INTRODUCTION

Diabetes is known to change the properties of soft tissues. Two methods have been used to model the effects of diabetes on soft tissues. The first model uses non-enzymatic glycation (NEG) of tissues in in vitro preparations. It is unclear how closely NEG mimics the changes in tissue properties caused by diabetes. The second model is chemical diabetes, utilizing animals made diabetic by treatment with streptozotocin or alloxan. These chemical agents adversely affect the health of the animals, stopping their normal growth and causing general emaciation. Thus the changes in tissue properties observed in these animals are potentially confounded by the actions of the chemical agents. Furthermore, many of the tests conducted on these tissues have been static tests yielding stiffness or failure properties. Since soft tissues are viscoelastic, static tests alone do not completely characterize the tissue properties.

Duquette et al. [1] addressed these problems by using a spontaneously diabetic, hyperglycemic rat model designated as BBZDP/Wor. These animals are spontaneously diabetic and maintain blood glucose levels in the range of 25 mmol/l. Sinusoidal stress inputs having frequencies between 0.1 and 2.0 Hz were applied to knee collateral ligaments. The complex compliance was calculated using a quasilinear method similar to that used by Stalnaker and Fleischman [2]. This method requires that each specimen be subjected to a sequence of harmonic stress inputs having the same amplitude and mean, but with each input having a different frequency. Results showed that the storage compliance in diabetic and control animals was similar, but that diabetes increased the loss compliance.

The goal of the present preliminary study was to compare the properties of normal and diabetic rat skin using a new experimental method whereby the frequency dependence of the complex compliance can be determined from a single test of short duration.

MATERIALS AND METHODS

Three skin specimens were obtained from the belly of a single BBZDP/Wor rat and similarly from a non-diabetic littermate. The specimens were 15 mm long, 1.2 mm wide and approximately 0.4 mm thick. Only collagenous tissue was considered to be load bearing. The load bearing area was determined to be load bearing. The load bearing area was determined at the conclusion of the experiment by fixing the specimen under slight tension in formaldehyde, mounting and slicing the specimen, and then viewing the cross section at several locations under a polarizing microscope.

Each specimen was mounted in an apparatus and preconditioned. Then a uniaxial pseudorandom Gaussian (PGN) stress stimulus with a mean of 15 kPa, a range of 5-25 kPa and a bandwidth of 0-5 Hz was applied for a period of 18 minutes. Preliminary creep tests showed that the longest time constant was 5-6 minutes. Data were sampled at 25 Hz and only the data collected in the last 2 minutes of test period were averaged to characterize the material. For each specimen, the strain response to the PGN stress stimulus was measured and the relationship expressed as a Volterra-Wiener series (Equation 1).

\[
\varepsilon(n) = h_0 + \sum_{j=0}^{R-1} h_1(j)\sigma(n-j) + \sum_{j_1=0}^{R-1} \sum_{j_2=0}^{R-1} h_2(j_1, j_2)\sigma(n-j_1)\sigma(n-j_2)
\]

where \(\varepsilon(n)\) is the strain at time increment \(n\), \(\sigma\) is the stress, \(h_i\) are the system kernels and \(R\) is the memory length. The first order kernels \(h_0(j)\) represent the linear viscoelastic response, while the second order kernels \(h_2(j_1, j_2)\) represent the nonlinear viscoelastic response. After the data had been adjusted to have zero mean, the kernels for each tissue specimen were determined from Equation 1 using the methods of Korenberg et al. [3]. The kernels for each type of tissue were then averaged \((n=3)\). Then using the average values for the kernels, Equation 1 was used to predict the response for each type of skin to several sinusoidal stress inputs. Finally, the complex compliance for each sinusoidal stress input was computed using the method described in Duquette et al. [1].
RESULTS

Equation 1 was used to predict the components of the complex compliance for sinusoidal stress inputs with a mean of 15 kPa and an amplitude of 12 kPa at frequencies between 0.5 and 2.5 Hz (Figure 1). The storage compliance of diabetic and non-diabetic skin was similar and decreased with increasing frequency. The loss compliance of both types of skin increased with increasing frequency, with diabetic skin exhibiting a greater loss compliance. The behavior of the phase angle as a function of frequency was similar to that of the loss compliance.

DISCUSSION

The results show that the differences in the viscoelastic behavior of diabetic and non-diabetic skin are due to differences in the viscous component of the response (loss compliance) rather than differences in the elastic component (storage compliance). Duquette et al [1] found similar results in a study of collateral knee ligaments from the same types of rats.

The first order kernels for both types of tissue were nearly identical, indicating that linear viscoelastic models of both tissues would predict almost identical values for the complex compliance. The differences in tissue behavior illustrated in Figure 1 are due to differences in the second order (i.e. nonlinear) kernel terms. Therefore, it is important to model skin as a nonlinear rather than a linear viscoelastic material.

There are several limitations to the present study. First, the tissue samples were obtained from a single animal of each type. Additional tests need to be conducted to determine if the difference in loss compliance is statistically significant. Second, computational limitations constrained the memory length to 0.52 seconds. The magnitude of the kernels did approach zero as the time lag approached 0.52 seconds, indicating that the memory length was adequate or nearly so. Currently we are implementing the use of Lysis 6.2 software [4] to calculate the kernels. This change will eliminate the computational restriction on memory length.

Using standard procedures to characterize soft tissues as shown in Figure 1, would require application of a series of sinusoidal stress inputs, each having a different frequency. Results of this study show that nonlinear viscoelastic tissues can be characterized in single test of short duration by using pseudorandom stress stimuli and Equation 1.

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REFERENCES
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Figure 1. Predicted components of the complex compliance for diabetic and non-diabetic skin. A. storage compliance; B. loss compliance; C. phase angle.