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

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Obesity is defined as an excessive body fat accumulation leading to various metabolic diseases, such as hypertension, cardiovascular disorders and type 2 diabetes [1]. Type 2 diabetes is characterized by an elevated plasma glucose content, insulin resistance and relative insulin deficiency. The most common diabetes-related skin lesions are the impaired/delayed wound healing, foot ulcers and premature aging of skin [2,3]. In obesity, subcutaneous adipose layer increases (hyperplasia) and adipocyte become enlarged (hypertrophy) [4]. They secrete free fatty acids (FFA) such as palmitic acid and inflammatory cytokines, which inhibit the effect of insulin, leading to insulin resistance. The palmitic acid, which produced by enlarged adipocytes also have similar effect. These data suggest that the increase of the subcutaneous adipose layer negatively control the function of dermal fibroblast cells [5]. These processes might take part in the development of chronic diabetic skin complications. Therefore, it is of primary importance to develop a fast and non-invasive diagnostic method, which allows early detection of these lesions. Nonlinear microscopy is one of the possible candidates, since it is capable of investigating the morphology and monitoring the physiological process in the skin by the use of femtosecond lasers operating in the near infrared spectral range (650-1300 nm). Two different nonlinear optical effects, such as second harmonic generation (SHG) and coherent anti-stokes Raman scattering (CARS) can be used for label-free imaging the collagen and lipid distribution of the skin [6,7]. The purpose of our study is to find correlations between structural alterations of collagen and morphological changes of adipocytes by means of *in vivo* SHG and *ex vivo* CARS imaging methods in ob/ob knockout and wild type mice.

The experimental setup is similar to that is described in details in Ref. 8. A broadly tunable, femtosecond pulse Ti-sapphire laser operating at 796 nm is used for SHG imaging of collagen. It is also used as a pump beam for our CARS measurements. An inherently synchronized [8], two-stage Yb-fiber amplifier unit generates the compressed Stokes pulses at 1028 nm for CARS imaging of adipocytes. A precision delay stage assures the zero delay difference between the tunable pump and fixed wavelength Stokes pulses.

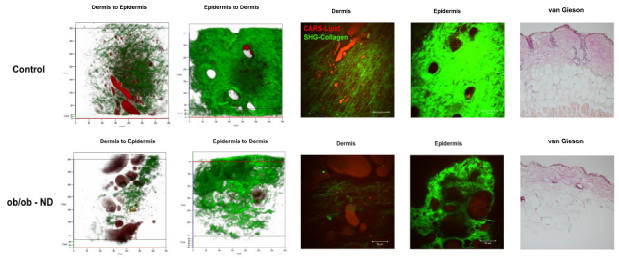

Fig. 1 Correlation between body mass, skin collagen content and the size of adipocytes

Summarizing our results we obtained by means of *in vivo* SHG and *ex vivo* CARS imaging of the skin samples of ob/ob knockout and wild type mice (see Fig. 1) we can say that *in vivo* combined SHG/CARS imaging might be an important tool for diagnosis of diabetes related skin disorders in the near future based on observed inverse relationship between the size of adipocytes and collagen content of the skin.

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