

EDITORIAL

Modeling fat cell stresses: paving the way for improved management of obesity and diabetes



Amit Gefen[†]

“...sustained static stretching delivered to adipocytes within a certain physiological range can stimulate them to produce lipids, whereas physiological dynamic (cyclic) stretching is able to inhibit lipid production ... [this] should be investigated very thoroughly ... it could revolutionize the way that we understand obesity and diabetes.”



Obesity and Type 2 diabetes, which are now well recognized as interdependent diseases, are leading preventable causes of mortality and morbidity worldwide, with increasing prevalences in adults as well as in children [1]. National health authorities now view both obesity and diabetes as being two of the most serious public health problems of the 21st century, especially given the inter-relationships between these diseases and hypertension, atherosclerosis and dyslipidemia. Understanding the function of adipose tissue is key to successfully addressing these problems.

Adipose tissue is a complex organ that, other than serving as a means for energy storage in the form of triglycerides, is able to secrete hormones and cytokines [2]. The increased mass of adipose tissue, which is characteristic of obesity, is due to hypertrophy of adipocytes and also an increase in the number of adipocytes, as is evident from tissue histology [3]. The number of adipocytes can increase through mitosis (mostly in adipocytes that have not yet started to produce lipids) or through differentiation from preadipocyte cells. Despite the fact that it is unknown which of these two

mechanisms actually functions, or which is more dominant in humans *in vivo*, it is often hypothesized that maturation of pre-adipocytes into adipocytes is an important cause of obesity [4,5]. Adipogenesis is initiated and regulated through the activation of key transcription factors such as peroxisome proliferator-activated receptor (PPAR)- γ_2 , which is an adipocyte-specific nuclear hormone receptor/adipogenic transcription factor, and through subsequent cascades of biochemical signaling involving members of the CCAAT/enhancer-binding protein family and the Kruppel-like factor family, as well as several extracellular-mediated signaling pathways [6]. Lipolysis, the breakdown of lipids stored in the adipocytes, occurs in response to signaling from other tissues that are energetically deprived, and involves hydrolyzation of triglycerides to glycerol and fatty acids (each triglyceride molecule breaks into one glycerol and three fatty acid molecules) [7]. Other than responding to stimuli caused by energy deprivation at other tissues in the body, adipocytes also go through a basal lipolytic activity, which is mediated primarily by expression of adipose triglyceride lipase [8].

“Our findings ... provide the motivation to look for a mechanism at the cell scale that drives the proliferation of fat tissue in immobilized SCI patients or ... in sedentary subjects, surmising that mechanical loads may play a role in both the anabolism and catabolism of lipid droplets in adipocytes...”

[†]Department of Biomedical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel; Tel.: +97 236 408 093; Fax: +97 236 405 845; gefen@eng.tau.ac.il

Clinical treatment of obesity as well as strategies to reduce the blood glucose levels are aimed at decreasing the amount of excessive adipose tissue by changing the balance between the intake and expenditure of energy via physical exercise, diet control or both. These guidelines are given to patients under the assumption that if there are no hormonal disorders present, the obesity is caused by calorie consumption that is not counterbalanced by sufficient calorie burn. However, other than this conventional thinking, there could also be a second mechanism that plays a role in the development of obesity, and later on, in the development of diabetes, based on the concept of mechanotransduction (conversion of mechanical stimuli into biochemical activity) as follows: sustained static mechanical loading of adipocytes, which is characteristic of a sedentary lifestyle, stimulates the cells to produce more lipids. This mechanism is hypothesized to exist based on research that has been conducted in the laboratory of the author over the last 5 years, which has led to the following findings: first, adipose tissue in the buttocks is subjected to large mechanical deformations during sitting or lying, with tensional, compressive and shear components. This is evident from a method combining open-MRI imaging and finite element (FE) analysis, which we developed and applied for studying control subjects as well as patients post-spinal cord injury (SCI) [9,10]. Second, patients post-SCI, that is, who are immobilized in their lower body, have substantially more fat in their buttocks, and their fat tissue is more intensively loaded than that of controls, particularly with respect to tension strains [10,11].

Our findings described at the organ/tissue scale provide the motivation to look for a mechanism at the cell scale that drives the proliferation of fat tissue in immobilized SCI patients or, in general, in sedentary subjects, surmising that mechanical loads may play a role in both the anabolism and catabolism of lipid droplets in adipocytes (e.g., the role that mechanical loads play in stimulating fibroblasts to synthesize procollagen [12]). Accordingly, we have recently developed a number of research tools to determine both the internal mechanical loads that develop in mechanically stimulated cultured adipocytes and the outcome of lipid production by such mechanically stimulated cells. In order to study mechanical loads at the cellular and subcellular levels in adipocytes, we developed a

method called ‘confocal-based cell-specific FE modeling’, which, in brief, is a methodology to study cellular deformations in realistic cell geometries based on confocal scanning of the cells that is followed by computer modeling of the scanned cells [13,14]. Cell-specific FE modeling is able to analyze deformations in the plasma membrane, cytoplasm and nucleus of the cells, as well as in the cytoplasmic surroundings of the lipid droplets within adipocytes [14], while simulating typical test configurations used in cellular mechanics studies (e.g., stretching or compression of cultures). Most importantly, cell-specific FE modeling allows extrapolation from controlled mechanical loads applied on a culture in laboratory experiments to deformations that occur in the actual cells, their distributions and inhomogeneity properties [13,14]. A second research tool that we have recently introduced in the field of adipocyte biomechanics is designed for the continuous and nondestructive monitoring of lipid production in biochemically or mechanically stimulated adipocytes over periods ranging from weeks to over a month. This is made possible by means of a novel image processing-based method for the identification and measurement of lipid droplets in maturing adipocytes, which does not require staining of the cells and, thus, causes no cytotoxic effects or chemical bias of the experiments [15]. These research tools will now allow systematic and rigorous testing of the aforementioned hypothesis, which, if proven correct, will provide a completely new understanding of the mechanisms that play a role in obesity as well as in Type 2 diabetes.

Indeed, the understanding that adipocytes respond to mechanical loading and that loading could be used to regulate lipid production is consistent with what we know regarding the effect of mechanical loads on other cell types (e.g., osteoblasts, which produce mineralized bone given a sufficient mechanical stimulation [i.e., strain/stress; although mostly dynamic strain/stress in the case of osteoblasts]) [16]. There is a considerable volume of literature that demonstrates the responsiveness of other cell types to mechanical loads, including cardiomyocytes, vascular endothelial cells, smooth muscle cells and skeletal muscle cells [17]. Adipocytes can therefore also be expected to respond to mechanical loads, and using some insight from the bone remodeling theory [16], it might further be expected that some loading

patterns will inhibit lipid production in adipocytes whereas other patterns will promote lipid production, much like how the static loading of bone induces bone loss, whereas dynamic loading at certain ranges of magnitude and frequency causes bone gain. In fact, there is already some evidence that the dynamic (cyclic) stretching of adipocytes to 130% of their undeformed length at a frequency of 1 Hz inhibits their differentiation, and this has been attributed to reduced expression of PPAR- γ , which is mediated by activation of an extracellular signal-regulated protein kinase/mitogen-activated-protein kinase pathway [18]. However, surprisingly there is very little information in the literature regarding the effects of static stretching on lipid production in adipocytes. Static loading was applied in the form of compressive forces in one study, using weights [5], and some inhibition of the adipogenesis post-loading was observed; however, it is a concern that this might have been an artifact of unintentionally squashing some cells. The two published studies, the one applying cyclic loading [18] and the one applying compressive loading [5], were also limited by the methodology of the destructive testing of lipid contents in the cultures, by either chemical staining (Oil-Red-O) [5,18] or western blotting [18], which not only limited the number of time points at which cells could be studied, but also compromised the statistical power (since cultures could not be their own controls). It is important to note here that our new method for monitoring lipid production in cultured adipocytes [16] is not limited by these issues. That being said, combining the aforementioned hypothesis with the results of the one reported study in which dynamic stretching was applied [18] allows further development of the hypothesis as follows: sustained static stretching delivered to adipocytes within a certain physiological range can stimulate them to produce lipids, whereas physiological dynamic (cyclic) stretching is able to inhibit lipid production. The author of this article believes that the above hypothesis should be investigated very thoroughly, since, if proven to be correct, it could revolutionize the way that we understand obesity and diabetes. Accordingly, efforts are now being taken by the author's research group to answer the following research questions: first, does static stretching promote lipid production in cultured adipocytes, and,

if so, to what levels of stretch do adipocytes respond? Second, does dynamic stretching suppress lipid production in cultured adipocytes, and, if so, at which levels and frequencies of stretch, and to which extent? Third, how do adipocytes respond to static and dynamic stretches in terms of proliferation versus differentiation behaviors?

The significance of successfully answering the above questions lies in both the basic science aspects and the applications side. First, providing the correct answers to these questions should lead to new insights regarding the mechanisms of being overweight, obesity and diabetes, with further implications on closely related conditions such as hypertension and dyslipidemia. In particular, examining adipocyte-level processes in the context of mechanotransduction will enable us to broaden our understanding of why obesity and diabetes occur beyond the simple, or perhaps simplified, explanation of balance of calories that are consumed and burned. In other words, it is likely that studying mechanotransduction in adipocytes will provide novel descriptions and explanations regarding how a sedentary lifestyle may lead to people becoming overweight, obese and diabetic from a cellular mechanics perspective rather than from a nutritional perspective, which opens a completely new path for research of obesity, diabetes and related morbidities. In terms of applications, correctly answering the aforementioned research questions might lead to the development of new means and tools to quantitatively predict how lipid production would be affected by static and dynamic deformations around and within adipocytes. This may then provide information that can later be employed in the design of new physical exercise protocols, technologies and devices that induce vibration and dynamic stretching of tissues to minimize lipid production in adipocytes.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Bibliography

- 1 Flegal KM, Carroll MD, Ogden CL, Curtin LR: Prevalence and trends in obesity among US adults, 1999–2008. *JAMA* 303, 235–241 (2010).
- 2 Kahn BB, Flier JS: Obesity and insulin resistance. *J. Clin. Invest.* 106, 473–481 (2000).
- 3 Coppack SW: Adipose tissue changes in obesity. *Biochem. Soc. Trans.* 33, 1049–1052 (2005).
- 4 Dicker A, Le Blanc K, Åström G *et al.*: Functional studies of mesenchymal stem cells derived from adult human adipose tissue. *Exp. Cell Res.* 308, 283–290 (2005).
- 5 Hossain MG, Iwata T, Mizusawa N *et al.*: Compressive force inhibits adipogenesis through COX-2-mediated down-regulation of PPAR γ 2 and C/EBP α . *J. Biosci. Bioeng.* 109, 297–303 (2010).
- 6 Rosen ED, MacDougald OA: Adipocyte differentiation from the inside out. *Nat. Rev. Mol. Cell Biol.* 7, 885–896 (2006).
- 7 Lafontan M, Langin D: Lipolysis and lipid mobilization in human adipose tissue. *Prog. Lipid Res.* 48, 275–297 (2009).
- 8 Miyoshi H, Perfield JW 2nd, Obin MS *et al.*: Adipose triglyceride lipase regulates basal lipolysis and lipid droplet size in adipocytes. *J. Cell. Biochem.* 105, 1430–1436 (2008).
- 9 Linder-Ganz E, Shabshin N, Itzhak Y *et al.*: Assessment of mechanical conditions in sub-dermal tissues during sitting: a combined experimental-MRI and finite element approach. *J. Biomech.* 40, 1443–1454 (2007).
- 10 Linder-Ganz E, Shabshin N, Itzhak Y *et al.*: Strains and stresses in sub-dermal tissues of the buttocks are greater in paraplegics than in healthy during sitting. *J. Biomech.* 41, 567–580 (2008).
- 11 Sopher R, Nixon J, Gorecki C *et al.*: Exposure to internal muscle tissue loads under the ischial tuberosities during sitting is elevated at abnormally high or low body mass indices. *J. Biomech.* 43, 280–286 (2010).
- 12 Breen EC: Mechanical strain increases type I collagen expression in pulmonary fibroblasts *in vitro*. *J. Appl. Physiol.* 88, 203–209 (2000).
- 13 Slomka N, Gefen A: Confocal microscopy-based three-dimensional cell-specific modeling for large deformation analyses in cellular mechanics. *J. Biomech.* 43, 1806–1816 (2010).
- 14 Or-Tzadikario S, Gefen A: Confocal-based cell-specific finite element modeling extended to study variable cell shapes and intracellular structures: the example of the adipocyte. *J. Biomech.* 44(3), 567–573 (2010).
- 15 Or-Tzadikario S, Sopher R, Gefen A: Quantitative monitoring of lipid accumulation over time in cultured adipocytes as function of culture conditions: toward controlled adipose tissue engineering. *Tissue Eng. Part C Methods* 16, 1167–1181 (2010).
- 16 Be'ery-Lipperman M, Gefen A: Contribution of muscular weakness to osteoporosis: computational and animal models. *Clin. Biomech.* 20, 984–997 (2005).
- 17 Wang JH, Thampatty BP: An introductory review of cell mechanobiology. *Biomech. Model. Mechanobiol.* 5, 1–16 (2006).
- 18 Tanabe Y, Koga M, Saito M *et al.*: Inhibition of adipocyte differentiation by mechanical stretching through ERK-mediated downregulation of PPAR γ 2. *J. Cell Sci.* 117, 3605–3614 (2004).