Characterization of Obesity in Murine Skin *in vivo* by CARS and SHG Microscopy Using a Cost Efficient, Fiber Laser Based Wavelenght Extension Unit

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Introduction

Obesity is defined as an excessive body fat accumulation leading to various metabolic diseases, such as hypertension, cardiovascular disorders and type 2 diabetes [1]. Beside these health problems almost half of the patients suffer from dermatological complications in the case of diabetes mellitus, such as delayed wound healing, foot ulcers and premature aging of the skin.

In obesity, subcutaneous adipose layer increases (hyperplasia) and adipocytes become enlarged (hypertrophy). They secrete free fatty acids (FFA) such as palmitic acid and inflammatory cytokines, which inhibit the effect of insulin leading to insulin resistance. It has been reported that the enlarged adipocytes decrease fibroblast proliferation and col(I)-alpha1 and elastin gene expression, and increase MMP13 gene expression. These results suggest that the increase of subcutaneous adipose layer is negatively control the function of dermal fibroblast cells. These processes might take part in the development of chronic diabetic skin complications.

There is need to find a fast and non-invasive diagnostic method being capable to detect these diseases at an early stage. Nonlinear microscopy is one of the possible candidates: it offers the possibility of investigating the morphology and monitoring the physiological process in the skin using different nonlinear optical effects. For instance, second harmonic generation (SHG) and coherent anti-stokes Raman scattering (CARS) has been respectively used for imaging the collagen network of the skin or detection of C-H-bond rich compounds like lipid droplets. Nonlinear microscopy uses femtosecond lasers operating in the red and the near infrared spectral range (650-1300 nm), which allows deep imaging (down to ~0.5 mm) and submicron spatial resolution.

The purpose of our present work is to identify the main features and structural changes of the diabetic skin using in vivo SHG and ex vivo CARS imaging in ob/ob knockout and wild type mice.

Methods

Animals and diet:

Six-week-old female B6.V-Lep ob/J mice were randomly divided in two groups and fed different diet during 30 weeks. One of the two groups received normal diet (ob/ob – ND) (n=3) with free acces to food and water , and the other group was fed calorie restricted diet (ob/ob – CRD) (n=3), thus they were given their chow (2g – one pellet) each day at the same time between 12 and 1 PM, five days a week, with double feeding on the sixth day and none on the seventh, as previously described. As a healthy group we used C57BL/6 mice kept on standard diet (control).

Imaging setup [2]:

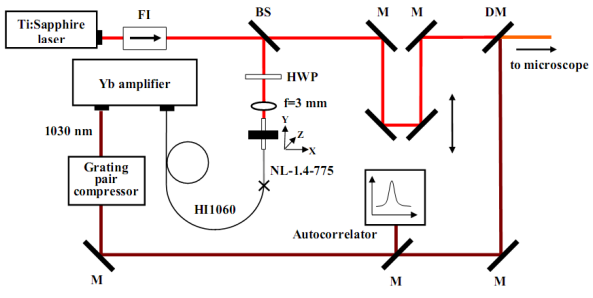


Fig. 1 Setup of the CARS wavelength extension unit. FI: Faraday isolator, BS: Beam splitter, M: Mirror, HWP: Half-Wave plate, DM: Dichroic mirror

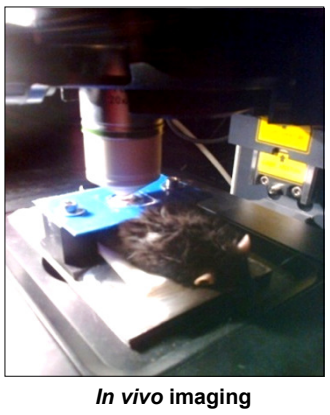
In vivo SHG imaging:

In vivo second harmonic generation (SHG) imaging was applied four times throughout the experimental period. Mice were anesthetized with 1,2% Avertin solution (0,23 ml/10 g) (Sigma Aldrich) intraperitoneally, then the dorsal surface was shaved. They were placed in a „fixing box" in order to minimalize the moving of examined skin area, then covered with a cover glass, put under the objective and examined as it described in „Imaging tools". For each mouse five „z-stack images" were analyzed.

Ex vivo CARS imaging:

Ex vivo second harmonic generation combined with coherent anti-Stokes Raman spectroscopy (SHG-CARS) techniques were used once at the end of experiment. Mice were euthanized and the dorsal surface was shaved, then a diameter of 4 mm full-thickness punch biopsies was removed and examined similarly to the abovementioned SHG imaging, however the images were acquired from hypodermis to the epidermis direction in order to obtain higher quality imaging.

Fixing of the examined skin area



In vivo imaging

Results

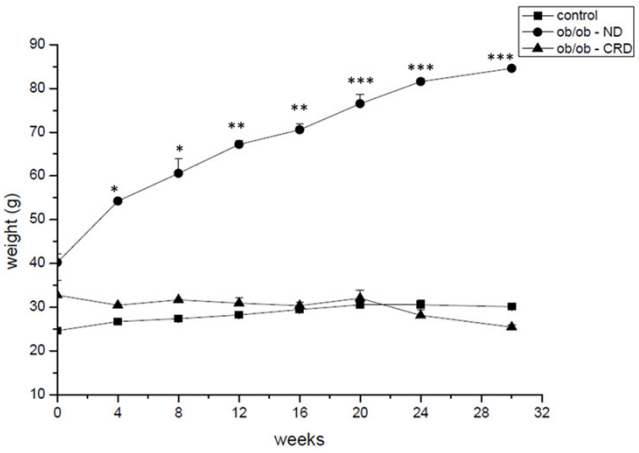


Fig.2

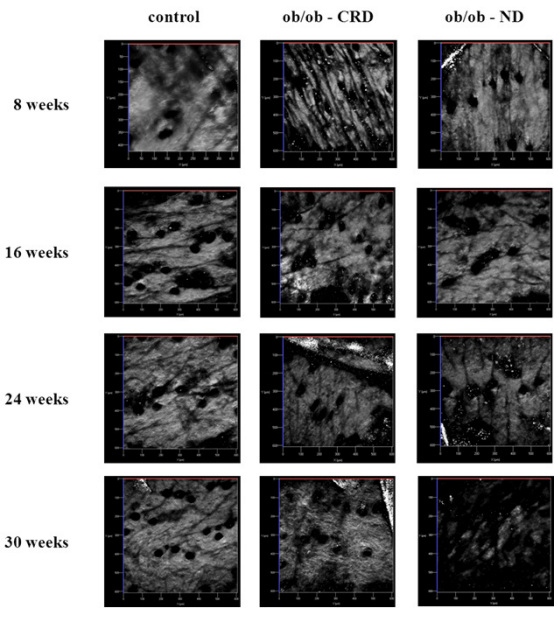


Fig.3a

Mice weight

The Ob/ob knockout group kept on standard diet (ob/ob – ND) during 30 weeks had a significantly higher body -weight compared to control and calorie restricted group (ob/ob – CRD). The weight increment in ob/ob – ND group was nearly 200%, while in other groups the body weight was relatively constant.

*Fig.2, Fig.3b Statistical significance level of differences was performed using Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001 compared to control group.*

Skin morphology changes – Collagen

During the 30 weeks, at the first three times we did not observed any singificant differences in SHG intensities between mice groups.
At the last evaluation (week 30), when the ob/ob – ND group had almost tripled body weight, the SHG intensity decreased significantly compared to control group (*Fig.3a,b*).

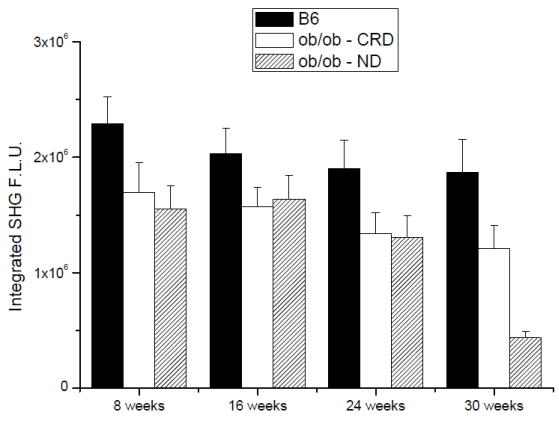


Fig.3b

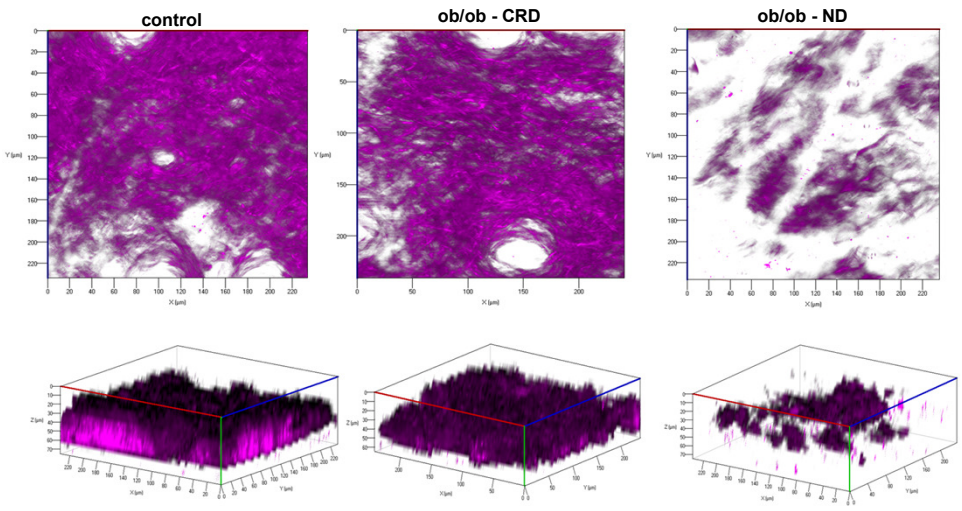
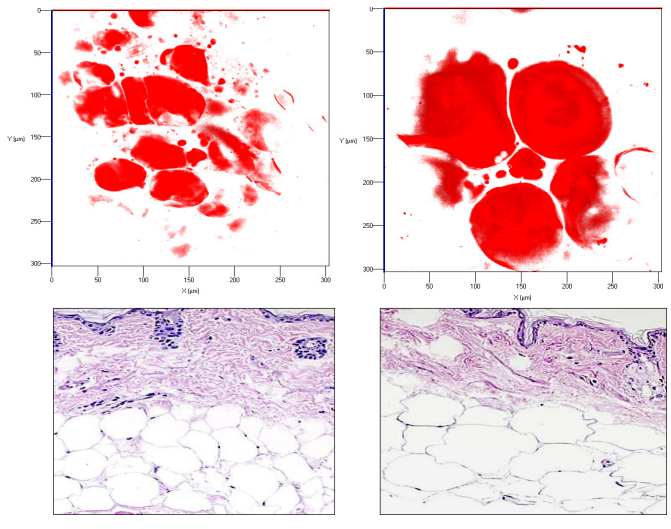


Fig.4

The ob/ob – ND group had reduced dermal collagen content and the SHG intensity decreased correlating with the degree of obesity (*Fig.4*).



Skin morphology changes – adipocytes

The subcutan adipocytes in the ob/ob – ND group measure significant larger diameter (84.07 ± 22.67 µm) than that of the control group (50.74 ± 12.47 µm).

Beside the enlarged adipocytes in the ob/ob – ND group, the dermal thickness significanty decreased, which is proved by the Van Gieson staining.

Discussion

We demonstrated that combined SHG/CARS imaging is capable of in vivo detection of various changes in diabetic skin including the collagen content and adipocyte enlargement. Our results indicated an anti-correlation between body weight and SHG intensity of dermal collagen content, as well as between the size of adipocytes and dermal thickness.

References

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2. A. Kolonics, D. Csáti, P. Antal, R. Szipőcs, "A simple, cost efficient fiber amplifier wavelength extension unit for broadly tunable, femtosecond pulse Ti-sapphire lasers for CARS microscopy," Proc. Biomedical Optics and Digital Holography and Three Dimensional Imaging (Miami, Florida, United States, April 28-May 2 2012); OSA Technical Digest Series; BSu3A.28 /1-3 (2012)