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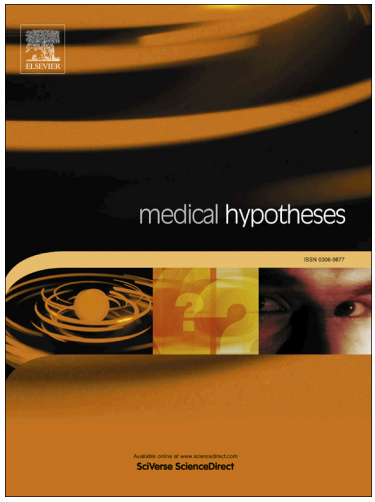


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PII: S0306-9877(17)30817-4
DOI: http://dx.doi.org/10.1016/j.mehy.2017.09.016
Reference: YMEHY 8682

To appear in: *Medical Hypotheses*

Received Date: 3 August 2017
Revised Date: 5 September 2017
Accepted Date: 18 September 2017

Please cite this article as: H. Weli, J. Cooper, Y. Yang, Skin advanced glycation content reflects vaginal tissue glycation level in relation to pregnancy, *Medical Hypotheses* (2017), doi: http://dx.doi.org/10.1016/j.mehy.2017.09.016

# Skin advanced glycation content reflects vaginal tissue glycation level in relation to pregnancy

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**Abstract**

A few studies have revealed that the advanced glycation content of the vaginal wall in pelvic organ prolapse tissues is elevated. This elevation makes advanced glycation a significant association with the disease. Early detection of vaginal wall glycation could therefore be relevant in the prevention and management of pelvic organ prolapse. A vaginal wall biopsy to detect this would be ideal, but is invasive. Therefore the use of a more accessible organ to access, such as skin, would be beneficial. Our previous independent study suggests that conditions such as pregnancy, can induce a change in the vaginal tissues' glycation content. The aim of this study was to assess whether the skin glycation undergoes similar changes as observed in vaginal tissue glycation in the same subjects in order to prove the hypothesis that skin advanced glycation content can predict vaginal tissue glycation. A rat model was used. The vaginal tissues from non-pregnant and E15-E18 pregnant rats and skin tissues from the same rats were taken for the measurement of advanced glycation content. The glycation marker, pentosidine, was quantified by a high performance liquid chromatography. Our results demonstrated that glycation content in vaginal wall tissues from pregnant rats was lower than the tissues from non-pregnant ones, and a strong positive association between skin and vaginal wall pentosidine level was observed. We conclude that skin pentosidine is reflective of vaginal wall pentosidine. Skin glycation may therefore be a potential tool in the prediction and management of pelvic organ prolapse.

## Introduction

Pelvic organ prolapse is present in up to 50% of women presenting for routine gynaecological visits (1), with symptoms found in 8.4% of the general population (2). It is associated with multiple risk factors including age, multiple vaginal deliveries, chronically increased pelvic pressure, traumatic vaginal deliveries and obesity (3–5). Ageing is a focus of recent studies because pelvic organ prolapse occurs mostly in women above 40 years of age (6). Glycation-associated ageing in particular is being investigated to obtain a better understanding of the cause and progression of pelvic organ prolapse (7–9).

Glycation is a process in which proteins, fats and nucleic acids in body tissues get modified non-enzymatically with reducing sugars, it typically occurs over months or years (10). It predominantly affects extracellular matrix (ECM) collagen and elastin because they have slow turnover rates within the ECM (11). Glycation results in stiffer, less digestible proteins and can permanently alter connective tissue properties (12). Advanced glycation is implicated in ageing and many diseases including gynaecological disorders such as pelvic organ prolapse and urinary incontinence (4, 5). Pelvic tissue glycation has been further studied since the observation of higher amounts of insoluble collagen or glycation products in prolapsed pelvic tissues by Jackson et al in 1996 (7).

## Hypothesis/Theory

There are currently no predictive tools for pelvic organ prolapse. The most common diagnostic techniques are based on physical pelvic examination (1,2,13,14). Due to the personal and private nature of pelvic problems and symptoms, diagnosis of pelvic organ prolapse often occurs when sufferers present with symptoms. As a result, more women have clinically evident prolapse than actually present with symptom to their health care provider (1).

The consistent finding of higher vaginal wall glycation in women with pelvic organ prolapse in previous studies (7,8) suggests that the identification of vaginal wall glycation may be a useful tool for prediction, diagnosis and further management of pelvic organ prolapse. Vaginal tissue is poorly accessible and obtaining a vaginal biopsy is invasive and inconvenient for patients. The skin, on the contrary, is easily accessible and skin fluorescence can be measured non-invasively to reflect its advanced glycation levels (15). Since ageing is a generalized body process, it is expected that the various processes involved in ageing such as advanced glycation should occur at similar rates in different body tissues. We therefore hypothesize that skin glycation would reflect vaginal tissue glycation.

## Evaluation of Hypothesis

Ageing increases advanced glycation in the body continuously. Other conditions can regulate glycation content of body tissues. For example, advanced glycation is facilitated by oxidative stress (16). As a result, increased oxidative stress such as smoking and inflammation leads to a higher glycation content (17). On the contrary, elevation of the female hormone, estrogen causes the up-regulation of several antioxidants including a glycation reducing antioxidant, glyoxalase I (18). As a result, a lower glycation content has been observed in pregnant vaginal tissues in a rat model (19). Furthermore, it has been previously shown that skin glycation is lower during pregnancy (15). It is therefore reasonable to directly investigate skin glycation in relation to vaginal tissue glycation between pregnant and non-pregnant subjects by studying advanced glycation content of both tissues simultaneously from the same animals because the different hormone levels in pregnant and non-pregnant states should lead to different glycation levels. Proving this hypothesis implies that an assessment of skin glycation in women may be useful in the management of women with pelvic organ prolapse and a relevant predictive and preventive tool.

## Materials and Methods

### *Tissue collections*

Full thickness skin and vaginal tissue from 6-8 month old pregnant (E15-E18) and 4 non-pregnant female Sprague Dawley outbred rats were obtained following local ethical approval by

the Animal Welfare & Ethical Body Review and in accordance with the 2006 Animal Act. Skin segments from the left dorsal surface of the rats were used (20).

***Advanced glycation detection***

The advanced glycation marker, pentosidine, was chosen for detection due to its fluorescence property (9). Samples were lyophilised and hydrolysed in 6 M Hydrochloric acid at 110$^{o}$C. High performance liquid chromatography (HPLC) separation and quantification was performed using a C-18 (250 mm (length) x 4.60 mm (width)) analytical column and ultraviolet (UV) detector. Standard pentosidine purchased from Caymann Chemical was used for the creation of a standard curve for quantification. Pentosidine was eluted at 9.6 minutes in a 30 minutes run using gradient ratios of 0.1% triflouroacetic acid and 80% acetonitrile. Detection of pentosidine occurred at 325 nm wavelength.

***Statistics***

Means of the pregnant and non-pregnant groups were compared using an unpaired T test. Significance level was set at a *p* value of 0.05. Data are expressed as mean and standard error of mean. The Pearson correlation test was performed to study the relationship between vaginal and skin pentosidine.

**Results**

The average skin pentosidine was obtained per gram dry tissue. Figure 1a shows that the average amounts of pentosidine were 0.007 mg/g and 0.014 mg/g in the pregnant and non-pregnant skin tissues respectively. There was minimal variability within groups and the difference was significant ($p$ = 0.0002). Pentosidine in non-pregnant rat vaginal tissues was also significantly higher than in pregnant rat vaginal tissues ($p$ = 0.042) as shown in Figure 1b. Although pentosidine levels in the skin were less than in the vaginal tissues, the pregnancy associated difference was more notable in the skin than in vaginal tissues.

Pentosidine was higher in the vaginal tissue than in the skin of the same rats. Raised vaginal pentosidine was associated with raised skin pentosidine. A strong correlation ($R^2$= 0.847) was present between the pentosidine content of vaginal and skin tissues of the same rats (Figure 2).

**Discussion/Consequences of Hypothesis**

Advanced glycation is a cause of ageing that has gained significant attention in recent times (8). Glycation considerably affects the skin. It results in increased skin stiffness, decreased elasticity and higher resistance to matrixmetalloproteinase enzyme degradation (21) which impedes connective tissue remodeling. Glycation induces inflammatory responses intracellularly and extracellularly (11). Advanced glycation end products can initiate DNA damage and stimulate cellular injury through their receptors, Receptor of Advanced Glycation End products, (RAGE) (11).

In our study, glycation was lower in the skin during pregnancy. This is congruent with a previous study noting reduced skin glycation-induced autoflourescence in pregnancy (15). Recently, vaginal tissue glycation was noted to be lower in pregnancy through influences on ER-α and glyoxalase I, an AGE lowering antioxidant (19). Another study has observed reduced glycation in vaginal tissues under oestrogen therapy (22). Oestrogen has also been found to influence blood vessel glycation. These, in conjunction with the present finding of reduced skin glycation in pregnancy suggest that pregnancy effects on glycation may be reflected in multiple body tissues and organs and the observation of known glycation-associated illnesses in one organ should warrant checks for others.

A positive relationship between vaginal and skin tissue glycation content was also noted in this study and supports the hypothesis that skin glycation reflects vaginal tissue glycation level. Advanced glycation content has been studied in different tissues in the body because it is believed to be a global phenomenon occurring at reasonably similar rates in various sites of the body. Animal studies and human clinical trials have confirmed changes of advanced glycation content in a few other body tissues to be reflected in the skin (23–25). Mikulikova *et al* quantified pentosidine in tissues from rats with a high fructose intake and found that pentosidine accumulation increases in tendon, skin and aorta occurred at different rates, with pentosidine increasing more in the skin and aorta than in tendons accumulation when 10% fructose was given (24). Skin advanced glycation level has been used to predict advanced glycation level

associated diseases in other tissues for example, in the retina and cardiovascular system (23,25,26) but no previous study has correlated advanced glycation level between skin and vaginal tissues. We investigated collagen glycation levels of vaginal and skin tissues in pregnancy, a physiological process, and found correlation between the collagen altering glycation product, pentosidine in both tissues in the same rats, implying that collagen changes in connective tissues across the whole body may provide vital information for physiological and pathological processes. The correlation of glycation products in skin and other tissues, and the ease of access of the skin tissue for sampling or biopsy, lays a foundation for future studies on the predictive value of skin glycation in relation to vaginal tissue glycation, and thus its potential role in the investigation, prevention and management of glycation related gynaecological diseases. Our hypothesis is also supported by a recent study which revealed that ultra-structural collagen changes in the skin of women with pelvic organ prolapse (a pathological condition) correlated with changes in pelvic tissues of the same women (27).

Further studies are required to determine whether the correlation of advanced glycation levels in skin and vaginal tissue holds in human subjects during physiological and pathological process. Furthermore, in pathological cases, it is important to note that changes in collagen metabolism may be the result or cause of a disease or physiological process. For example, in pelvic organ prolapse, the alteration of collagen metabolism in the vaginal wall may be a result of prior tissue weakening or stretching but not necessarily the cause of the prolapse (28). In such a condition,

collagen metabolism alterations or glycation levels may be more expressed in the vaginal wall than in skin tissues.

## Conclusion

Our animal model study reveals that skin glycation correlates with vaginal wall glycation as both tissues respond similarly to a state of elevated oestrogen. This, in addition to previous literature demonstrating similar changes within the skin in response to changes in levels of the hormone, strongly suggests that skin glycation is reflective of vaginal tissue glycation, which is implicated in pelvic floor disorders. Skin glycation is a potentially useful marker in the prediction and diagnosis of glycation-associated pelvic floor diseases, with a view towards promoting preventive lifestyle.

## Conflict of interest statement

All authors confirm that there are no financial and personal relationships with other people or organisations that have inappropriately influenced this work.

## Acknowledgement

Rivers State Government, Nigeria, NHS charitable fund of the University hospital of North Midlands, UK and Keele University Animal House.

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**Figure captions**

Figure 1: Measurement of AGE mark, pentosidine, by HPLC. (a) Presented in skin tissues of pregnant and non-pregnant rats P=0.000; (b) presented in vaginal tissues from the same pregnant and non-pregnant rats. The data were re-presented from the publication (21) with the permission.

Figure 2: Correlation of pentosidine expression between skin and vaginal tissues from the same

objects. $R^2$=0.847.

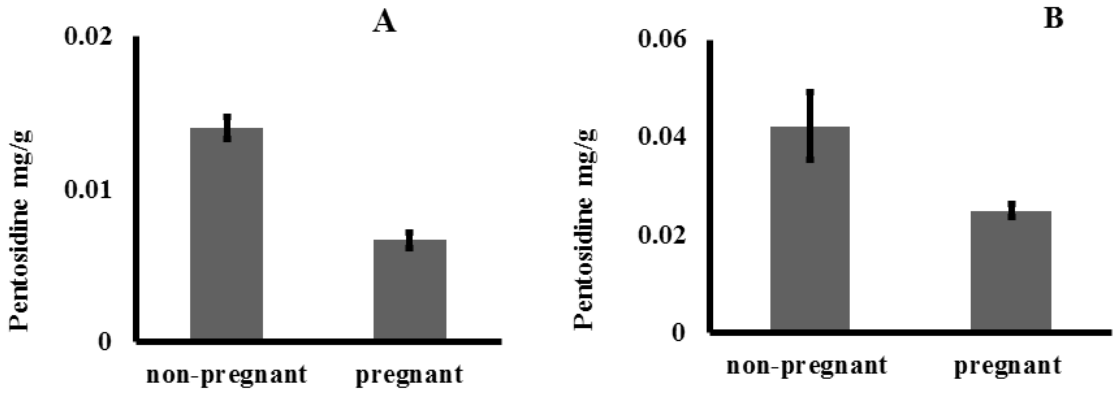


**Figure 1**

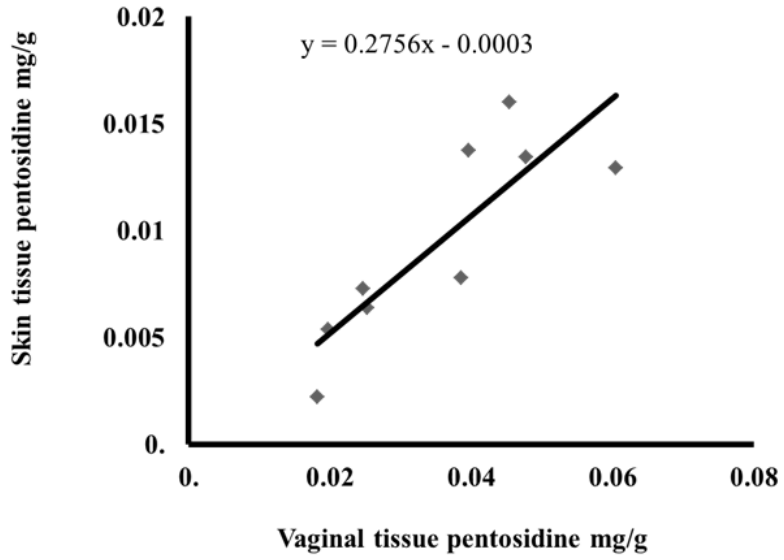


**Figure 2**