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# Mechanobiology and Mechanotherapy of Adipose Tissue-Effect of Mechanical Force on Fat Tissue Engineering

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**Summary:** Our bodies are subjected to various mechanical forces, which in turn affect both the structure and function of our bodies. In particular, these mechanical forces play an important role in tissue growth and regeneration. Adipocytes and adipose-derived stem cells are both mechanosensitive and mechanoresponsive. The aim of this review is to summarize the relationship between mechanobiology and adipogenesis. PubMed was used to search for articles using the following keywords: mechanobiology, adipogenesis, adipose-derived stem cells, and cytoskeleton. In vitro and in vivo experiments have shown that adipogenesis is strongly promoted/inhibited by various internal and external mechanical forces, and that these effects are mediated by changes in the cytoskeleton of adipose-derived stem cells and/or various signaling pathways. Thus, adipose tissue engineering could be enhanced by the careful application of mechanical forces. It was shown recently that mature adipose tissue regenerates in an adipose tissue-engineering chamber. This observation has great potential for the reconstruction of soft tissue deficiencies, but the mechanisms behind it remain to be elucidated. On the basis of our understanding of mechanobiology, we hypothesize that the chamber removes mechanical force on the fat that normally impose high cytoskeletal tension. The reduction in tension in adipose stem cells triggers their differentiation into adipocytes. The improvement in our understanding of the relationship between mechanobiology and adipogenesis means that in the near future, we may be able to increase or decrease body fat, as needed in the clinic, by controlling the tension that is loaded onto fat. *(Plast Reconstr Surg Glob Open 2015;3:e578; doi: 10.1097/GOX.0000000000000564; Published online 15 December 2015.)*

## MECHANOBIOLOGY AND MECHANOTHERAPY

All organisms living on the earth are subjected to various mechanical forces, such as gravity, tension, and compression. This, in turn, affects the morphology and function of our bodies. Mechanical forces have been shown to operate at all levels of the body, namely, at the molecular, cellular, tissue, and organ levels. Signaling pathway convert the mechanical forces into signals that regulate multiple cellular events, such as proliferation, differentiation, spreading, and gene expression. These events, in turn, influence the development, growth, repair, and

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regeneration of tissue and organs.[1] Mechanobiology is the study of these effects of mechanical forces on cells, tissues, and organs. Currently, these is increasing interest in the usefulness of mechanical forces for promoting the proper development and function of tissue replacement constructs in the tissue engineering, especially those that will bear mechanical loads in vivo.[2]

Traditionally, mechanotherapy[3] has been defined as a treatment with medical devices, such as massage and orthopedic machines. However, given our expanding understanding of mechanobiology, we have proposed that the word "mechanotherapy" should be redefined as medical treatments that control the mechanical forces on cells, tissues, and organs.[3] This allows the plastic surgeon, for example, to refer to the common procedure of soft tissue expansion as a mechanotherapy. Soft tissue expansion can be achieved by either an invasive or a noninvasive expander. An invasive expander causes the skin to overstretch, forcing it to generate new skin to accommodate the expander. As a direct or indirect result of this mechanical force, the expanded skin and subcutaneous fat layer become thinner.[4] By contrast, the noninvasive external volume expansion devices provide negative pressure that causes the volume of soft tissue to expand. For example, the external volume expansion device (BRAVA, LLC, Miami, Fla.) is a nonsurgical and noninvasive alternative for breast enlargement.[5] It can also be used for preexpansion for breast autologous fat transplantation.[6] A murine study showed that external volume expansion increased both the thickness of the subcutaneous fat layer and the number of adipocytes in expansion-treated areas.[7] These observations suggest that mechanotherapy on adipose tissue could be used to augment for cosmetic or reconstructive purposes. It may also be possible to use mechanotherapy to or reduce the fat tissues in obesity. It seems likely that these approaches will start to be used in the clinical in the near future.

## CHARACTERISTICS OF ADIPOSE TISSUES

### Basic Characteristics of Adipose Tissue

Adipose tissue is an organ with multiple functions, including stocking energy, buffering external forces to protect the body, and secreting cytokines. There are 2 types of adipose tissue, namely, subcutaneous and visceral adipose tissue. Subcutaneous adipose tissue is mainly distributed in the abdominal wall, the femoral and gluteal region, and the back.[8,9] Adipose tissue has a unique structure; although adipocytes constitute 90% of the adipose tissue volume, they only account for approximately 15% of all cells in the tissue.[10] Most of the cytoplasm of adipocytes is occupied by a lipid droplet, which is responsible for the roughly spherical shape of adipocytes. There shape of adipocytes changes greatly during differentiation from the stem cell. It is know that stem cells are more adipogenic when they are round.

Adipose tissue also contains other types of cells besides adipocytes, including adipose-derived stem cells (ASCs), endothelial cells, mural cells, and others.[10] It also contains extracellular matrix (ECM), which consists of stromal ECM and the basement membrane.[11] The latter is a thin layer that surrounds and mechanically supports the adipocytes. Thus, the ECM and adipocytes maintain the structure of adipose tissue. Each adipocyte is in close proximity to at least one capillary in adipose tissue,[12] which indicates that adipocytes are fragile and sensitive to hypoxia. Thus, the adipogenesis is always accompanied with angiogenesis.

### Stem Cells and Adipogenesis

The ASCs that are harvested from adipose tissue and are located around large vessels[10] resemble bone marrow-derived mesenchymal stem cells (MSCs). They have a high proliferative capacity and can differentiate into multiple cell lineages, including osteogenic, chondrogenic, and adipogenic lineages. The fate of ASCs is regulated by both chemical and mechanical factors; however, in vitro studies suggest that mechanical factors are particularly potent regulators as they are effective even in the absence of chemical factors. By appropriately manipulating the chemical and mechanical environments, the ASCs can become dysregulated and progress preferentially toward adipocyte lineage differentiation.[13]

Initially, it was thought that under normal physiological conditions, adults do not undergo adipose regeneration. However, a recent study showed that, although the total number of mature adipocytes in postadolescent humans remains constant, approximately 10% of adipocytes are renewed annually.[14] The new adipocytes derive from the stromal vascular fraction of adipose tissue, which is where the ASCs are preferentially located. This suggests that the ASCs are a crucial cell source for adipose regeneration.

Notably, a normal physiological phenomenon is the progressive replacement of bone marrow tissue with adipose tissue with aging. There are 2 types of progenitor cells in the bone marrow, namely, hematopoietic and mesenchymal precursor cells. The latter can differentiate into osteoblasts, adipocytes, or

chondrocytes, depending on the conditions.[15] Significantly, the mesenchymal precursor cells exhibit an inverse relationship between osteoblastogenesis and adipogenesis; if adipogenesis is promoted, osteoblastogenesis will be inhibited.[16,17] The adipogenesis in bone marrow can be regulated by physiological factors, such as aging, mechanical force, and cytokines, as well as pathophysiological factors, including diseases such as anorexia nervosa and dyslipidemia and pharmacological agents such as thiazolidinediones and statins.

## MECHANOBIOLOGY AND ADIPOGENESIS

### Effect of Internal Mechanical Forces on Adipogenesis

Internal mechanical forces are exerted by cytoskeletal tension, which is generated by actomyosin contractility and the reaction forces that are generated by the ECM in vivo or the underlying substrate in vitro.[18] The cytoskeletal tension is then transmitted to ECM via focal adhesions, and thereby directs ECM assembly.[19] Different substrate stiffness and micropatterns can affect the cytoskeletal tension, which, in turn, can profoundly influence cell behaviors such as cell shape, differentiation, and gene expression.

Substrate stiffness can by itself direct the specific differentiation of MSCs; when MSCs are cultured on soft substrates that mimick the elasticity of brain tissue (0.1–1 kPa), neuronal precursors are generated.[20] By contrast, stiffer substrates that mimic muscle (8–17 kPa) induce myogenic commitment.[21–23] When ASCs are cultured on gels that mimic the native stiffness of adipose tissue (2 kPa), adipogenic markers are significantly upregulated, even in the absence of exogenous adipogenic growth factors and small molecules. As substrate stiffness increases, the cells spread more, lose their rounded morphology, and failed to upregulate adipogenic markers.[24]

Similarly, surface nanotopography also affects the differentiation of MSCs toward adipocytes; a round nanogroove patterns, which allows the cell to remain rounded, promotes adipogenesis, whereas straight grooves and grids, which cause the cells to elongate, increased osteogenesis.[25] MSCs are also more adipogenic when they are confined to micropatterned squares. By contrast, when they are confined to rectangles, they are more osteogenic. This phenomenon associates with actomyosin contractility, which is higher in the cells on the rectangles than in the cells on the squares.[26]

Thus, it appears that there is a very closed relationship between the cell tension exerted by the cytoskeleton and the lineage commitment of stem cells. Because one study showed that disrupting the cytoskeleton with cytochalasin increases the adipogenesis of MSCs,[25] it seems that stem cells that have a less organized cytoskeleton and low tension are more prone to differentiate into adipocytes.

Three-dimensional culture environments mimic the in vivo environment more closely than 2-dimensional environments. They are also considered to be ideal systems for regulating cell tension so that stem cells differentiate into the desired direction.[27] Cells behave very differently in 2- and 3-dimensional environments; in particular, when MSCs are cultured in 3-dimensional spheroids, their differentiation into adipocytes is greatly increased compared with when they are cultured in 2-dimensional cultures.[28] Moreover, to culture mature adipocytes, the ceiling culture method is need.[29] Thus, 3-dimensional culture environments are more suitable for adipogenesis and indeed have already been used in adipose tissue engineering.

### Effect of External Mechanical Forces on Adipogenesis

External mechanical force, including stretch, compression, and shear stress, plays a key role in stem cell adhesion, spreading, proliferation, migration, and differentiation. External mechanical forces that act on ECM are transmitted into the cell via integrin-regulated adhesion.[30] The integrin-regulated adhesion then transmit the mechanical signal to the cytoskeleton and nucleus,[31] or directly activate the extracellular regulated protein kinase (ERK)/mitogen-activated protein kinase (MAPK),[34,35] rho-rho-kinase,[25] and other signaling pathways[32] or mechanosensitive ion channels.[33]

## STUDIES ON THE EFFECT OF *IN VITRO* MECHANICAL FORCE ON ADIPOGENESIS

Several studies have investigated the effects of mechanical forces on adipogenesis *in vitro* because it is increasingly being understood that various adipose tissue cells, including adipocytes and preadipocytes, are mechanosensitive and mechanoresponsive. Different kinds of mechanical forces loaded on stem cells, and preadipocytes and adipocytes show totally different effect on adipogenesis. A recent study showed that when mature adipocytes (ie, 12 days after induction of differentiation) undergo 120% uniaxial static stretching for 72 hours, the MAPK/ERK kinase signaling pathway is activated, and adipocyte hypertrophy ensues.[36] However, when mouse preadipocyte 3T3-L1 cells undergo uniaxial cyclic stretching (130%, 1 Hz, for 15 or 45 hours), adipogenic differentiation is inhibited.[34] In general, static

stretching promotes adipogenesis, whereas dynamic mechanical forces such as cyclic stretching or vibration and static compression inhibit adipogenesis. Different signaling pathways are involved in these phenomena.

## STUDIES ON THE EFFECT OF *IN VIVO* MECHANICAL FORCE ON ADIPOGENESIS

About 80% of all body fat is subcutaneous fat, which tends to be located in specific regions including abdominal and gluteofemoral regions, especially in obese individuals, depending on sex and hormonal status.[9] However, we believe that this pattern of subcutaneous fat distribution may also reflect the direct effects of mechanical forces on fat tissues. Although this hypothesis needs to be addressed in further studies, it seems clear that direct massage or stretching of fatty regions may decrease fat tissue volume. "Fatty infiltrate" responses to microgravity, prolonged bed rest, or botulinum toxin A injection is good example of how fat can increase locally as a result of the low tension associated with muscular atrophy.[37–39]

Repairing large-volume soft tissue deficiencies such as breast deficiency and Romberg's disease is a great challenge in plastic surgery. The traditional treatment is autologous flap transfer, which causes donor-site morbidity and a cosmetically imperfect recipient site. Although tissue engineering provides an alternative technique for generating new autologous tissue, the volume of engineered adipose tissue is limited. To solve this problem, Findlay et al[40] subcutaneously implanted polycarbonate tissue-engineering chambers (78.5-mL volume) into the groins of pigs. Each chamber included a pedicled fat flap (5 mL). This approach increased the volume of the fat flaps by more than 5-fold. Although the precise mechanism that yield fat flap growth in the chambers remains uncertain, the authors listed a number of factors that play an important role in supporting and controlling new tissue growth. One of these may be the chamber-mediated loss of mechanical forces on the flap because these forces are known to regulate the function of most tissues and organs.[3]

Tension of adipose tissue balances the mechanical forces derived from the fibrous connective tissue surrounding adipose tissue. By embedding the fat flap in a spacious chamber, not only is space created for adipose tissue regeneration, the tension of the fat flap from the surrounding tissue is also decreased. These extracellular forces including matrix strain, compression, flow shear force, and hydrostatic pressure are dynamically loaded to the cytoskeletal tension in native tissue. The cytoskeletal tensional state depends on the balance between the intracellular actomyosin contractility and the reaction forces exerted the ECM. The balance internal contractility and external forces are a key determination of cell fate.[41] The decrease of tension derived from adipose tissue results in reduction of external forces of cells at varying spatial orientations, which maintains a round morphology of cells and promotes adipogenesis of stem cells.[42] We hypothesize that the embedding of chamber separate the fat flap from surrounding tissue and provide a space, which decreases the mechanical forces derived from surrounding tissue, thereby lowering the cytoskeletal tension, which triggers and promotes the adipogenesis of ASCs and adipose regeneration.

## FUTURE DIRECTIONS IN MECHANOBIOLOGY AND MECHANOTHERAPY IN THE ADIPOGENESIS FIELD

Our increasing understanding of adipose mechanobiology is illuminating the potential of mechanotherapy for treating obesity and improving adipose tissue engineering. With regard to mechanotherapy for obesity, the traditional ways to lose fat are reducing total calorie intake and taking more exercise. However, at the cellular level, obesity is considered to be the result of an increase in the number and/or the size of adipocytes.[43] It is possible that the development and progression of obesity may be the result of loss of ASCs and adipocytes regulatory controlled by mechanical forces. We could recontrol the cells by loading appropriate mechanical forces to treat obesity at cellular level. For example, because mechanical stimuli can decrease lipid droplet accumulation,[36] mechanical forces such as vibration[44] acting synergistically with changes in diet may decrease fat by reducing the accumulation of lipid droplets. This treatment could also decrease fat by inhibiting the adipogenic differentiation of stem cells.[32]

The mechanisms of action of BRAVA have been reported previously.[45,46] The application of controlled noninvasive suction by external volume expansion devices determines macroscopic tissue stretch and induces local ischemia and the accumulation of edema; all 3 factors can trigger inflammation, and inflammation-dependent and -independent effects are known to activate pathways that lead to cell proliferation, vessel remodeling, and adipogenesis.[45,46] Thus, they speculated that adipogenesis accelerated by the device is not the direct result of mechanical force on adipocytes but an indirect effect of edema and ischemia. This conjecture will need to be tested further in a future study.

A greater understanding of adipose mechanobiology will also help us to determine which mechanical variables in tissue engineering will most effectively create functional tissue. Mechanical stimuli could affect 3 elements of tissue engineering, namely, the stem cells, the scaffold, and the microenvironment. A good example of the importance of mechanical forces in tissue engineering is the fact that enclosing a pedicled fat flap in a spacious adipose tissue-engineering chamber markedly increased fat flap growth.[40] We speculate that the loss of mechanical forces loading on fat flap contributed to this fat flap growth. Because this phenomenon was discovered only recently, further research is needed to elucidate the mechanism or signaling pathway that is involved. There are also many other questions that need to be addressed. For instance, how can the tension that is loaded on fat in vivo be measured? How much tension change is needed to trigger the adipogenesis? Are there any other types of mechanical force that, like tension in vivo, can promote adipogenesis? Despite the many questions that remain, we believe that in the near future, we will be able to increase or decrease body fat, as needed in clinic, by controlling the tension that is loaded onto fat.

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