payment Methods
- 1. Payment enclosed. Bank draft made payable to Marllor Biomedical Srl
  Banco Popolare filiale di Cattolica (RN) - IBAN: IT16 L 05034 67750 0000000000372
  The journal will be sent to confirm the transfer.
- 2. Please send me an invoice
- 3. Please charge □ American Express □ Visa □ Eurocard □ MasterCard
  Card Number ____________________________ Expire Date ____________ Date ____________
  Signature ____________________________

Please send the journal to
Name ____________________________________
Address ____________________________________
Post/Zip code ____________________________ Country ____________________________
Phone ____________________________ E-mail ____________________________

Please return order form to:
Marllor Biomedical Srl - Via Don Minzoni, 12 - 47842 San Giovanni in Marignano (RN) Italy
Fax.: +39 0541 1572570 - E-mail: media@ejamed.com
INSTRUCTIONS TO AUTHORS

regular articles

Submissions should be submitted via EJAMeD online Manuscript Submission System at www.ejamed.com

All submissions must be marked as:
• Original Articles (2,000–4,000 words, including references and figures/tables)
• Book Reviews
• Case Reports (1,000–1,500 words, including references and figures/tables)
• Commentary and Discussion (1,000–2,500 words, including references and figures/tables)
• Historical Notes
• Innovative Techniques (2,000–4,000 words, including references and figures/tables)
• Review Articles (2,000–4,000 words, including references and figures/tables)
• Letter To The Editor
  (800 words max, 5 references max, no figures/tables)

Questions about submissions may be submitted to info@ejamed.com.

Manuscripts: document format .doc only.

Abstract: 200–300 words.

Figures/drawings: Illustrator EPS, version CS2 or older; CMYK or grayscale color space — do not use spot (PMS) colors; do not convert fonts to outlines.

Photographs: TIFF, JPEG, JPG or PNG with a 300 dpi or higher resolution.

Tables: Document format (.doc) only.


Manuscript preparation and submission and Ethical requirements please consult guideline of Uniform Requirements for Manuscripts submitted to Biomedical Publication at http://www.icmje.org/

Conflict of interest: Authors are responsible for recognising and disclosing financial and other conflicts of interest that might bias their work. They should acknowledge in the manuscript all financial support for the work and other financial or personal connections to the work.

COPYRIGHT INFORMATION

Submission of a manuscript implies that the work described has not been published before (except in form of an abstract or as part of a published lecture, review or thesis); that it is not under consideration for publication elsewhere; that its publication has been approved by all co-authors, if any, as well as - tacitly or explicitly - by the responsible authorities at the institution where the work was carried out. Transfer of copyright to Marlbor Biomedical srl becomes effective if and when the article is accepted for publication. The copyright covers the exclusive right to reproduce and distribute the article, including reprints, translations, photographic reproductions, microform, electronic form (offline, online) or other reproductions of similar nature.

All articles published in this journal are protected by copyright, which covers the exclusive rights to reproduce and distribute the article (as offprints), as well as all translation rights. No material published in this journal may be reproduced photographically or stored on microfilm, in electronic data bases, video disks, etc., without first obtaining written permission from the publisher. The use of general descriptive names, trade names, trademarks, etc., in this publication, even if not specifically identified, does not imply that these names are not protected by the relevant laws and regulations.

An author may make his/her article published by Ejamed available on his/her personal home page, provided the source of the published article is cited and Ejamed is mentioned as the copyright owner. Authors are requested to create a link to the published article in Ejamed’s internet service. The link must be accompanied by the following text: ”The original publication is available at www.ejamed.com”.

The author warrants that this contribution is original and that he/she has full power to make this grant. The author signs for and accepts responsibility for releasing this material on behalf of any and all co-authors.

After submission of this agreement signed by the corresponding author, changes of authorship or in the order of the authors listed will not be accepted by Ejamed.