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

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KEY WORDS obesity; skin; collagen; adipocyte; in vivo nonlinear microscopy; label-free imaging methods

ABSTRACT Nonlinear microscopy techniques are being increasingly used to perform *in vivo* studies in dermatology. These methods enable us to investigate the morphology and monitor the physiological process in the skin by the use of femtosecond lasers operating in the red, near-infrared spectral range (680–1,300 nm). In this work we used two different techniques that require no labeling: second harmonic generation (SHG) for collagen detection and coherent anti-Stokes Raman scattering (CARS) to assess lipid distribution in genetically obese murine skin. Obesity is one of the most serious public health problems due to its high and increasing prevalence and the associated risk of type 2 diabetes and cardiovascular diseases. Other than these diseases, nearly half of patients with diabetes mellitus suffer from dermatological complications such as delayed wound healing, foot ulcers and several other skin changes. In our experiment we investigated and followed the effects of obesity on dermal collagen alterations and adipocyte enlargement using a technique not reported in the literature so far. Our results indicate that the *in vivo* SHG and *ex vivo* CARS imaging technique might be an important tool for diagnosis of diabetes-related skin disorders in the near future. *Microsc. Res. Tech. 78:823–830, 2015*.

INTRODUCTION

The ability to follow the changes and distribution of various biological compounds in the skin is essential for better understanding the mechanism of diseases. Nonlinear microscopy offers a noninvasive, high-resolution and deeply penetrating optical imaging technique with high sensitivity (Denk et al., 1990). The epidermis and dermis both contain numerous endogenous chromophores, such as NADH, melanin, keratin, elastin, and collagen that can be visualized by various excitation wavelengths, without the need of exogenous contrast agents (Breunig et al., 2012). The combination of different modalities such as two-photon absorption fluorescence (TPAF), second harmonic generation (SHG) or coherent anti-Stokes Raman spectroscopy (CARS) are promising techniques to obtain detailed morphological and structural information about living tissue (Breunig et al., 2010; Han et al., 2005).

Obesity is defined as an excessive body fat accumulation that leads to various metabolic diseases, such as hypertension, cardiovascular disorders and type 2 diabetes (Kopelman, 2000). The most common diabetes-related skin lesions are impaired wound healing and foot ulcers (Reiber, 2002). In obesity, the subcutaneous adipose layer increases and adipocytes grow in size. It has been previously described that an increase of subcutaneous adipose layer has a suppressive effect on certain functions of fibroblasts that results in unfavorable changes in the structure of the dermis. This feedback may play an important role in the development of chronic diabetic skin complications, such as pressure ulcers (Ezure and Amano, 2011; Ezure et al., 2009). For this reason, there is a need to find a fast and

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Received 16 January 2015; accepted in revised form 21 June 2015

REVIEW EDITOR: Alberto Diaspro

Conflict of Interest: AK and RS hold shares in R&D Ultrafast Lasers Ltd. Other authors declare no conflict of interest.

Author Contributions: DH prepared the samples for SHG and CARS images, DH, KA and RS recorded the microscope images, DH, KL evaluated the data, GM, GT and RS optimized the microscope and laser setup for CARS and SHG imaging, DH, KA, NW, SK and RS wrote the manuscript.

Contract grant sponsor: Hungarian Development Agency (NFÜ); Contract grant number: TECH-09-A2-2009-0134; Contract grant sponsor: Hungarian Brain Research Program; Contract grant number: KTIA_13_NAP-A-I/16; Contract grant sponsor: R&D Ultrafast Lasers Ltd.

DOI 10.1002/jemt.22545
Published online 24 July 2015 in Wiley Online Library (wileyonlinelibrary.com).