Master's thesis submitted for obtaining the degree Master of Medicine

Daily Habit Skin Study:

Daily habits influencing skin barrier function measurements: a prospective study

Lisa Huygen

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Promotor: Prof. Dr. Jan Gutermuth, MD PhD

Co-promotor: Prof. Dr. Inge Kortekaas, PhD

Medicine and Life Sciences

Glossary

1. Abstract

Background: Measurement of electrical skin impedance (EI) is a new non-invasive method for the assessment of skin barrier function. Different factors, such as age, anatomical location, water exposure, perspiration, and skin hydration, have already been found to influence measurements of other biophysical properties of the skin. The effect of these factors was mostly examined in highly controlled settings and was never evaluated for EI. Therefore, the main aim of this study was to evaluate the effect of daily habits on EI measurements in healthy adults.

Methods: Non-smoking healthy adults (n=31) were equally divided into 3 age groups (18-29, 30-49, and ≥50). The influencing effect of body cream application, skin washing, walking, stair climbing, and caffeine intake was evaluated at different time intervals. Additionally, measurements were performed on both the volar forearm and abdomen to investigate the influence of anatomical location on EI.

Results: EI values decreased at 15 and 90 minutes after body cream application and skin washing on both the forearm and abdomen. Moderate and heavy physical activity and caffeine intake did not influence EI. EI was not correlated to participants' age. Lastly, no difference in EI was found between the different anatomical locations.

Conclusions: To obtain reproducible data, participants should avoid skin washing and hydration with body cream at least 90 minutes before measurements of EI. Participants should not restrain from exercising or drinking coffee prior to their visit. EI may also be a reliable tool for the evaluation of skin barrier function, but further research is necessary.

Keywords: Electrical skin impedance, skin barrier function, water exposure, caffeine intake, exercise, skin hydration, Nevisense System

2. Glossary of term

Transepidermal water loss	**TEWL**	The quantity of condensed water (in grams) that diffuses across a fixed area of skin (in cubic meters) per unit time (in hours). [2-4]
Electrical skin impedance	**EI**	Is the response of a specific skin region to an externally applied low electrical current at a variety of frequencies (in Ohm). [5-7]
Magnitude index value	**MIX value**	The ratio of total impedance magnitudes measured at two predetermined frequencies of 20kHz and 500kHz (MIX = $\lvert Z_{20kHz}\rvert$ / $\lvert Z_{500kHz}\rvert$). [5, 6, 8, 9]
Stratum corneum	**SC**	The upper layer of the skin that is composed of flattened, anucleate corneocytes interconnected by corneodesmosomes and surrounded by multiple lamellar lipid sheets of ceramides, cholesterol, and free fatty acids. This skin layer contributes for 90% to the skin barrier function. [10, 11]
Atopic dermatitis	**AD**	Chronic relapsing inflammatory skin disorder characterized by eczematous rash, pruritus, excoriation, dry skin, and susceptibility to cutaneous infections. [12]

3. Article

3.1. Introduction

As is widely known, the skin is responsible for many important protective functions of the human body. These functions are composed of controlling the unregulated loss of water and solutes through the skin, preventing invasion of pathogens, protecting against chemical and physical assault, thermal regulation, the sensation of vibration, pressure, and touch, storage of water and energy, biosynthesis of vitamin D and melanin and absorption and excretion of different products. (11, 13-17) The skin is composed of three different layers which can again be divided into multiple sublayers. (10) The epidermis is the most superficial layer of the skin and can be divided into the stratum corneum (most superficial), stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale (deepest layer). (10) The second layer of the skin is the dermis, which is separated from the epidermis by the basement membrane. The dermis can be divided into an upper papillary layer and a profound reticular layer. (10) The third layer of the skin is composed of adipose tissue and is called the hypodermis or subcutis. (10)

The barrier function of the skin is mainly formed by the stratum corneum (SC), accounting for up to 90% of the functionality of the skin. (18) The SC is composed of flattened, anucleate corneocytes interconnected by corneodesmosomes and surrounded by multiple lamellar lipid sheets composed of ceramides, cholesterol, and free fatty acids which form a hydrophobic matrix (Figure 1). (15, 19, 20) Corneocytes are produced by a process called keratinisation. During this process, keratinocytes from the lamina basalis progressively mature in the stratum spinosum and granulosum to eventually form the flattened, anucleated, and protein-enriched cells of the SC, which are loaded with keratin filaments. (15, 20, 21) Filaggrin, a matrix protein, plays an important role in the final stage of corneocyte differentiation. This protein is responsible for the connection of keratin filaments into tight bundles. This will cause the cells to assume their typical flat shape. (15) Loss of normal profilaggrin and filaggrin is associated with a less effective skin barrier function. (22) The corneocytes in the SC are mainly responsible for the protection against chemical and physical trauma. Together with their intercellular lipid layer the corneocytes also contribute to the water impermeability of the skin. (15) The nucleated epidermal layers (all layers of the epidermis except for the stratum corneum) do also play an important role in the formation of the skin barrier due to the presence of tight junctions, adherence junctions, and desmosomes. [15, 23] The antimicrobial function of the skin barrier is provided by the presence of acids, hydrolytic enzymes, and antimicrobial peptides. [13, 14, 20, 24] The keratinocytes and Langerhans cells in the epidermis have a crucial role in the activation of the immune system upon skin damage or invasion of pathogens via the production of alarmins (cytokines). [23] Once the microbes or other exogenous particles (allergens, pollution, chemicals) have passed the skin barrier, the immune system may take over the defense by activation of innate immune cells, such as macrophages, and components of the adaptive/humoral immune system. [23]

A disruption or impairment of the skin barrier function contributes significantly to the pathophysiology of many common skin diseases, such as contact dermatitis, ichthyosis, and atopic dermatitis (AD). [14, 20, 25-27] The evaluation of the skin barrier function is therefore an important parameter in the assessment of general skin health and the extent of skin diseases.

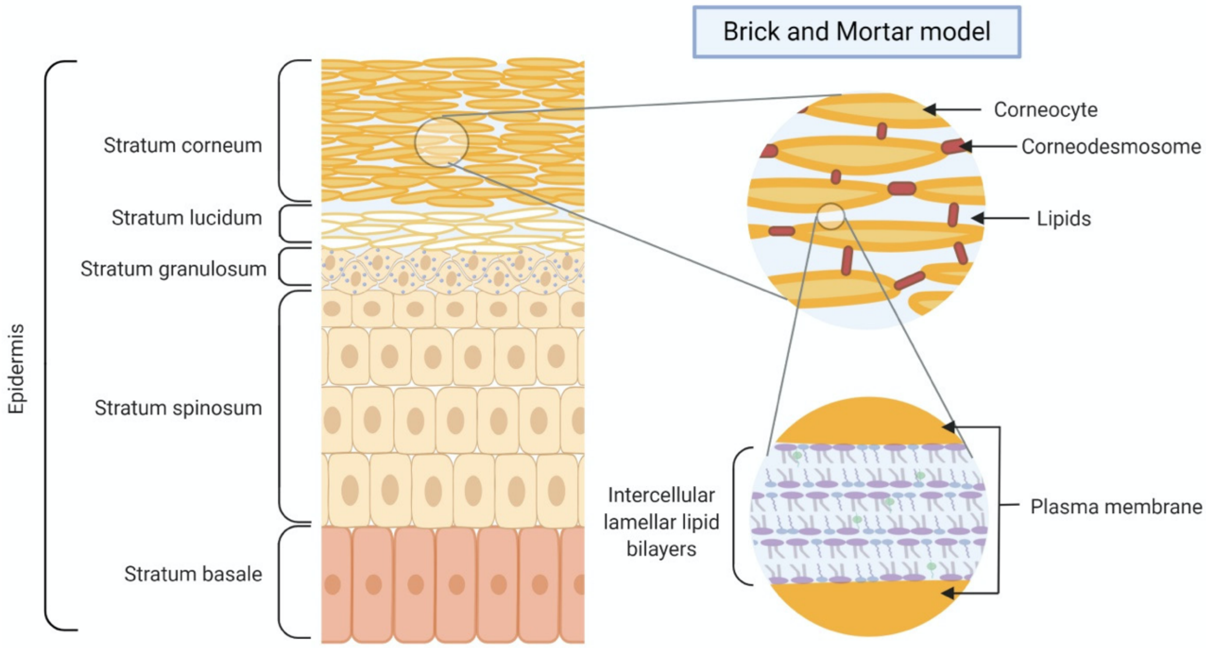


Figure 1. Schematic structure of the epidermis. The epidermis consists of several layers of keratinocytes which are closely interconnected by corneodesmosomes and surrounded by multiple lamellar lipid sheets to form a tight barrier. [20]

The 'golden standard' for the assessment of the skin barrier function is microscopy studies of skin biopsies. [19] Unfortunately, this is an invasive technique that cannot easily be used in the general population. As an alternative, measurements of biophysical properties of the skin can be used to evaluate the function of the skin barrier. A well-known clinical and most frequently used method is the measurement of transepidermal water loss (TEWL). TEWL is defined as the quantity of condensed water that diffuses across a fixed area of skin per unit of time. [2] This measurement is a marker for the permeability (inside-out barrier function) of the skin. A disruption of the skin barrier leads to an increase in permeability and therefore an increase in TEWL. [28] It has been shown that in most cases the inside-out skin barrier function correlates with the outside-in barrier. [15]

An emerging new method for the evaluation of skin barrier function is the measurement of the electrical skin impedance (EI). The EI technique measures the resistance and capacitance of material using alternating low electrical currents at various frequencies. [29, 30] It mainly reflects the extent of skin hydration, the thickness of the stratum corneum, and the condition of water channels through the skin. [31, 32] Impedances at low frequencies reflect the electrode-skin interface and the resistive properties of the extracellular environment. [5, 7, 9] The EI at lower frequencies is also largely influenced by the integrity of the stratum corneum. [5, 9] EI measurements at higher frequencies are mainly influenced by viable skin (epidermis and dermis) and represent the resistive properties of both the intra- and extracellular environments and the capacitive properties of the cell membranes. [1, 7] While EI measurements at lower frequencies (below approximately 10kHz) can be useful in the evaluation of the degree of hydration of the stratum corneum, a significant inter-individual and inter-site variation was observed. [6, 9] This is caused by the fact that measurements between one and approximately 10kHz are susceptible to the degree of contact between the electrode and the skin surface. [8] To avoid these shortcomings indices of the four different aspects (magnitude, phase, real part, and imaginary part) of the EI can be used. [6, 9] One of the most commonly used indices is the magnitude index (MIX) value. [5, 6, 9] This index represents a ratio of impedance magnitudes measured at 2 predetermined frequencies (20kHz and 500kHz). [6, 8, 9] It was shown that these indices can be used in the differentiation between different causes of irritant contact reactions of the skin. [6] The characteristics of these biophysical parameters also showed to be a highly useful tool in the assessment of the skin barrier function in patients with AD. [30, 33] Although, this method has not yet been validated for diagnostic use.

Different factors have been found to influence the results of these biophysical measurements. Among these factors, the most important are age, anatomical location, water exposure, perspiration, smoking, and skin hydration. [2, 4, 32, 34-42] Aging has shown to lead to a generalised thinning of the epidermis, an increase in the size of corneocytes, a decrease in the intercellular lipid layer, and a diminishment in hydration of the stratum corneum. [43] However, the role of aging in the skin barrier function is still controversial. Some studies show an impairment of the skin barrier function with increasing age, while others show no change. [43-46] S. Luebberding et al. (44) demonstrated a slightly diminished function of the skin barrier in the elderly population due to a decrease in sebum production and an increase in skin surface pH. TEWL and SC hydration did not change, or improved slightly, with advanced age. [44] I. Nicander et al. (43) investigated the relationship between EI measurements at various anatomical locations and ages. A significantly higher MIX value was observed in the elderly participants (>60 years old) compared to the younger participants (20-40 years old). [43] Anatomical location also has a significant influence on biophysical parameters due to differences in eccrine sweat gland activity, the SC thickness, and the extent of skin hydration depending on the local skin function. [38, 43] The highest TEWL and skin hydration values have been obtained from the palms and soles with lower values at the abdomen, back, and lower legs. In addition, high skin pH values are registered at sites with physiologic occlusion, such as the axilla, inguinal and submammary folds, and finger webs. [38] In general, the skin is exposed daily to an important influencer of skin barrier function, namely water. Firooz et al. (39) has shown that a daily water exposure of 30 minutes for 5 consecutive days increases the TEWL and pH of normal skin. Extended water exposure leads to severe disruption of the intercellular lipid lamellae of SC, swelling of corneocytes, and facilitated the penetration of external substances. [39, 40, 47, 48] The direct increase in TEWL seen after water exposure is mostly due to the presence of remaining water on the skin. [39] Strenuous efforts and heating of the body result in arteriolar vasodilatation and an increase in capillary blood pressure and blood flow. Additionally, there is a stimulation of eccrine and apocrine sweat glands resulting in perspiration. It has been shown that these effects result in a significant increase in stratum corneum capacitance and TEWL. [42] Finally, the application of body cream or lotion is a very frequently used method by the general population to improve general skin health. The overall effects of body lotion or cream application are skin hydration, improvement of the skin barrier function, and avoidance of transepidermal water loss and invasion of pathogens. (8, 49) Summarized, all the above-mentioned factors can either alter TEWL and EI itself or influence the measurements of these properties. It is important to mention that the effect of most of these factors was studied in a highly controlled, and sometimes artificial setting. Therefore the biggest shortcoming in current studies is the lack of analysis of the effects of people's daily habits on measurements of TEWL. For the novel EI method, little data exist on the effects of daily habits in healthy individuals so far.

Guidelines were developed for the assessment of TEWL and stratum corneum hydration with electrical methods by the European Expert Group on Efficacy Measurement of Cosmetics and Other Topical Products (EEMCO) group with the purpose to limit the influencing effects of external factors and to standardise measurement results in the context of a clinical study. (4, 29) To ensure reliable and comparable measurements, all influencing factors should be standardised (e.g. a standard room with controlled room temperature, and humidity). Therefore these guidelines advise considering a period for acclimatisation and resting of 15-30 minutes in a room with a temperature of 20-22°C and relative humidity of 40-60%. It is also advised to avoid the consummation of hot food and/or caffeine/theine-containing drinks, exertion and sweating as a result, usage of skin products which can affect the measurements, and smoking just before and during the measurement. (4, 29) These influencing factors are largely similar to those listed above. The factors cited above resulted in a list of restrictions for the patients prior to these measurements which can be experienced as inconvenient. As many of these situations were examined in a highly controlled and sometimes artificial setting, the relevance of these restrictions can be a point for discussion. To our knowledge, the effect of caffeine intake on TEWL and EI measurements has also not yet been studied.

The main purpose of this study is therefore to examine the effect of common daily habits of people on measurements of skin barrier function. This will make it possible to meet the shortcomings of previous, more artificial research on which current guidelines are based. In addition, this study will be one of the first to investigate the effects of various influencing factors on measurements of EI. Common daily habits that will be examined during this study are hot caffeine intake, physical exercise, washing of the skin with warm water and soap, and application of body cream. The effect of these daily habits on measurements of TEWL and EI will be investigated. The goal is to mimic the daily routine of a person as well as possible to give correct and relevant instructions to the patient in the future. Additionally, the correlation between the age of the participant and the TEWL and EI measurements will be examined. To obtain an adequate distribution in ages, participants were equally divided into three age groups (18–29, 30–49, and ≥50). Lastly, it will be examined if measurements on 2 different commonly used anatomical locations, namely the volar forearm and abdomen, vary significantly. This study may contribute to the current guidelines around TEWL and EI measurements to further standardise these measurements. This study may also provide novel insights into the use of EI to objectively evaluate the skin barrier function for future use in the clinic or research purposes.

During this research, measurements of both TEWL and EI were performed. In this master's thesis, only the results of the EI measurements will be analysed and discussed. The measurements of TEWL will be discussed in the master's thesis of Pauline Thys, a final year master's student of Pharmaceutical Sciences at the VUB and a member of this research team.

3.2. Methods

3.2.1. Study design

An academic, prospective study was undertaken to examine the effect of body cream application, skin washing with warm water and soap, physical activity, and hot caffeine intake on EI measurements in healthy non-smoking adults. In addition, it was investigated if age had a significant influence on measurements of EI. Furthermore, differences in EI measured on both the left and right volar forearm and abdomen were examined to investigate the possible influence of anatomical location on these measurements

3.2.2. Study population

A total of 31 participants were recruited by Pauline Thys and Lisa Huygen from September 2021 until December 2021. The targeted study population was overall healthy non-smoking adults. Participants were equally divided into three different age groups, being 18-29, 30-49, and ≥50 years old, with a targeted goal of 10 subjects per group. Eventually, 11 participants were included in the age group of 18-29 years old, and 10 participants were included in both of the other two age groups. Subjects who understood the content of the study – and signed the informed consent – and who were able to walk independently were eligible for inclusion in this study. The exclusion criteria concerned: smoking, age <18 years old, pregnancy, the inability to speak or fully understand Dutch or French, the inability to fully understand the content of the study, and the presence of a skin disease (AD, contact dermatitis, urticaria, …).

3.2.3. Study procedure

Study subjects were made aware of the study through flyers or were invited to participate via networking. Participants were enrolled based on the inclusion and exclusion criteria. Afterward, two halve-days were scheduled on which the measurements took place. It was not a necessity for these dates to be two consecutive days.

Figure 2. Setup of the room humidifier and thermometer. The room temperature was kept at 22˚C with a relative humidity of around 55%.

Participants were instructed to refrain from drinking hot and/or caffeine/theine-containing beverages, taking a shower, washing their forearm and abdomen, applying body cream or lotion on the test areas, or making strenuous efforts for at least 1 hour before their visit. The measurements took place in a relative humidity-controlled room with a relative humidity of around 55% and a temperature of 22 ˚C. These features were provided by central heating or an air conditioner and a humidifier with a hygrometer (Figure 2). At the beginning of each half-day, the participants had to acclimatise for at least 30 minutes in this controlled room. [4]

EI measurements were determined at baseline and after each action on both the volar forearm and abdomen. Each measurement took less than 10 seconds to perform. Measurements were repeated at least once and the average of two reproducible measurements was calculated and used as the final result. [1, 50] Current guidelines advise calculating the average of 3 reproducible measurements to obtain the most reliable results possible. [29] This recommendation was initially followed at the beginning of this study. Due to financial consideration and the observed high reproducibility of the measurements, it was decided to continue with the average of only 2 measurements.

At the start of the first visit, baseline measurements were initially obtained on the right side after 30 minutes of acclimatization in the relative humidity-controlled room. Thereafter, the effect of body cream application was tested. The product used was Emollient Cream of the brand Dexeryl® which is composed of the following ingredients: glycerol, vaseline, liquid paraffin, glycerol monostearate, stearic acid, dimethicone, macrogol 600, trolamine, acrylamide/acryloyldimethyl taurate copolymer, isohexadecane, polysorbate, glycol pentylene, ethylhexylglycerine, carbomer, and purified water. A quarter of a finger top unit of body cream was applied on both the participant's right volar forearm and abdomen. Measurements of EI were repeated on both anatomical locations after a time interval of 15 and 90 minutes (Table 1).

Then, second baseline measurements were performed on the left volar forearm and abdomen. The effect of skin washing with warm water and soap was measured. The product used was the Pure Care Shower Cream, Creme Soft of the brand Nivea®. This soap is composed of the following ingredients: aqua, sodium laureth sulfate, cocamidopropyl betaine, glycol distearate, decyl glucoside, parfum, glycerin, prunus amygdalus dulcis oil, sodium chloride, citric acid, laureth-4, sodium benzoate, linalool, limonene, citronellol, benzyl alcohol, and geraniol. The left volar forearm and abdomen of the participant were washed with warm water and a predetermined amount of soap. Measurements of EI were repeated on both anatomical locations after 15 and 90 minutes (Table 1). For each participant, the same amount and type of neutral body cream and common soap were used.

Table 1. **Time schedule measurements day one**

Time	Action
+0’	Baseline measurements at right volar forearm and right side of abdomen
	Application of body cream on right volar forearm and right side of abdomen
+15’	Measurement of the effect of body cream application after 15 minutes at right volar forearm and right side of abdomen
+90’	Measurement of the effect of body cream application after 90 minutes at right volar forearm and right side of abdomen
+0’	Baseline measurements at left volar forearm and left side of abdomen
	Washing of the left volar forearm and left side of abdomen with warm water and soap
+15’	Measurement of the effect of washing of the skin after 15 minutes at left volar forearm and left side of abdomen
+90’	Measurement of the effect of washing of the skin after 90 minutes at left volar forearm and left side of abdomen

During the second visit, the effects of moderate and intense physical activity and intake of a hot, caffeinated beverage (a cup of coffee) were measured. Baseline measurements were obtained on the right volar forearm and abdomen after an acclimatisation period of at least 30 minutes in the relative humidity-controlled room. The participant was then instructed to walk for 5 minutes at a normal pace. The effect of moderate exercise was examined by repeating measurements of EI after 5, 30, and 60 minutes. Afterward, the participant was instructed to climb stairs for 5 minutes at an energetic pace (as if they were late for their doctor’s appointment). Measurements of EI were also repeated after 5, 30, and 60 minutes. Finally, one hour after performing physical exercise, a cup of coffee (Douwe Egberts Lungo 6 Dessert) was served to the participant. The effect of the intake of a hot, caffeinated beverage was measured at 5, 20, and 60 minutes after finishing the drink. [51] For all participants the same type of coffee capsule and coffee machine was used to ensure that each participant had the same amount of caffeine intake.

<u>Table 2.</u> **Time schedule measurements day two**

Time	Action
+0'	Baseline measurements at right volar forearm and right side of abdomen
	Walking for 5 minutes in a normal pace
+5'	Measurement of the effect of walking after 5 minutes at right volar forearm and right side of abdomen
+30'	Measurement of the effect of walking after 30 minutes at right volar forearm and right side of abdomen
+60'	Measurement of the effect of walking after 60 minutes at right volar forearm and right side of abdomen
	Climbing stairs for 5 minutes in an energetic pace
+5'	Measurement of the effect of climbing stairs after 5 minutes at right volar forearm and right side of abdomen
+30'	Measurement of the effect of climbing stairs after 30 minutes at right volar forearm and right side of abdomen
+60'	Measurement of the effect of climbing stairs after 60 minutes at right volar forearm and right side of abdomen
	Drinking a cup of hot coffee
+5'	Measurement of the effect of hot caffeine intake after 5 minutes at right volar forearm and right side of abdomen
+20'	Measurement of the effect of hot caffeine intake after 20 minutes at right volar forearm and right side of abdomen
+60'	Measurement of the effect of hot caffeine intake after 60 minutes at right volar forearm and right side of abdomen

3.2.4. *Measurements/measured parameters*

Electrical impedance spectroscopy

Skin electrical impedance (kiloOhm) is the response of a specific skin region to an externally applied low-voltage electrical alternating current at various frequencies. [7, 31] Skin hydration, the thickness of the stratum corneum, the condition of water channels through the skin, and different properties of the cells in the skin such as size, shape, orientation, compactness, and structure of the cell membranes influence the conductive and reactive behaviour of the cells, alternating their response to the externally applied current. The EI was measured using the Nevisense system (SciBase AB, Figure 3). [50] This device uses a non-invasive method consisting of a gold-plated electrode with small pins for the assessment of the skin impedance of different skin regions (Figure 4, Appendix 6.1.1.). Measurements are performed at 35 different frequencies ranging from 1kHz to 2.5MHz, at four different depths, and 10 different permutations. The applied voltage and resulting current are limited to 150mV and 75μA, respectively. In general, this will cause no harm nor pain sensation for the patient, but it can cause some local redness of the skin. [1] The Nevisense device was initially developed as a non-invasive tool for the diagnosis of skin cancer based on EI, afterwards, the function for the evaluation of the skin barrier function was added. [1, 50]

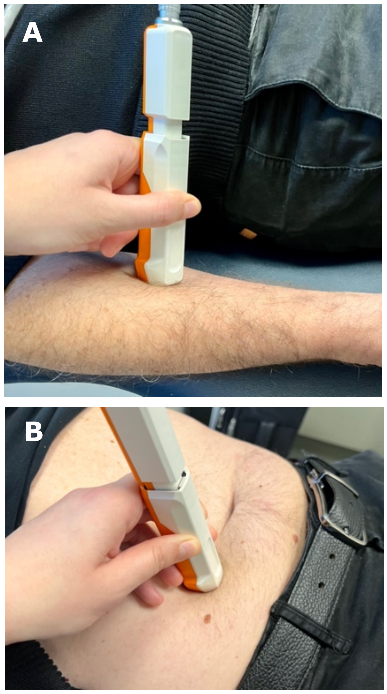


Figure 3. Electrical Impedance measurements. EI measurements taken on the skin of (A) the volar forearm and (B) the abdomen.

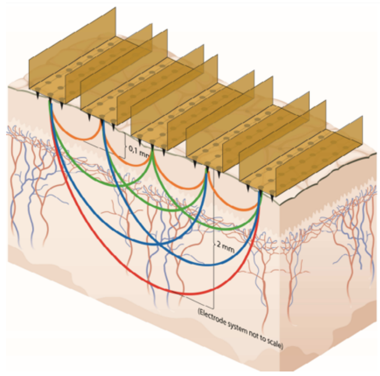


Figure 4. Schematic of the currents generated by the Nevisense electrode: measurements at 10 permutations and four different measurement depths [1]

One measurement with the Nevisense system generates up to 700 variables. For the assessment of the skin barrier function, in particular, this device uses the MIX value. This value is defined as the ratio of the total magnitude of the impedance at two fixed frequencies of

20kHz and 500kHz (MIX = $|Z_{20kHz}| / |Z_{500kHz}|$). [8] The impedance at 20kHz primarily reflects the extracellular properties of the epidermis, while the impedance at 500kHz reflects both the intra- and extracellular properties and the capacitive properties of the cell membranes (Figure 5). [1] Directly measured values of EI at low frequencies can also be used, but these have been shown to increase the inter-individual as well as inter-site variation. It was found that the use of indices can significantly decrease these shortcomings. [5, 6] The MIX value is generated automatically and shown on the display of the Nevisense system (Figure 6).

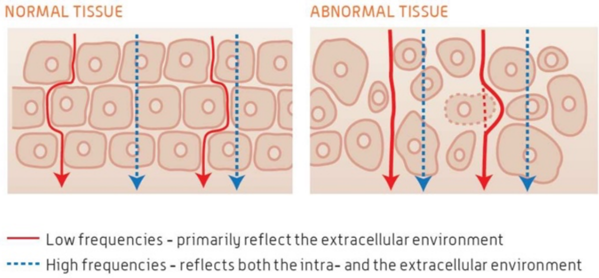


Figure 5. Schematic overview of current pathways generated by the Nevisense system at relatively low and high frequencies

Measurements with the EI probe of the Nevisense device were performed according to the manufacturer's instructions. First, the test area was cleaned and hydrated with a wound cleansing wipe (Salvequick®) by smoothly rubbing the skin 5 times and then pressing the wipe on this area for 30 seconds. The countdown was displayed on the device itself. Afterward, the cloth was squeezed to use the remaining droplets on the test surface and then this area was carefully dried with a compress. Next, the spring-loaded measuring probe was pressed down against the skin until a sound produced by the device indicated that the measurement was finished (approximately 10 seconds). Finally, the MIX value was automatically calculated and the results of the measurement were displayed on the screen of the device (Figure 6). At each time interval, a total of 2 measurements were performed and values were recorded on a paper case report form. The final value was determined by calculating the average of the 2 obtained reproducible values. This was done to avoid random measurement errors.

Besides the EI, also the TEWL, another biophysical property for the evaluation of skin barrier function, was examined during this study. For the evaluation of this property, the Multi Skin Test Center MC 1000 (Courage + Khazaka electronic GmbH) was used. [52]

<u>Figure 6.</u> Overview of the study setup. Set up of the Multi Skin Test Center MC 1000 open-chamber device (left) connected to a laptop and the Nevisense system (right).

3.2.5. Statistical analysis

<u>Sample size calculation</u>

Calculation of the sample size is based on the primary research objective, which is to evaluate if different daily habits influence TEWL and EI measurements. The calculation was made using an effect size of 0,57 (with a difference of interest of 3 $g/m^2/h$ and a standard deviation of both groups of 5,3 $g/m^2/h$), a type 1 error level of 0,05 and a power level of 0,8. A sample size of 27 patients was obtained with a power analysis using the program G*Power 3.1.

In this study, we used a TEWL index score expressing the TEWL with a score from 1 (low TEWL) to 20 (high TEWL). This score has not yet been used to evaluate the effects of daily habits on TEWL measurements. Therefore, an estimate of the sample size was calculated using absolute measurements of TEWL expressed in $g/m^2/h$.

Data analysis

Statistical analysis was performed using SPSS statistics version 28 and GraphPad Prism 9 with the help of statisticians of the Interfaculty Center Data Processing and Statistics (ICDS) of the Vrije Universiteit Brussel.

For the evaluation of the first research objective, a Repeated-Measures one-way ANOVA or Friedman test was performed. Prior, QQ-plots were generated and multiple normality tests were performed to determine whether or not the data sets were normally distributed. The normality tests performed were Anderson-Darling, D'Agostino-Pearson omnibus, Shapiro-Wilk, and Kolmogorov-Smirnov. Datasets not meeting at least one of the normality tests (P ≤0,05) were considered not normally distributed and were analysed using a nonparametric test (Friedman test). When all normality tests were satisfied, a parametric test was performed (Repeated-Measures one-way ANOVA).

To analyse the relationship between age and EI, correlation testing was performed. In advance, multiple normality tests were performed to determine if the datasets followed a Gaussian distribution. Most of the datasets were normally distributed and a simple linear regression with the calculation of the Pearson's correlation coefficient was performed. One dataset was not normally distributed and a nonparametric Spearman correlation was performed.

Lastly, to evaluate the differences in measurements of EI at different anatomical locations, a nonparametric Friedman test was performed. This test was used because not all datasets were normally distributed.

For all the aforementioned statistical analyses a level of significance of $P \leq 0,05$ was used.

Measurements were performed multiple times on the same participant at different time intervals after performing a particular activity. Therefore, the data of this study is composed of repeated measurements, and the chance of a type I error is increased. This issue was discussed with statisticians of the ICDS and, despite these repetitions, it was decided not to apply a correction for these repeated measures. Instead, it was decided to very carefully handle the results and *P*-values obtained to conclude the research objectives taking into account the higher chance of a type I error.

Some missing data were found to be completely at random. If data were missing, they were excluded from the related statistical analysis.

3.3. Results

3.3.1. Demographic data

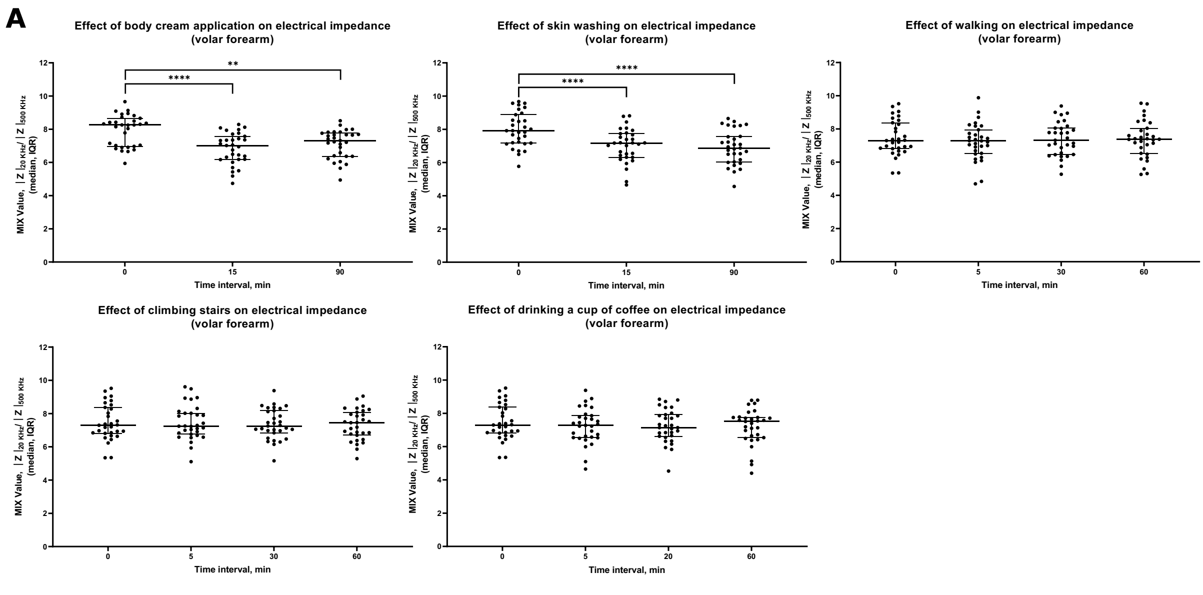
During the study period (19/10/2021-12/12/2021) a total of 31 participants met the inclusion criteria and were included in the study. Table 3 displays the relevant demographic characteristics of the included study population.

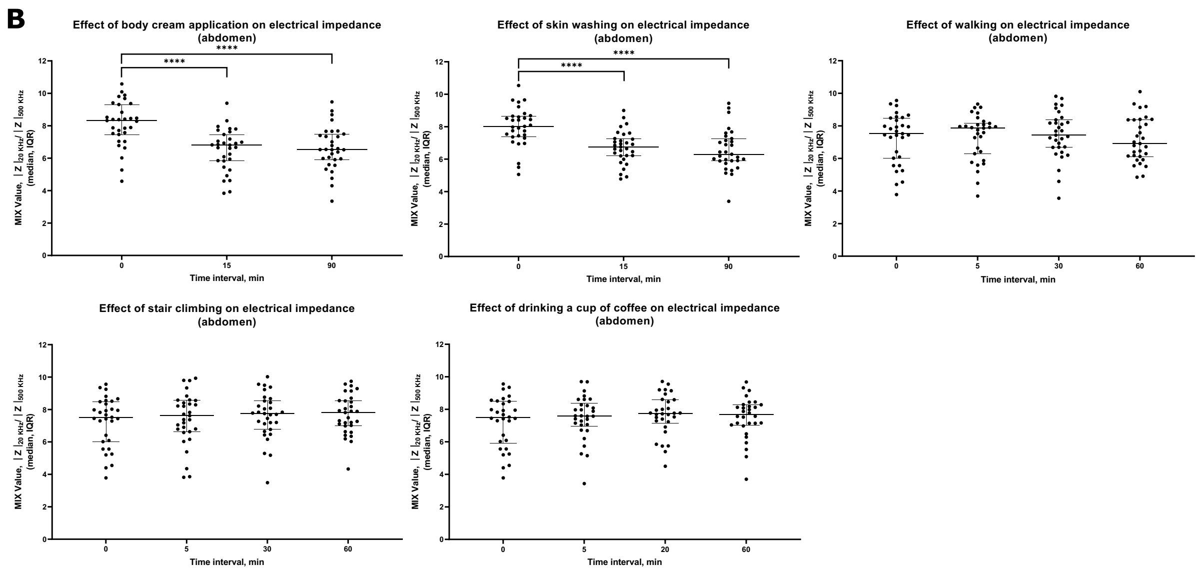
Table 3. Demographic characteristics of the study population

	Group 1 (18-29), n: 11	**Group 2 (30-49), n: 10**	**Group 3 (≥50), n: 10**	**Total, n: 31**
Age, median (IQR)	23 (22-25)	37,5 (31,75-44,5)	56,5 (55,5-60)	36 (24-56)
Gender: M/F (%)	45,46/54,54	40,00/60,00	40,00/60,00	41,94/58,06
Fitzpatrick Skin Type				
Type 1, n	0	0	0	0
Type 2, n	10	8	8	26
Type 3, n	1	1	2	4
Type 4, n	0	1	0	1
Type 5, n	0	0	0	0
Type 6, n	0	0	0	0

3.3.2. Application of body cream and skin washing affects measurements of electrical skin impedance

The first research objective was to study the effect of daily habits on measurements of EI. A significant decrease in EI was observed at 15 and 90 minutes after body cream application on both the volar forearm (15’: P <0,0001; 90’: P = 0,0015) and the abdomen (15’: P <0,0001; 90’: P <0,0001) compared to baseline measurements (Figure 7, Appendix 6.2.1, Appendix 6.2.2). Also, skin washing with warm water and soap showed a significant decrease of the EI after 15 and 90 minutes on the volar forearm (15’: P <0,0001; 90’: P <0,0001) and abdomen (15’: P <0,0001; 90’: P <0,0001) compared to baseline (Figure 7, Appendix 6.2.1., Appendix 6.2.2.). Moderate and heavy physical activity did not influence the EI after 5, 30, or 60 minutes on either one of the anatomical locations. Also drinking a cup of coffee did not significantly influence measurements of EI on the volar forearm or abdomen after 5, 20, or 60 minutes (Figure 7, Appendix 6.2.1., Appendix 6.2.2.).





<u>Figure 7.</u> **The effect of daily habits on measurements of electrical skin impedance**. Electrical skin impedance was measured in healthy subjects (n=31) at baseline and at different time intervals after body cream application, skin washing, walking, stair climbing, or drinking a cup of coffee at two different locations of the body: **A**; the volar forearm and **B**; the abdomen. Repeated-Measures one-way ANOVA and nonparametric Friedman tests were performed. Data are shown as median with interquartile range (IQR). **: P ≤0,01, ****: P ≤0,0001.

3.3.3. No correlation between age and measurements of electrical skin impedance

As the second research objective, we aimed to investigate whether there was a correlation between age and the measurements of EI. No significant relationship was observed between the age of the participants and the measurements of EI. This was the case for all anatomical locations (Figure 8). Note that the weak negative correlation between age and EI on the left volar forearm is almost significant (P = 0.0511, r = -0.3535). A slight decrease in EI is found with increasing age. The same trend, albeit not significant, is seen at all locations.

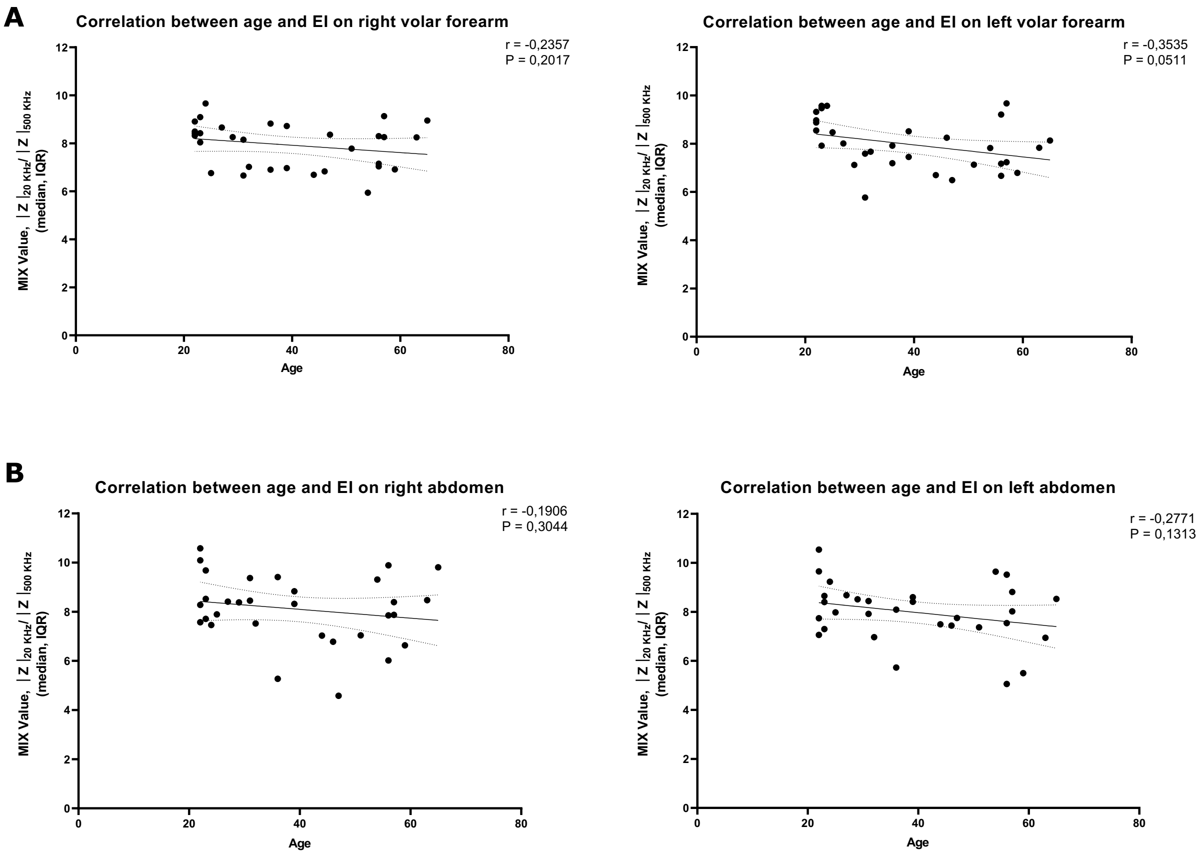


Figure 8. Correlation between age and measurements of electrical skin impedance. Correlations were calculated to investigate the correlation between age and electrical impedance in healthy subjects (n=31) on **A**; the right and left volar forearm and **B**; the right and left abdomen. A simple linear regression with the calculation of the Pearson's correlation coefficient and a nonparametric Spearman correlation were used. Data are presented as median and interquartile range (IQR).

3.3.4. No differences in electrical skin impedance measurements in left and right volar forearm and abdomen

Finally, the influence of the anatomical location on the measurements of EI was evaluated. There was no significant difference observed between EI measurements at the different anatomical locations being the left and right volar forearm and abdomen (Figure 9).

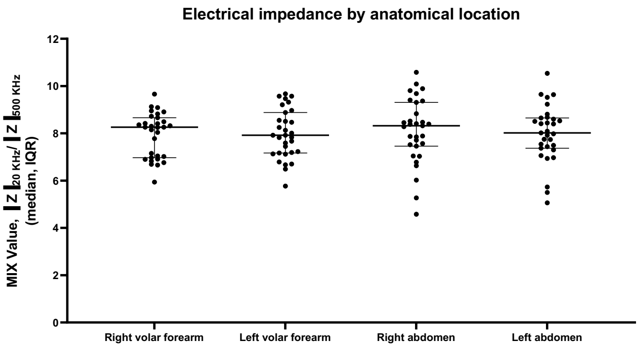


Figure 9. Effect of anatomical location on measurements of electrical skin impedance. Baseline measurements of EI were performed on the left and right volar forearm and abdomen in healthy subjects (n=31). A nonparametric Friedman test was performed. Data are shown as median and interquartile range (IQR).

3.4. Discussion

In this study, the effect of multiple daily habits on electrical impedance measurements, reflecting the skin barrier function, was examined. Among them, some were shown to have a significant influence on these measurements. Primarily, the application of body cream and skin washing with warm water and soap resulted in decreased values of EI after both 15 and 90 minutes. The decrease was seen on both the volar forearm and the abdomen. These differences can be explained by the fact that application of body cream and skin washing directly alters the resistive properties of the extracellular environment of the more superficial layers of the skin (measured mainly at 20kHz) by filling the pre-existing air-filled voids such as hair follicles, sweat glands, and furrows. [8] Skin hydration decreases the impedance of the skin to an applied current at all frequencies. [8] The resistive properties of the intracellular environment and the capacitive properties (measured mainly at 500kHz) are influenced to a lesser degree by skin hydration. Therefore, the numerator (low frequency) of the MIX value decreases at a higher level than the denominator (high frequency), which leads to an eventual decrease in MIX value as seen in this study. These results are in contrast with those of a recently conducted study by Morin et al. (8) published in 2020. In this study, the effect of hydration time on EI properties of the skin was evaluated using both *in vivo* (n=4) and *in vitro* (n=4) experiments. With increasing hydration time, an increase in MIX value was observed both *in vivo* and *in vitro*. This nonagreement can possibly be explained by the small sample size of this study and the slightly different research objective. While the aforementioned study examines the correlation between skin hydration time and EI, this current study examines the effect of one-time skin hydration on EI after different time intervals. Further research will therefore be necessary to clarify the correlation between skin hydration and the MIX value. For body cream application and skin washing, a significant effect on measurements of EI was still observed after 90 minutes. As we aimed to investigate multiple daily habits, time intervals were chosen arbitrarily to make the duration of the study feasible for the participants. To understand exactly how long the effect of these daily habits would last, additional research with measurements at more frequent time intervals and for a longer period will have to be conducted.

Exercise, both moderate and heavy, did not have a significant impact on measurements of EI on the volar forearm and abdomen after 5, 30, and 60 minutes. In contrast to EI, measurements of TEWL are influenced by exercise. (42, 53) This influence is due to perspiration that happens during activity. The TEWL probe cannot make the difference between water evaporation from deeper skin layers and water evaporation derived from the sweat gland. Measurements of EI will probably not be significantly influenced by activity and perspiration because the filling of the sweat glands alone will not be enough to significantly alter the electrical properties of the skin. Additionally, measurement of EI using the Nevisense device requires cleaning of the skin region to be tested and hydration with a wound cleansing wipe. This ritual will remove any excess sweat or other substances present on the surface of the skin.

Also, the intake of a hot, caffeine-containing beverage, in the form of a cup of coffee, had no significant effect on measurements of EI on the volar forearm and abdomen after 5, 20, and 60 minutes. This factor was specifically investigated because guidelines advise avoiding drinking caffeine/theine-containing and/or hot beverages before measurements of TEWL. (4) The effect of this daily habit on skin barrier function measurements can be explained by a slight rise in body temperature due to the intake of a hot beverage which can lead to an increase in perspiration and also the effect of caffeine itself on the blood supply to the skin. Caffeine causes vasoconstriction of the blood vessels and will thereby lead to an increase in intra-arterial pressure. [54] Additionally, caffeine stimulates the sympathetic nervous system which leads to an increase in sweat gland activity. [55] Despite the speculated effect of hot and/or caffeine-containing beverage intake on measurements of TEWL, this effect could not be observed during measurements of EI in this study.

When evaluating the correlation between the age of the participants and the results of measurements of EI, a non-significant, weak, negative correlation could be found at all anatomical locations. This is in contrast with the study conducted by I. Nicander et al. (43) which showed a significantly higher MIX value in the elderly participants (>60 years old) compared to the younger participants (20-40 years old). [43] As also mentioned in the introduction, the effect of increasing age on the skin barrier function is still controversial. [43-46] Even though no significant correlation between age and EI was found during this study, it is important to point out that the age in this study ranged only from 22 to 65 years. No children or elderly people (>65 years old) were included in this study. Additional large-scale research including participants originating from a broader age range is needed to investigate if increasing age does in fact has an impact on skin barrier function and EI properties of the skin.

Lastly, no significant differences in measurements of EI could be found between the left and right volar forearm and abdomen. Research has shown the existence of significant differences in skin barrier function measured by TEWL at different anatomical locations due to variations in the composition of the regional skin. [35, 38] Among these different anatomical locations, measurements on the volar forearm and abdomen generally led to comparable results. [35, 38] This trend was also observed in this study.

In this study measurements at different anatomical locations were only confined to the left and right volar forearm and left and right sides of the abdomen. These locations were chosen because literature research has shown that these were the most used locations for the evaluation of the general skin barrier function of an individual. As shown in this study, no significant differences were found and both anatomical locations can be used with comparable results. From experience, we suggest using the volar forearm due to practical advantages.

All measurements were performed after 30 minutes of acclimatisation in a relative humidity-controlled room with a relative humidity of around 55% and a temperature of 22 °C. This was recommended by the guidelines for the measurement of TEWL because research has shown that TEWL is highly dependent on these environmental factors. [3, 4, 29] It was shown that EI also can be influenced by the humidity of the surroundings due to an alteration in skin hydration. [56] Also the temperature of the room can influence skin hydration and skin temperature and can lead to an alteration in EI. [57] Therefore, measurements of EI should preferentially also be performed in a controlled room after 20-30 minutes of acclimatisation. [29] The influences of room temperature and humidity were not examined in this study.

In this study, measurements of both TEWL and EI were examined. While both of them provide information on the barrier function of the skin, the relationship between these properties is still not clear. This can possibly be explained by the fact that these two techniques measure very different properties of the skin barrier function. TEWL is based on measurements of water evaporation from deeper skin layers and therefore mainly reflects the permeability of the skin. [2, 28] This permeability depends on the intercellular lipid layer of the stratum corneum and the tight junctions at a lower level in the epidermis. [15] As mentioned above, measurements of EI do not only reflect the extracellular environment but also the intracellular environment, structure of the cell membrane, and other properties of the skin cells such as size, shape, orientation, and compactness. EI is also not only confined to the epidermis but can be measured at different depths down to the subcutis (Figure 4).

The use of EI and related to this the MIX value is a relatively new approach for the evaluation of skin barrier function that has not yet been extensively researched. The results of this study may initiate further research on the use of this biophysical property of the skin under different circumstances and in multiple skin diseases.

This study is also one of the first to specifically focus on the effect of multiple common daily habits of people on measurements of skin barrier function. Therefore, this study will contribute to the development of more targeted guidelines to be followed before measurements of TEWL and the electrical properties of the skin barrier. This study shows that besides the application of body cream and skin washing, other daily habits did not influence measurements of EI. Also, age and anatomical location did not have a significant influence. The present study also demonstrates that EI can be a reliable method for the assessment of skin barrier function in healthy individuals (with a normal skin barrier). Further research including participants with both healthy and diseased skin should be performed to further investigate the applicability of this biophysical property in skin barrier function measurement. The development of new algorithms using more than 2 impedances obtained by one measurement can also be an important further step in the application of EI for the assessment of skin barrier function.

3.5. Conclusion

This study shows that the application of body cream and skin washing with warm water and soap should be avoided at least 90 minutes before initiating the measurements of EI on the volar forearm and abdomen. On the contrary, exercise, both moderate and heavy, did not influence EI measurements. Therefore, participants should not particularly restrain from exercising before their appointment. Also, coffee intake did not influence measurements of EI and should therefore not be avoided. No significant relationship was found between the age of the participants and the EI. Lastly, no differences in MIX values were found between the left and right volar forearm and abdomen.

Some clear influences of the effect of daily habits on skin barrier function measurements were proven in this study. Nevertheless, this study can only be regarded as a good pilot study for further research in a better understanding of the influences of these daily habits. Additional research is necessary to determine more exactly the duration of the influence of body cream application and skin washing and to investigate other habits like drinking tea, alcohol intake, smoking, …

3.6. Disclosures

The authors declare that they have no competing interests.

3.7. Conflict of interest

The authors declare that they have no conflict of interest.

3.8. Funding

There was no funding source.

3.9. Ethical approval

This study was completed in compliance with national legislation and the guidelines of the ethical principles of the Declaration of Helsinki. [58] The study was approved by the local Committee of Ethics at the UZ Brussel/VUB (study number: EC-2021-303).

3.10. Informed consent

There was a written informed consent obtained from all individual participants included in the study.

4. Acknowledgment

To complete this research, I actively collaborated with Pauline Thys, a final year master's student of Pharmaceutical Sciences at the VUB. She collaborated on this topic as part of her master's thesis. This resulted in us being able to divide the various tasks of this research among ourselves. I initially started this subject by performing a thorough analysis of the current literature, working out the research questions, and drawing up a protocol and informed consent for approval by the Ethics Committee. In addition, I was responsible for leading this research in the right direction and was the point of contact for physicians, researchers, and participants. The tasks of recruiting and including were divided among all the members of the research team. In total, Pauline Thys was able to include 21 participants and I was able to include 10 participants. Finally, the data obtained during this study was divided among us so we could individually write our master's theses. In this master's thesis, the main focus is the EI as measurement of the skin barrier function. Results of the TEWL measurements will be discussed in more depth in the master's thesis of Pauline Thys. I would therefore like to thank her for her contribution to this master's thesis and for her help in including the participants.

I would also like to thank my promotor Prof. Dr. Jan Gutermuth and co-promotor Prof. Dr. Inge Kortekaas for their support and guidance in conducting this study and writing this master's thesis. Without them, it would not have been possible to execute this research. They have guided me in my growth as a scientist and future medical doctor.

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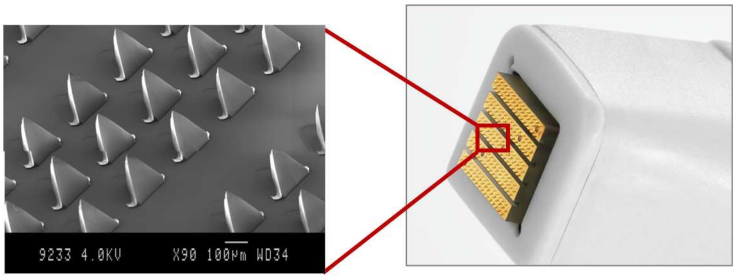
6. Appendix

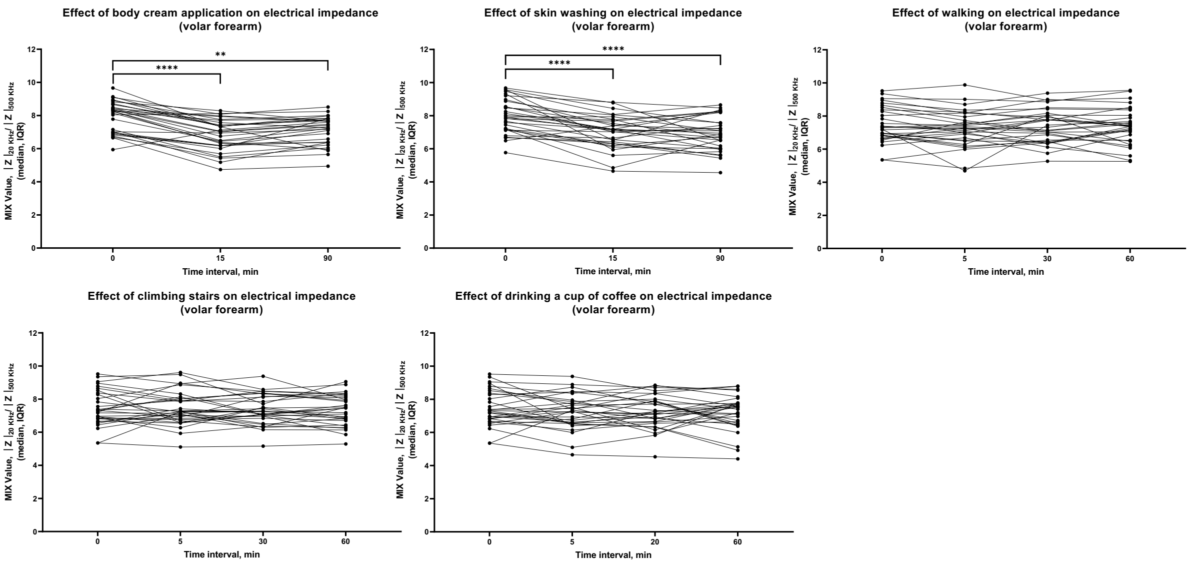
6.1. The Nevisense Device

6.1.1. Nevisense microinvasive electrode



6.2. The effect of daily habits on measurements of electrical skin impedance

6.2.1. EI measurements on the volar forearm



6.2.2. EI measurements on the abdomen