Studies on Cutaneous Water Distribution and Structure

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On June 9, 2000, Monika Gniadecka defended her thesis entitled 'Studies on cutaneous water distribution and structure' at the Department of Dermatology, Bispebjerg Hospital. The examiners chosen by the Copenhagen University were Professor Torkil Menne, Dr Tove Agner, both from the Department of Dermatology, Gentofte Hospital, Copenhagen and Professor Howell GM Edwards from the Department of Chemical and Forensic Sciences, University of Bradford, United Kingdom. The viva voce was led on behalf of Copenhagen University by Dr Ole Badsgaard, Gentofte Hospital.

The purpose of the thesis has been to elucidate the distribution and physico-chemical structure of water in healthy skin and selected pathologic skin conditions. High-frequency ultrasonography was applied for studying water distribution in the dermis and NIR-FT Raman spectroscopy was used to determine the instantaneous structure of water and interactions between water and proteins in the skin.

Skin water comprising approximately 70% of cutaneous mass is easily exchangeable and therefore plays an important role in the fluid homeostasis in the body. Water is essential for the well-being of the skin by interacting and influencing the other skin components (e.g. fibrous components responsible for visco-elastic properties). Interactions between macromolecules in connective tissue and water are possible due to the unique physico-chemical structure of water. Water is however, difficult to study and the knowledge about skin water is mostly derived from clinical observations on oedema and dehydration and fragmentary experiments aiming to determine water content in various layers of the skin, mostly in stratum corneum.

Celebrating after the viva. Standing from the left: Dr Tove Agner, Dr Monika Gniadecka, Dr Ole Badsgaard, Professor Howell GM Edwards, Professor Torkil Menne.

Ultrasonography has already been established for determining skin thickness and evaluating the changes in various skin diseases. Raman spectroscopy has been employed for water structure studies in chemistry for the last two decades but has only recently been introduced for studying the chemical structure of the tissues, including the skin. Having validated skin ultrasonography and NIR-FT Raman spectroscopy for determination of water compartment in normal skin, skin ageing and different forms of skin oedema have been studied.

It has been found that skin ageing is connected with changes in water distribution in the dermis. In photoageing water accumulates in the upper dermis while it seems to be depleted in the lower dermis in chronologically aged skin. Not only water distribution but also water structure changes have been found in photoaged skin. In young and chronically aged skin the majority of water molecules remains bound to other macromolecules. In contrast, in photoaged skin the surplus of water resists in the non-protein bound, tetrahedral form. This may be due to increased hydrophobicity and compactness of proteins. Thus, photoaged skin is overhydrated by means of the absolute amount of water but is seems to be functionally dehydrated (less interaction between water and proteins).

Here, for the first time diurnal changes in water distribution in the skin have been monitored *in vivo* in young and aged healthy individuals and in patients with skin oedema. A profound dermal oedema increase has been observed in patients with lipodermatosclerosis (sequel of chronic venous insufficiency). Moreover, oedema removal has been visualised by ultrasonography in the course of compression therapy in these patients. It has been documented that dermal oedema in lipodermatosclerosis is alleviated by 18–36 mmHg compression. Thus, efficacy of compression treatment in patients can be objectively measured *in vivo*. Moreover, it has been found that water distribution differs in the skin in different forms of skin oedema: lipodermatosclerosis (mostly upper dermis), lymphoedema (uniform distribution in the dermis) and cardiac insufficiency (predominantly lower dermis). This may be of pathogenic significance especially with regard to skin ulceration.

This study has documented the usefulness of high-frequency ultrasonography and NIR-FT Raman spectroscopy for assessment of the water compartment in the skin. These methods cannot only be employed for studying normal skin, skin ageing or oedema but may also be useful in other skin diseases, e.g. skin cancer or inflammatory reactions.

Howell GM Edwards is a Professor of molecular spectroscopy with life experience in vibrational spectroscopy. His group has initiated Raman spectroscopy of human skin and has been interested mostly in stratum corneum and penetration in the skin. Professor Edwards was the first examiner. He focussed on articles I, II and IV referring to nuclear magnetic resonance spectroscopy and Raman spectroscopy applied for studies of water structure. Professor Edwards was interested in background for the selection of the above-mentioned techniques. Moreover he enquired about the possibilities of the future application of Raman spectroscopy and vibrational spectroscopies in medicine. Differences between infrared spectroscopy and Raman spectroscopy were described. The technical improvements nessesary for Raman spectroscopy to be applied videly in biomedicine were discussed too.

Consultant Tove Agner has previously used skin ultrasonography for investigation of contact dermatitis and skin oedema formation. She discussed articles III, V, VI–IX and the review article. She focused on patient recruitment and data analysis. Different methods of image analysis of ultrasound images (quantification of echogenicity) were discussed. Future applications of Raman spectroscopy for skin cancer detection were drawn.

The thesis was based on nine original articles:

I. Gniadecka M, Quistorff B. Assessment of dermal water by high-frequency ultrasound: comparative studies with nuclear magnetic resonance. Br J Dermatol 1996; 135: 218–224.

II. Gniadecka M, Nielsen OF, Christensen DH, Wulf HC. Structure of water, proteins, and lipids in intact human skin, hair, and nail. J Invest Dermatol 1998; 110: 393–398.

III. Gniadecka M, Gniadecki R, Serup J, Søndergaard J. Ultrasound structure and digital image analysis of the subepidermal low echogenic band in aged human skin: diurnal changes and interindividual variability. J Invest Dermatol 1994; 102: 362–365.

IV. Gniadecka M, Serup J, Søndergaard J. Age-related diurnal changes of dermal oedema: evaluation by high-frequency ultrasound. Br J Dermatol 1994; 131: 849–855.

V. Gniadecka M, Jemec GBE. Quantitative evaluation of chronological ageing and photoageing *in vivo*: studies on skin echogenicity and thickness. Br J Dermatol 1998; 139: 815–821.

VI. Gniadecka M, Nielsen OF, Wessel S, Christensen DH, Heidenheim M, Wulf HC. Water and protein structure in photoaged and chronically aged skin. J Invest Dermatol 1998; 111: 1129–1133.

VII. Gniadecka M. Dermal oedema in lipodermatosclerosis: distribution, effects of posture and compressive therapy evaluated by high-frequency ultrasonography. Acta Derm Venereol 1995; 75: 120–124.

VIII. Gniadecka M, Karlsmark T, Bertram A. Removal of dermal edema with class I and II compression stockings in patients with lipodermatosclerosis. J Am Acad Dermatol 1998; 39: 966-970.

IX. Gniadecka M. Localization of dermal edema in lipodermatosclerosis, lymphedema, and cardiac insufficiency. J Am Acad Dermatol 1996; 35: 37–41.