Diabetic Skin Complications: A Need for Reorganizing the Categories of Diabetes-Associated Complications

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Skin diabetic pathologies are one of the most devastating complications resulting from prolonged diabetes. These pathologies are associated with high morbidity and mortality rates. However, due to an existing misconception, the research of the pathophysiology leading to the development of these complications is neglected and unfocused.

Under the current misconception, all skin diabetic complications are included under the entity of Diabetic Foot [for recent reviews, 1–6]. This category is based on two incorrect concepts. Firstly, it focuses on impaired wound healing as the only skin pathology associated with diabetes. Secondly, this category named "diabetic foot" is based on the current leading theory that relates to the abnormality in wound healing in the diabetes patient as a consequence of other complications associated with the disease. For example, impaired wound healing is believed to result from denervation and decreased sensations due to nerve damage, decreased blood supply due to blood vessel damage, and abnormal function of the immune system and frequent infections.

These two concepts are not accurate. Among the diabetes-associated skin pathologies, the most devastating and common pathology is impaired wound healing. However, there are several other skin pathologies, with which most physicians and researchers are less familiar, that are associated with diabetes and that cause patients much aggravation [reviewed in 7–12]. These include:

- **Acanthosis nigricans,** characterized by dark, thicker skin seen in body folds such as neck and axilla, a pathology currently being used as an early marker for insulin resistance screening even in children
- **Necrobiosis lipoidica diabeticorum,** characterized by irregular multiple oval-shaped plaques developing especially on the pretibial skin, usually on both legs
- **Granuloma annulare,** characterized by the appearance of flesh-colored erythematous or violaceous papules and plaques on dorsa of hands and arms or feet and legs
- **Bullosis diabeticorum,** a condition in which bullous lesions develop rapidly, commonly overnight, without preceding trauma, in diabetic patients
- **Limited joint mobility,** which consists of two major components: limited mobility, primarily painless, of the extension of the small joints of the hands, and thickening and stiffness of the overlying skin
- **Hirsutism,** associated with insulin resistance.

There are several other pathologies, all summarized in recent reviews.

Furthermore, even when following the research conducted on diabetes-impaired wound healing, the current leading theory directs the research in a narrow-minded approach. Following this misconceived path that attributes diabetic skin pathologies as secondary to the other diabetes-associated complications, research conducted in the field focuses exclusively on understanding the microvascular and neuropathic changes associated with diabetes [1–6]. Little or no attention is given to the skin pathologies per se and to the possible direct effects that impaired insulin signaling and the developing hyperglycemia might have on skin function.

In the present review I summarize the research conducted in my laboratory over the last few years, focusing on the role of insulin in the regulation of skin physiology. We are able to clearly demonstrate that insulin signaling is essential for normal skin proliferation and differentiation, as well as skin metabolism, and that lack of insulin signaling results in abnormalities in these processes and leads directly to impaired wound healing. In the light of these results we suggest that the misconceived category titled Diabetic Foot will no longer be used and that skin pathologies associated with diabetes will be grouped under a more appropriate and accurate title – namely, Diabetic Skin Complications, or perhaps Diabetic Dermopathy.

Skin structure and function

For better understanding of skin lesions associated with diabetes, one should be familiar with the histologic structure of the skin as well as with the processes essential for its function [13–15]. The skin is the largest single organ of the body, accounting for 16% of total body weight. It is composed of the epidermis and the underlying dermis and subcutis [Figure 1]. The epidermis consists essentially of stratified squamous keratinized epithelium (or, in simple terms, a multi-layered epithelium that produces and secretes keratin), and is organized into distinct cell layers [Figure 1] that differ in their morphologic and biochemical characteristics [2,3]. This differentiation process is dependent on a calcium gradient that exists throughout the epidermis and the entire process is controlled by sophisticated and complex regulation. The differentiation process can also be induced in cultured epidermal keratinocytes by increasing the calcium concentration, mimicking the differentiation process *in vivo* [Figure 1]

The main role of the epidermis is to act as a barrier between the body and the hostile surrounding environment. The other component of skin, the dermis, is composed of connective tissue that supports the epidermis and binds it to the underlying subcutis.

It provides the elasticity of the skin and participates in the wound-healing process, as well as in the defense function of the skin.

Intact skin offers protection against the penetration of biological or microbial pathogens and against loss of metabolites, as well as physical protection and visco-elasticity to withstand mechanical constraints imposed upon the skin by environmental factors. In order to fulfill its function, the skin must remain intact at all times and keep its mechanical properties and elasticity. Several factors help maintain these functions: rapid renewal of the entire epidermis every 15–30 days; the existence of desmosomes and hemidesmosomes between the cells that maintain the integrity of the layers; secretion of substances from the cells that help maintain the barrier quality of the skin; and secretion of factors from both the epidermis and the dermis that influence the properties of the skin cells, such as cellular proliferation and migration, as well as differentiation, cellular senescence and apoptosis. Any process that affects these properties might result in the compromise of the skin barrier that could lead to illness and even death.

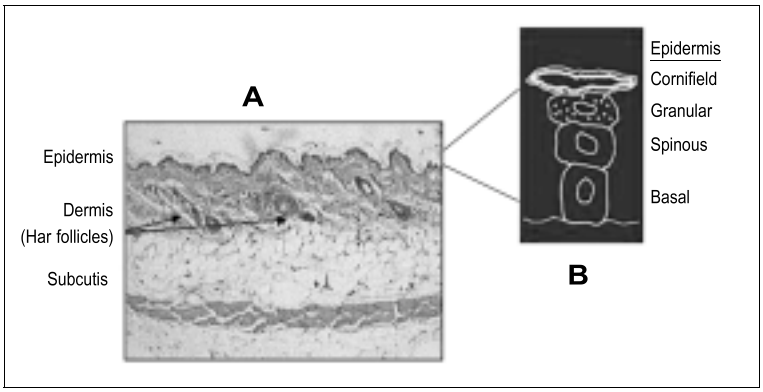

Figure 1. [A] Skin section stained with hematoxylin & eosin. **[B]** Diagram depicting epidermal histology.

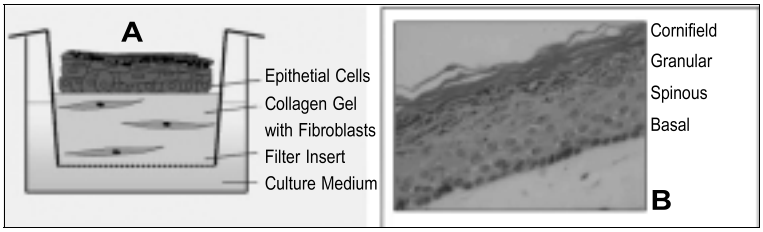

Figure 2. [A] Diagram depicting the co-culture set-up. **[B]** Section of a co-culture stained with hematoxylin & eosin.

Insulin and skin

The first scientific indication that insulin may be important for skin and skin cells derives from the fact that insulin is an essential component in cultured human keratinocyte growth culture [16–18], even though investigators thus far have no explanation as to why insulin and not insulin growth factor-1 is added.

In the light of clinical and scientific facts linking insulin and skin, my laboratory began to investigate the possible effects or roles that insulin may have on skin. Initially we found that the insulin receptor and all the proteins of the insulin-mediating signaling pathway are expressed in skin cells and are activated in response to insulin signaling [19]. Interestingly, we found that activation of the IR, in response to insulin, changed during induction of skin epidermal differentiation with no change in its expression. It was found that the highest levels of insulin activity was in proliferating or differentiating cells, whereas there was minimal activation of the IR in fully differentiated keratinocytes [19]. This was a possible indicator that insulin might be involved in epidermal differentiation. To test this hypothesis we induced differentiation of cultured primary epidermal cells by increasing the calcium concentrations as described above, but in the presence of insulin. It was found that insulin enhanced or accelerated the differentiation process, further supporting the initial findings. However, the most direct evidence supporting the importance of insulin in the differentiation process came from studies of skin cells lacking the expression of the insulin receptor. We found that if the IR is not expressed, the differentiation process cannot progress normally [20]. Certain cytoskeletal proteins that serve as differentiation markers due to the induction in their expression during differentiation were not induced in the IR null skin cells. In addition, other effects of insulin on skin keratinocytes in the IR null cells were abolished as well [21]

Another important finding was that insulin growth factor-1, in contrast to insulin, not only did not induce or support the differentiation process but actually inhibited it, demonstrating that insulin has a unique role in skin that is different from the role of insulin growth factor-1 in this tissue [19].

Evidence is accumulating in our laboratory demonstrating the involvement of insulin in the regulation of other cellular processes, including proliferation, apoptosis, metabolism and more (E.W., unpublished data). Furthermore, from our results it seems that unique signaling pathways mediate each of the insulin effects in skin [22]. Nonetheless, we were looking for further support for the role of insulin in skin, and therefore developed two additional model systems. One is the organotypic skin co-culture model and the other is the skin-specific insulin receptor knockout (SIRKO) mouse. I will describe these models in short, and will mention the preliminary results we have obtained thus far using these models.

The organotypic skin co-culture model is based on creating skin *in vitro*. The model is composed of the dermal equivalent made of fibroblasts embedded in a collagen gel, and the epidermal equivalent is composed of skin keratinocytes [23]. Under certain culture conditions, the cells are organized into a structure histologically similar to skin *in vivo* [Figure 2]. The main advantage of this model is that it enables the researcher to manipulate the growth conditions as well as the phenotype of the various layers of the skin. For example, one can add insulin or glucose and follow the effects on skin organization and skin function, or one can establish each of the skin equivalents from cells genetically manipulated to over- or under-express various proteins. From the initial results we

IR = insulin receptor

have obtained thus far it is clear that adding insulin to these cultures at early stages of skin organization leads to better or enhanced differentiation, further supporting our results.

Another model we are currently studying is the SIRKO mouse. In this mouse model, the insulin receptor is inactivated, using the Cre-Lox system [24,25], only in the epidermal compartment of the skin, while all other body tissues express the insulin receptor normally. The main advantage of this model is that lack of epidermal IR does not compromise the mouse. This is in contrast to the total inactivation of the IR in the IR null mouse which is incompatible with life. The SIRKO mouse enables us to investigate the involvement of the IR in the wound-healing process and it seems thus far that lack of IR expression affects the wound-healing process.

Conclusions

Insulin is a multifunctional hormone involved in regulating a wide range of cellular processes. While the classical insulin target tissues were considered in the past to be muscle, fat and liver, it is clear today that many other tissues require insulin for maintaining their normal physiology. One of these tissues is skin, and lack of insulin leads to devastating pathologies in skin and in the wound-healing process. Changing the official approach toward skin pathologies resulting from impaired insulin signaling and diabetes may hopefully direct research into new innovative approaches that could lead to development of new treatments and even preventive treatments against these complications. We suggest that the first step toward that direction should be changing the title of Diabetic Foot to Diabetic Skin Complications or Diabetic Dermopathy.

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The notes I handle no better than many pianists. But the pauses between the notes – ah, that is where the art resides

Artur Schnabel (1882-51), German-born U.S. pianist who made his concert debut at the age of eight; he became an international celebrity, specializing in Beethoven, Mozart and Schubert. Fleeing the rise of Nazism in Germany, he moved to Switzerland, finally settling in the USA in 1939.