Imbalance between Types I and VI collagen promotes skin fragility in human and experimental diabetes

Jurandir Tomaz De Miranda¹, Veronica Protocevich Toledo¹, Zelita Aparecida Queiroz¹, Lizandre Keren Ramos da Silveira¹, Antonio dos Santos Filho¹, Ana Paula Pereira Velosa¹, Sérgio Catanozi², Vera Luiza Capelozzi³ and Walcy Rosolia Teodoro¹

¹Rheumatology Division, Discipline of Endocrinology and ³Department of Pathology of the Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil
**INTRODUCTION: DIABETES**

- Diabetes mellitus is a chronic metabolic disease, characterized by a relative or absolute lack of insulin resulting in hyperglycemia.

- One of the most common complications in these patients is the skin ulcers associated with peripheral neuropathies.

- Diabetic neuropathies cause changes in metalloproteininases, oxidative stress, deficient neoangiogenesis, inadequate concentrations of growth factors, regulators of gene expression, excessive formation of advanced glycation products (AGEs).
OBJECTIVE:

This study compared the expression of the type I collagen and type VI collagen of human skin and experimental diabetes.
**METHODS: Experimental protocol in RATS**

- **Animals:** Males *Wistar* rats (n=40; weighing 200-250g).
- **Diabetes Induction:** Streptozotocin injection (STZ; 35 mg/kg)

**Sample collection**

**Blood collect - glycose measurement**

**Induction of Diabetes**

Acute intravenous infusion of STZ via the tail vein

**Confirmation of Diabetes**

Blood collect - glycose measurement
**METHODS:** Experimental protocol in RATS

**Euthanasia Skin collect**

- **Diabetes Induction**
  - 1 Day
- **Euthanasia Group G1**
  - 7 Days
- **Euthanasia Group G2**
  - 30 Days
**METHODS: HUMAN / RATS biopsies**

- **Human skin biopsy**
  - Surgically removed

- **Rat Skin Biopsy**
  - Euthanisia: 7th and 30th days after diabetes induction.

- **Immunofluorescence (IF)**
  - Analyzed:
    - Anti-Human Collagen I and VI
    - Anti-Rat Collagen I and VI

- **Histomorphometry (IF)**

- **Statistical Analysis**
RESULTS: Morphologic evaluation

Control

Diabetic

7th day Rat

30th day Rat

Human

DP

Epi

Anex

DP

Epi

Anex

DP

Epi

TP

Epi

50 µm

50 µm

50 µm

50 µm

TOMAZ DE MIRANDA. J 2019
RESULTS: RAT COLLAGEN TYPE I

![Graph showing % Rat collagen type I for Control 7th, Diabetic 7th, Control 30th, and Diabetic 30th groups.](image)

- **Control 7th** group shows a higher % Rat collagen type I compared to other groups.
- **Diabetic 7th** group has a lower % Rat collagen type I compared to Control 7th.
- **Control 30th** group shows a significant increase in % Rat collagen type I compared to 7th group.
- **Diabetic 30th** group has the lowest % Rat collagen type I among all groups.

**Significance Levels:**
- **Two-tailed t-test (p < 0.05)**: **Control 7th** vs. **Diabetic 7th**
- **Two-tailed t-test (p < 0.01)**: **Control 30th** vs. **Diabetic 30th**
RESULTS: HUMAN COLLAGEN TYPE I

Human collagen type I

% Human collagen type I

Control
Diabetic

***

Control
Diabetic
RESULTS: HUMAN and RAT COLLAGEN TYPE I

Human collagen type I

% Human collagen type I

- Control
- Diabetic

***

Rat collagen type I

% Rat collagen type I

- Control 7th
- Diabetic 7th
- Control 30th
- Diabetic 30th

**

30th day rat

200µm

200µm

TOMAZ DE MIRANDA. J 2019
**RESULTS: RAT TYPE VI COLLAGEN**

![Graph showing Rat collagen type VI comparison between Control and Diabetic groups at 7th and 30th days. The graph includes error bars and indicates significant differences with *** symbols.](image-url)
RESULTS: HUMAN TYPE VI COLLAGEN

Human collagen type VI

% Human collagen type VI

Control

Diabetic

Control

Diabetic

200 μm

***
**RESULTS:** HUMAN and RAT TYPE VI COLLAGEN

### Human collagen type VI

- **Graph:** Bar chart showing % Human collagen type VI for Control and Diabetic groups. The Diabetic group shows a significantly lower percentage compared to the Control group (***p***-value).

### Rat collagen type VI

- **Graph:** Bar chart showing % Rat collagen type VI for Control 7th, Diabetic 7th, Control 30th, and Diabetic 30th day rat groups. The Diabetic 7th and Diabetic 30th day groups show a significantly lower percentage compared to the Control groups (***p***-value).

Both images show immunofluorescent images of rat tail skin sections stained with anti-human and anti-rat collagen type VI antibodies, indicating the presence of collagen VI in the dermal layer.

TOMAZ DE MIRANDA. J 2019
CONCLUSIONS:

Our study showed:

• The fragility of diabetic skin that may be associated with reduced collagen type I by altering the structures of collagen fibers

• *In situ* imbalance between Col VI and Col I modulates skin fibrosis in human and experimental diabetes emerging as a promising therapeutic option for recovering skin fragility
Thank you for your attention

Jurandir Tomaz De Miranda  
PhD Student

Verônica Protocevich  
MSc Student

Zelita A. Queiroz  
MSc Student

Lizandre K. R. Silveira  
MSc Student

Ana Paula P. Velosa  
PhD

Vera L. Capelozzi  
MD PhD

Walcy R. Teodoro  
PhD