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CONCISE COMMUNICATION

Voluntary exercise improves murine dermal connective tissue status in high-fat diet-induced obesity

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Abstract Obesity is a risk factor for several cardiovascular and metabolic diseases. Its influence on the skin is less obvious, yet certain negative effects of adipose tissue inflammation on the dermis have been suggested. Excess weight is closely associated with sedentary behavior, so any increase in physical activity is considered beneficial against obesity. To investigate the effects of obesity and physical exercise on the skin, we established a mouse model in which mice were kept either on a high-fat diet or received standard chow. After the two groups achieved a significant weight difference, physical exercise was introduced to both. Animals were given the opportunity to perform voluntary exercise for 40 min daily in a hamster wheel for a period of 8 weeks. We evaluated the status of the dermis at the beginning and at the end of the exercise period by in vivo nonlinear microscopy. Obese mice kept on high-fat diet lost weight steadily after they started to exercise. In the high-fat diet group, we could detect significantly larger adipocytes and a thicker layer of subcutaneous tissue; both changes started to normalize after exercise. Nonlinear microscopy revealed an impaired collagen structure in obese mice that improved considerably after physical activity was introduced. With the ability to detect damage on collagen structure, we set out to address the question whether this process is reversible. With the use of a novel imaging method, we were able to show the reversibility of connective tissue deterioration as a benefit of physical exercise.

Keywords Obesity · Adipose tissue inflammation · Matrix degradation · Physical exercise · In vivo nonlinear microscopy

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Introduction

Sedentary lifestyle accompanied by the consumption of food rich in saturated fat and carbohydrates is increasingly more common that leads to the “obesity epidemic”. The fat tissue is now considered a complex network of cells; a much more complicated tissue than a great amount of adipocytes that simply store the excess fat [23]. The matrix of the adipose layer contains additional types of cells such as preadipocytes, fibroblasts, endothelial, and immune cells. It is known that an excess in fat tissue leads to a systemic inflammation. The subcutaneous fat layer is infiltrated with neutrophils, regulatory T-cells, and macrophages, where a shift from M2 to M1 subset is seen [14]. Through release of inflammatory cytokines and reactive oxygen species along with proteases and matrix degrading enzymes, adipose tissue inflammation leads to a remodeling of the surrounding connective tissue, a process needed for the local expansion of fat tissue [14, 23].

A clinical sign of the systemic inflammation in obesity is that it is strongly associated with an increased rate of metabolic diseases, such as insulin resistance, type 2 diabetes, dyslipidemia, and hypertension [9]. Obesity and diabetes-related dermatological complications are now commonly seen with the increased incidence of metabolic syndrome. The most severe consequences on the skin include impaired

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wound healing and formation of ulcers [18, 25, 26]. Additionally, neuropathy, macro- and microangiopathy, and disturbed immune functions are also key elements of the pathophysiological events. It has been shown recently in mice that high-fat diet and an increased subcutaneous fat content contribute to ulcerative skin lesions that implicate the role of inflammation in subcutaneous adipose tissue (SAT) [27]. The role of subcutaneous fat has been underestimated; it became obvious that its accumulation is more than just a cosmetic issue [6, 7]. Sedentary lifestyle is a significant factor that leads to obesity. Numerous studies have shown that physical exercise (PE) can attenuate the adipose tissue inflammation that will then improve the patients' metabolic status, can lower the serum glucose level and blood pressure. Besides, regular exercise has other benefits, among other improved cognitive functions and prevention of cancer [3, 8, 16, 19]. Strong evidence exists that PE improves wound healing in different experimental setups [21, 22, 24, 28].

In our previous experiments, we demonstrated that obesity-related skin changes can be visualized by nonlinear microscopy. We have shown that weight gain is accompanied with substantial changes in the skin: thinning of the dermis, a reduction in collagen content, and adipose tissue accumulation that can be detected with various techniques [11, 12]. We hypothesized that these changes may be reversed with the introduction of PE. For this reason, we set out to visualize the improvement in the status of the dermis after PE in high-fat diet mice. We utilized in vivo laser scanning nonlinear microscopy for monitoring skin changes, a novel method with proven potential in research and clinical diagnostics [1].

Materials and methods

Experimental animals, diet, and physical exercise

6-week-old C57BL/6N female mice (Charles River, Germany) were kept under standardized conditions in a 12h light–dark cycle. They were divided into two groups. Mice in the High-fat group (HF, $n=5$) were kept on a diet that contained 30% fat (SSNIFF, Germany) and the animals in the Control group (CO, $n=4$) received ordinary chow with only 10% fat (SSNIFF).

High-fat diet was introduced when mice became 8-week-old, then voluntary exercise was initiated when the mice reached the age of 40 weeks. Mice in both groups spent 40 min daily in a hamster wheel during the light phase for 8 weeks. Weight measurements were taken weekly. After each exercise, distances covered by the mice have been recorded by a calibrated bicycle computer (Rolson Tools Ltd, UK).

In vivo nonlinear microscopy

The condition of the dermis was assessed on each mouse before the start of the PE period (Week 0) and at the end of the experiment (Week 8) by in vivo second harmonic generation (SHG) imaging. Images were taken in the same sessions under i.p. anesthesia with the use of 1.2% Avertin (Sigma Aldrich, Hungary). Before measurements, hair was removed from the dorsal areas. During evaluation, mice were placed into a restrainer to prevent movement. 6×6 mm large regions of interest (ROI) were marked on both sides on the back of the mice, 5 mm away from the spine. Images captured at Week 0 from one ROI were used as baseline; then at Week 8, images with an identical microscopic setup were taken from the opposite side as described above (Fig. 2c). A Ti-sapphire laser operating at 796 nm was utilized for SHG imaging and images were captured by an LSM 7-MP (Carl Zeiss, Germany) laser-scanning microscope. Computer-controlled positioning of the objective along the z-axis was utilized to record SHG images ("z-stack images") to achieve tissue imaging in various depths. For each mouse, five z-stack images were recorded. The quantification of collagen damages was carried out by measuring the intensity of SHG signal converted to integrated optical density (IOD) by ImageJ software (NIH, USA). The amount of unimpaired collagen in the skin correlates with the proportion of IOD. Further details can be found in references [11, 12].

Histopathological analysis

After SHG imaging of each ROI, 6 mm large skin samples were obtained by punch biopsy under general anesthesia. 4-µm sections of formalin-fixed and paraffin-embedded samples were stained with collagen-specific Weigert van Gieson's (WvG) staining. The thickness of dermis, subcutaneous adipose tissue, and the size of adipocytes were examined in 5 fields of view along with the analysis of the collagen structure.

Thickness of the dermis was assessed by measuring the distance between the epidermal–dermal junction and the dermal–subcutaneous–fat junction. SAT thickness was evaluated similarly, based on the distances between subcutaneous fat junction and panniculus carnosus. The physical dimensions of adipocytes were assessed by measuring their longest diameters [5, 11]. Damage in the collagen structure was quantified by measuring the collagen density using a color deconvolution technique [2], adapted for WvG staining. All measurements were carried out using ImageJ software.

Statistical analysis

Normal distribution was confirmed by Shapiro-Wilks test. Two-way repeated measures ANOVA and Tukey post hoc test was used to analyze the data. Graphs display arithmetic means and error bars represent standard deviations (SD). Statistical analysis was performed by Prism software (GraphPad, USA).

Results

Weight change and physical activity

The weight of obese mice was significantly higher than controls at the beginning of the exercise period. A significant weight loss during the PE from week 5 on in the HF group has been noted. This significant weight difference between the two groups disappeared by Week 8, despite the significantly lower activity of obese mice compared to controls. The shorter distance covered by the obese mice was due to their lower speed, since we did not observe any obvious rest during the 40 min exercise. In addition, there was no increase in the activity of the HF group over the 8-week experiment. On the contrary, the activity of control mice increased gradually (Fig. 1).

Changes in the status of the dermal connective tissue

Before PE, a significant difference in SHG intensities was seen between the two groups. SHG images revealed a decreased collagen density in the HF group. After PE, this difference disappeared and the SHG intensity increased significantly in HF mice compared to baseline. The structure of the dermis and SHG intensities did not change in CO mice (Fig. 2).

Histopathology

We found a significantly lower collagen fiber density in the HF group compared to CO, which showed a remarkable improvement after the PE period. In line with that finding, the dermis became significantly thicker in the HF group. Furthermore, SAT became thinner and adipocytes shrunk significantly in the HF group as a result of exercise. In the HF group, all parameters examined changed to be similar to the control group after the 8-week exercise (Fig. 3).

Discussion

An alarming rate of increase in the incidence of obesity is witnessed worldwide. It has become a major health concern as it leads to cardiovascular and metabolic diseases. These together pose a vast economic burden on healthcare systems that could be prevented with lifestyle changes. Complications and effects of obesity that manifest on the skin have received little attention so far even though skin is a great model to study consequences of altered tissue microenvironment, such as extracellular matrix and connective tissue damages. Lately, the central role of adipose tissue inflammation, due to the increased amount of fat, has been proposed as a key element of connective tissue destruction. Neutrophil granulocytes

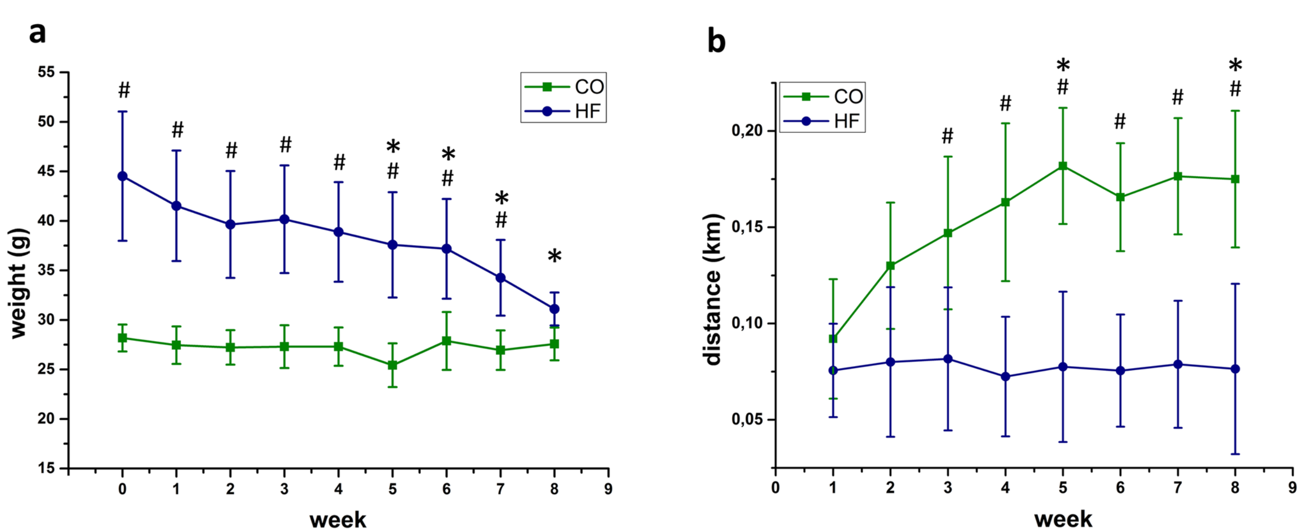


Fig. 1 **a** Change of body weights during 8 weeks of exercise in CO ($n=4$) and HF ($n=5$) groups. $^{\#}P<0.05$ compared to controls; $^{*}P<0.05$ compared to week 1 in the same group. **b** Average distance covered by CO ($n=4$) and HF ($n=5$) groups each week. $^{\#}P<0.05$ compared to controls; $^{*}P<0.05$ compared to week 1 in the same group

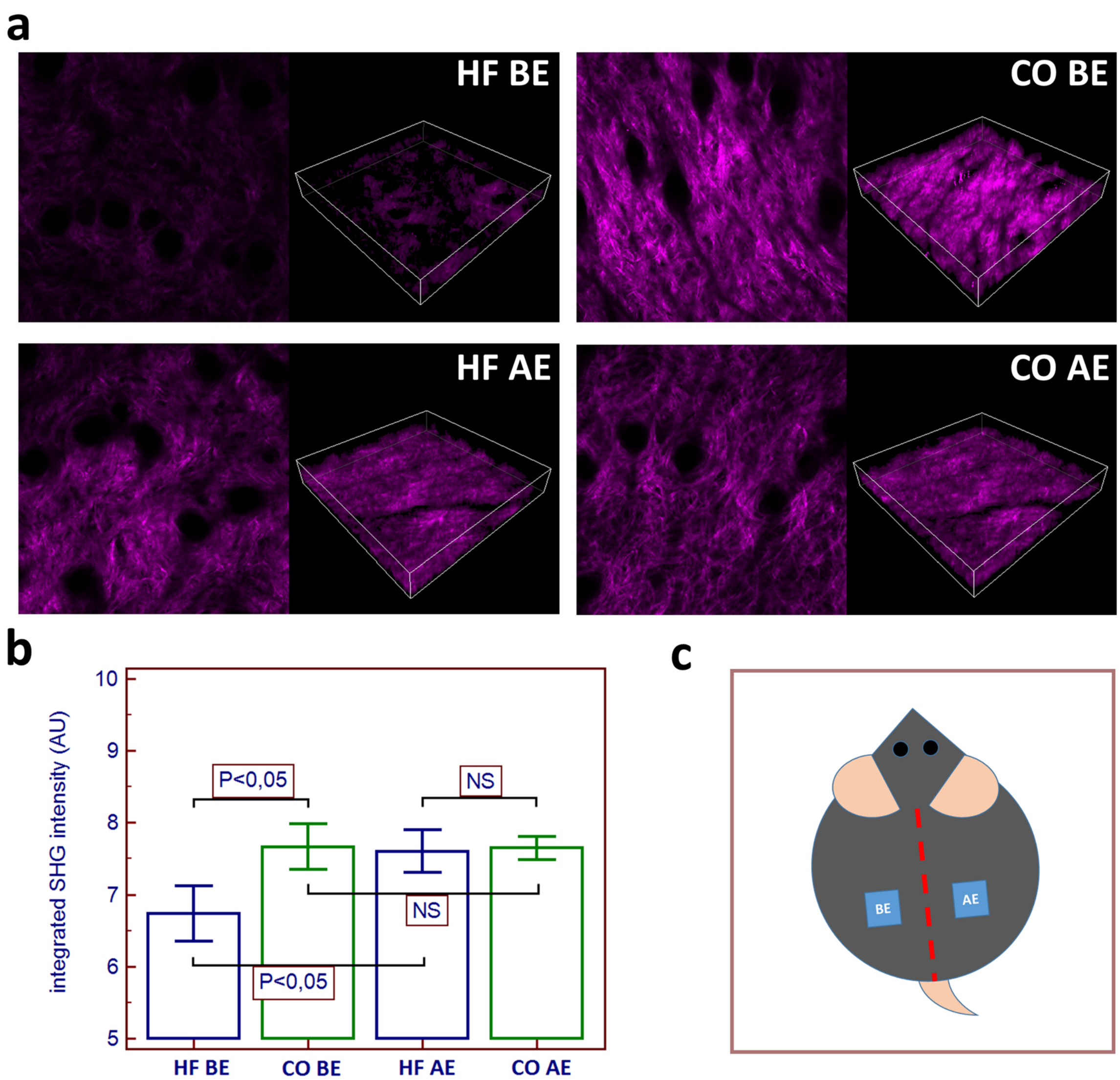
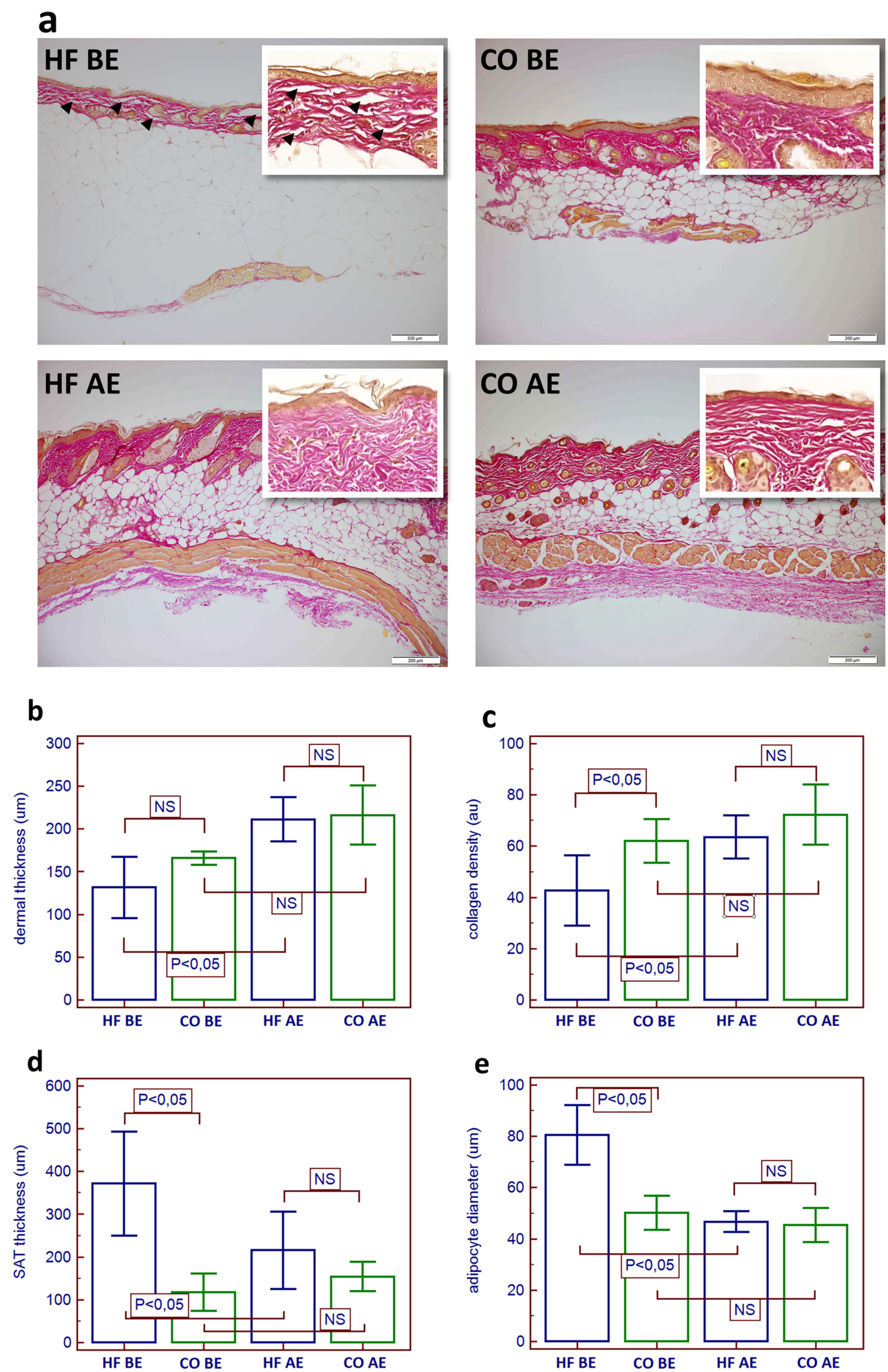


Fig. 2 **a** The *first column* displays SHG images of one representative animal of the HF group before (BE) and after exercise (AE). The *second column* shows images of a CO mouse. In each column, the *left panel* is a horizontal section of the Z stack, while *panels* on the *right* represent the Z stacks converted to 3D images. **b** Average SHG intensity expressed in arbitrary units based on integrated optical densities. Statistical comparison was made between HF ($n=5$) and CO ($n=4$) groups before (BE) and after exercise (AE). **c** Schematic mouse illustration displaying ROI's before (week 0, BE) and after exercise (week 8, AE)

are crucial components in AT inflammation through the production of neutrophil elastase that stimulates M1 macrophage accumulation in visceral adipose tissue. Kawanishi et al. demonstrated that 60 min of treadmill exercise daily for 16 weeks reduced the elevated counts of neutrophil granulocytes and macrophages in HF mice. Similarly, increased levels of cytokines MCP-1, TNF-a, IL-6, and IL-8 became significantly lower after PE [14]. This was demonstrated in the visceral adipose tissue, where inflammation is strongly related to metabolic consequences such as insulin resistance, dyslipidemia, and hypertension [10, 13, 17, 20]. Indeed, beside metabolic issues, the AT inflammation and connective tissue alterations in dermatologic complications are highly significant. Recently, Zhang et al. reported the occurrence of ulcerative skin lesions in obese C57BL/6 mice on HF diet. They

Fig. 3 **a** *First column* of WvG-stained sections displays images of a representative HF mouse before (BE) and after exercise (AE). The *second column* shows the images of a CO mouse. On WvG-stained sections, damage in the collagen structure was apparent in HF mice indicated by *black arrowheads*. On the *right upper corner* of each picture, a magnified image of the dermis is shown to demonstrate collagen structure. **b–e** *Bar graphs* display dermal thickness (**b**), collagen density (**c**), SAT thickness, (**d**) and adipocyte diameter (**e**). Statistical comparison was made between HF ($n=5$) and CO ($n=4$) groups before (BE) and after exercise (AE) ▶


a
HF BE
CO BE
HF AE
CO AE
b
dermal thickness (um)
300
250
200
150
100
50
0
NS
NS
NS
P<0,05
HF BE
CO BE
HF AE
CO AE
c
collagen density (au)
100
80
60
40
20
0
P<0,05
NS
NS
P<0,05
HF BE
CO BE
HF AE
CO AE
d
SAT thickness (um)
600
500
400
300
200
100
0
P<0,05
NS
P<0,05
NS
HF BE
CO BE
HF AE
CO AE
e
adipocyte diameter (um)
100
80
60
40
20
0
P<0,05
NS
P<0,05
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HF BE
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HF AE
CO AE

have shown that increased consumption of fat stimulates CD11c+ macrophages to migrate into the skin and produce IL-1β and IL-18 pro-inflammatory cytokines [27]. In our experiments, although we used the same mouse strain, we did not observe formation of any ulcers. The apparent lack of these lesions might indicate the role of PE to reduce skin inflammation. Previous experiments proved that 18-month-old BALB/cByJ mice who spent 30 min daily on a motorized treadmill for nine consecutive days showed accelerated wound healing [15]. Also, the same exercise resulted in faster wound healing in 22-week-old HF diet-fed obese mice [21]. Therefore, the anti-inflammatory effect of physical activity may prevent early changes that may lead to formation of ulcers and improve wound healing capabilities. Taken together, results from different experiments indicate that less than one hour daily PE can significantly ameliorate AT inflammation, which is in line with our findings. In fact, PE has remarkable effects not only on metabolism and wound healing, but skin aging as well. 22-month-old C57/BL6 mice after 1 h of daily treadmill exercise for 33 consecutive days not only showed decreased levels of inflammatory cytokines MCP-1 and IL-6, but also had features of young skin: higher dermal collagen content and thicker stratum spinosum. Production of IL-15, an AMP-activated protein kinase (AMPK)-dependent factor, by skeletal muscle after PE is hypothesized to be responsible for the slower rate of degeneration of dermal fibroblasts which ameliorate skin aging [4].

Thus, one can see that there is a growing body of evidence that high-fat intake and adipose tissue inflammation are important factors in the initiation of skin diseases and disturbances in wound healing. Additionally, beneficial effects of PE to ameliorate AT inflammation and aging and to improve skin metabolism have also been shown. In line with the literature, we saw similar results that confirmed the damage of collagen structure in obese mice. We demonstrated a decrease in collagen fiber density in obese mice by quantitative histological analysis and in vivo SHG imaging. By introducing PE, we were able to show a significant improvement in the quality of connective tissue along with the decrease in the amount of fat tissue and size of adipocytes. The novelty in this study was that favorable effects of daily physical exercise on collagen fibers of the dermis could be demonstrated in vivo by SHG imaging.

We think that our findings further support results from previous studies to underline the importance of proper nutritional habits and exercise in connective tissue homeostasis. In conclusion, it appears that skin bears with great potential to return to its original state once positive changes take place. It also shows that from the skin's point of view it is never too late to start to exercise.

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Compliance with ethical standards

All procedures were approved by the institutional Ethical Committee of Semmelweis University (PEI/001/800-6/2015).

Conflict of interest The authors declare no competing interests.

References

1. Alex A, Weingast J, Weinigel M, Kellner-Hofer M, Nemecek R, Binder M, Pehamberger H, Konig K, Drexler W (2013) Three-dimensional multiphoton/optical coherence tomography for diagnostic applications in dermatology. J Biophoton 6:352–362. doi:10.1002/jbio.201200085
2. Brianezi G, Grandi F, Bagatin E, Enokihara MM, Miot HA (2015) Dermal type I collagen assessment by digital image analysis. An Bras Dermatol 90:723–727. doi:10.1590/abd1806-4841.20153331
3. Conney AH, Lou YR, Nghiem P, Bernard JJ, Wagner GC, Lu YP (2013) Inhibition of UVB-induced nonmelanoma skin cancer: a path from tea to caffeine to exercise to decreased tissue fat. Top Curr Chem 329:61–72. doi:10.1007/128_2012_336
4. Crane JD, MacNeil LG, Lally JS, Ford RJ, Bujak AL, Brar IK, Kemp BE, Raha S, Steinberg GR, Tarnopolsky MA (2015) Exercise-stimulated interleukin-15 is controlled by AMPK and regulates skin metabolism and aging. Aging cell 14:625–634. doi:10.1111/acel.12341
5. Distler JH, Jungel A, Huber LC, Schulze-Horsel U, Zwerina J, Gay RE, Michel BA, Hauser T, Schett G, Gay S, Distler O (2007) Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. Arthritis Rheum 56:311–322. doi:10.1002/art.22314
6. Ezure T, Amano S (2010) Increased subcutaneous adipose tissue impairs dermal function in diet-induced obese mice. Exp Dermatol 19:878–882. doi:10.1111/j.1600-0625.2009.00970.x
7. Ezure T, Amano S (2015) Increment of subcutaneous adipose tissue is associated with decrease of elastic fibres in the dermal layer. Exp Dermatol 24:924–929. doi:10.1111/exd.12816
8. Firth J, Stubbs B, Rosenbaum S, Vancampfort D, Malchow B, Schuch F, Elliott R, Nuechterlein KH, Yung AR (2016) Aerobic exercise improves cognitive functioning in people with schizophrenia: a systematic review and meta-analysis. Schizophr Bull. doi:10.1093/schbul/sbw115
9. Gkogkolou P, Bohm M (2012) Advanced glycation end products: key players in skin aging? Dermatoendocrinol 4:259–270. doi:10.4161/derm.22028
10. Grundy SM (2016) Metabolic syndrome update. Trends Cardiovasc Med 26:364–373. doi:10.1016/j.tcm.2015.10.004
11. Haluszka D, Lorincz K, Molnar G, Tamas G, Kolonics A, Szipocs R, Karpati S, Wikonkal NM (2015) In vivo second-harmonic generation and ex vivo coherent anti-stokes raman scattering microscopy to study the effect of obesity to fibroblast cell function using an Yb-fiber laser-based CARS extension unit. Microsc Res Tech 78:823–830. doi:10.1002/jemt.22545
12. Haluszka D, Lőrincz K, Kiss N, Szipőcs R, Kuroli E, Gyöngyösi N, Wikonkál NM (2016) Diet-induced obesity skin changes

monitored by in vivo SHG and ex vivo CARS microscopy. Biomed Opt Express 7:4480–4489. doi:10.1364/BOE.7.004480
13. Head GA (2015) Cardiovascular and metabolic consequences of obesity. Front Physiol. doi:10.3389/fphys.2015.00032
14. Kawanishi N, Niihara H, Mizokami T, Yada K, Suzuki K (2015) Exercise training attenuates neutrophil infiltration and elastase expression in adipose tissue of high-fat-diet-induced obese mice. Physiol Rep. doi:10.14814/phy2.12534
15. Keylock KT, Vieira VJ, Wallig MA, DiPietro LA, Schrementi M, Woods JA (2008) Exercise accelerates cutaneous wound healing and decreases wound inflammation in aged mice. Am J Physiol Regul Integr Comp Physiol 294:R179–R184. doi:10.1152/ajpregu.00177.2007
16. Kim TW, Choi HH, Chung YR (2016) Treadmill exercise alleviates impairment of cognitive function by enhancing hippocampal neuroplasticity in the high-fat diet-induced obese mice. J Exerc Rehabil 12:156–162. doi:10.12965/jer.1632644.322
17. Lopes HF, Correa-Giannella ML, Consolim-Colombo FM, Egan BM (2016) Visceral adiposity syndrome. Diabetol Metab Syndr 8:40. doi:10.1186/s13098-016-0156-2
18. Mirmirani P, Carpenter DM (2014) Skin disorders associated with obesity in children and adolescents: a population-based study. Pediatr Dermatol 31:183–190. doi:10.1111/pde.12271
19. Murphy EA, Enos RT, Velazquez KT (2015) Influence of exercise on inflammation in cancer: direct effect or innocent bystander? Exerc Sport Sci Rev 43:134–142. doi:10.1249/JES.0000000000000054
20. Mykkanen OT, Huotari A, Herzig KH, Dunlop TW, Mykkanen H, Kirjavainen PV (2014) Wild blueberries (*Vaccinium myrtillus*) alleviate inflammation and hypertension associated with developing obesity in mice fed with a high-fat diet. PLoS One 9:e114790. doi:10.1371/journal.pone.0114790
21. Pence BD, DiPietro LA, Woods JA (2012) Exercise speeds cutaneous wound healing in high-fat diet-induced obese mice. Med Sci Sports Exerc 44:1846–1854. doi:10.1249/MSS.0b013e31825a5971
22. Pence BD, Woods JA (2014) Exercise, obesity, and cutaneous wound healing: evidence from rodent and human studies. Adv Wound Care (New Rochelle) 3:71–79. doi:10.1089/wound.2012.0377
23. Rosen ED, Spiegelman BM (2014) What we talk about when we talk about fat. Cell 156:20–44. doi:10.1016/j.cell.2013.12.012
24. Seitz O, Schurmann C, Hermes N, Muller E, Pfeilschifter J, Frank S, Goren I (2010) Wound healing in mice with high-fat diet- or ob gene-induced diabetes-obesity syndromes: a comparative study. Exp Diabetes Res 2010:476969. doi:10.1155/2010/476969
25. Shipman AR, Millington GW (2011) Obesity and the skin. Br J Dermatol 165:743–750. doi:10.1111/j.1365-2133.2011.10393.x
26. Yosipovitch G, DeVore A, Dawn A (2007) Obesity and the skin: skin physiology and skin manifestations of obesity. J Am Acad Dermatol 56:901–916. doi:10.1016/j.jaad.2006.12.004 **(quiz 917–920)**
27. Zhang Y, Li Q, Rao E, Sun Y, Grossmann ME, Morris RJ, Cleary MP, Li B (2015) Epidermal fatty acid binding protein promotes skin inflammation induced by high-fat diet. Immunity 42:953–964. doi:10.1016/j.immuni.2015.04.016
28. Zogaib FG, Monte-Alto-Costa A (2011) Moderate intensity physical training accelerates healing of full-thickness wounds in mice. Braz J Med Biol Res 44:1025–1035. doi:10.1590/s0100-879x2011007500115