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Titel der Masterarbeit / Title of the Master's Thesis

## „Effects of nutrition, lifestyle and intervention with equol on structure and absolute telomere length in skin“


verfasst von / submitted by

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# List of abbreviations

| | |
|---|---|
| aTL | absolute telomere length |
| bp | base pair |
| °C | degree Celsius |
| DNA | deoxyribonucleic acid |
| e.g. | exempli gratia; for example |
| enc. | encapsulated |
| f. | forward |
| kbp | kilobase pair |
| min. | minute |
| ml | millilitre |
| neg. | negative |
| NFW | nuclease free water |
| nr. | number |
| PBS | phosphate-buffered saline |
| PCR | polymerase chain reaction |
| pmol | picomol |
| pos. | positive |
| qPCR | quantitative real-time polymerase chain reaction |
| r. | reverse |
| ROS | reactive oxygen species |
| SELS | surface evaluation of living skin |
| sec. | second |
| µl | microliter |

# 1. Introduction

Aging has become an increasing relevance in our daily life. There are many studies performed on this topic, because it is linked with a lot of environmental factors. Further, lifestyle e.g. alcohol, smoking, nutrition and sport influence the structure of the skin.

The masterwork was done in a project, where another colleague was involved. We investigated the effect of equol, a metabolite of isoflavone in soy, on skin aging. Analysis regarding to structure parameter by the means of skin analyse equipment and investigation of cells on molecular level, were implemented. The work of project was separated into two parts, thereby my colleague, Christina Urbanek, focused on DNA methylation with LINE-1 and me myself on absolute telomere length measurement. In addition, the relationship between aging and lifestyle was evaluated, accordingly a Food and Lifestyle Questionnaire was included in the intervention study.

## 1.1. Equol

The isoflavones in soybeans are found in form of glycosides or as aglycones. Daidzein and genistein represent the aglycones whereas genistin, daidzin and glycitin represent the substances with a glycosidic bond. Soybeans contain mainly genistein approximately 75,8 mg per 100g followed by malonylgenistein with an amount of 74 mg per 100g and daidzein with 62 mg per 100g. But it is important to mention, that storage and converting influence the content of those isoflavones [Knasmüller et al., 2014].

Equol is a metabolite of the isoflavones, daidzin and daidzein, contain in soy. Equol was found 1932 by isolating from equine urine and 50 years later it was first identified in human urine. The substance is the product of biological metabolism from intestinal bacteria [Setchell K. D. R. et al., 2010; Setchell K. D. R., et al., 2002].

An interesting fact is, that only 1/3 of the European population is able to produce equol from soy daidzein, related to individual differences in the composition of the intestinal flora.

### 1.1.1. Chemistry and formation

From a chemically point of few, equol [7-hydroxy-3-(49-hydroxyphenyl)-chroman] is a nonsteroidel estrogen. The biotransformation from daidzein to equol includes 3 main steps. First of all daidzin is reduced by hydrolyse to the aglykon daidzein. This is necessary, because the conjugated forms are not bioavailable.

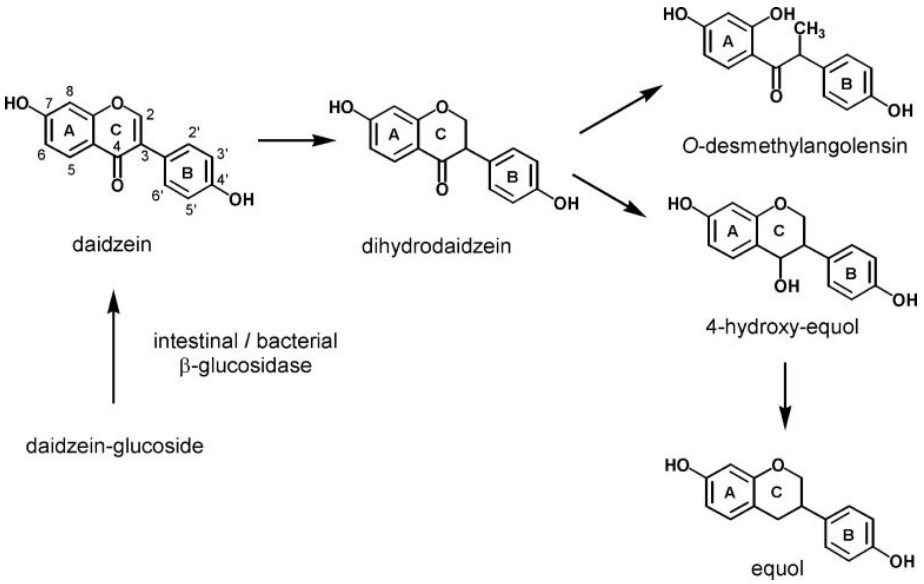


**Figure 1 Biotransformation from daidzein to equol [Rüfer et al., 2006]**

The hydrolysis is induced by bacterial glycosidase or brush border membrane β-glucosidase in the proximal intestine. The next step is the reduction from daidzein to dihydrodaidzein, which further is converted to either O-desmethylangolensin or 4-hydroxy-equol, the precursor of equol [Setchell K. et al., 2010; Rüfer C. et al., 2005].

### 1.1.2. Antioxidant and epigenetic activities of equol

Equol has, like a lot of other isoflavones, antioxidant properties. Thereby it acts as a free radical scavenger [Yuan J-P. et al., 2007]. Mitchell and his colleagues showed in their study, that equol has a more antioxidative activity than genistein and daidzein [Mitchell et al., 1998]. This effect is due the nonplanar structure, which impart equol with a better flexibility for conformational changes [Yuan J-P. et al., 2007]. Further,

equol has a strong antioxidative effect against UV irradiation, which may affect skin carcinogenesis, inflammation and immunosuppression positively [Widyarini S. et al., 2006]. Isoflavones and further equol are linked with epigenetic mechanisms, which regulate gene expression, including histone modifications, DNA methylation noncoding RNAs and nucleosome positioning [Pudenz M., 2014].

### 1.1.3. Equol and dermal aging

Chronological aging and photo-aging are the result of Reactive Oxygen Species (ROS) production. Therefore it is important that botanicals like equol combat skin aging.

Chronological aging: free radicals are produced by aerobic cellular reactions and lead to mitochondrial damage and in further way to cellular death. During these processes it is important that botanicals like equol repair damaged skin cells, otherwise mutations occur in premature aging.

Extrinsic aging, caused by solar UV radiation or ultra violet light, is called photo-aging. There are two main types of UV radiation: UVA and UVB. On the one hand, UVB with just an amount of 2-5% of the sun's emissions, already able to damage DNA, irrupts epidermis cells and may trigger photo-aging. On the other hand there are 95-98% of UVA radiations on earth. It damages the epidermis as well and further deteriorates collagen and elastin fibres, because of deeper irruption into the dermis.

Photo-aging depends on different skin types and time of exposure without skin protection [Lephart E. D., 2016].

Equol may have an impact on skin regeneration and health. Lephart found out that the isoflavone equol significantly stimulates collagen and elastin gene expression, which is associated with an optimal dermal health. Further it can decrease wound healing and decelerate neoplastic growth [Lephart E. D., 2013].

### 1.1.4. Biological properties of equol

Equol has the ability, because of its equality to estradiol, to bind to the estrogen receptor (ER). Equol is present in the form of R and S isomers, in which S- equol seems to be the biologically active form. The isoflavone shows in binding assays, a strong affinity for ER$\alpha$ and ER$\beta$. The binding preference of equol clearly lies with ER$\beta$. Although equol may be an interesting pharmaceutical agent for hormone-dependent disorders, it has a number of other appreciable biological properties like cancer, osteoporosis, menopausal symptoms and cardiovascular diseases [Setchell et al., 2010].

## 1.2. Skin structure and function

Skin is the largest organ of the human body and is adjacent to the organism from the environment. The skin fulfils sensory operation, as well as contact and protective function. It comprises two main layers. The first layer is called epidermis, which portrays the thin outer layer consisting of plate-like cells. Second, the underlying dermis comprising elastic and fibrous connective tissue. This under layer contains blood vessels, nerve fibres, hair follicles and glands [Dorling Kindersley, 2002; Fritsch P., 2004]. Epidermis includes stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale.

In epidermis there are keratinocytes, which make up the biggest part of cells. The regeneration mainly takes place in stratum basale. Within 30 days, the keratinocytes are pushed from the bottom up, and finally the dead keratinocytes flake off on the surface of stratum corneum. Between basale cells, Merkel cells and melanocytes are located. Merkel cells act like secondary sensory cells and melanocytes are pigment cells. In the cytoplasm of melanocytes, brown-black eumelanin and yellow-reddish phaeomelanin are formed through inducible tyrosinase, which is caused by UV radiation. By accumulation of these pigments, melanosomes are formed, which are taken up by the surrounding keratinocytes. The melanonsomes absorb UV rays. With higher sun exposer, the skin becomes tanned by an increase of pigments in the melanosomes. In stratum granulosum, the keratinization of the cells is introduced and

they lose their mitochondria. The cell organelles are depleted and forming into flat organisms. In stratum lucidum, only cells with complete keratinization (cirnification) are found. In these organisms, no nucleus or organelles are located. Stratum corneum contains only dead keratinocytes [Jastrow H., 2016].

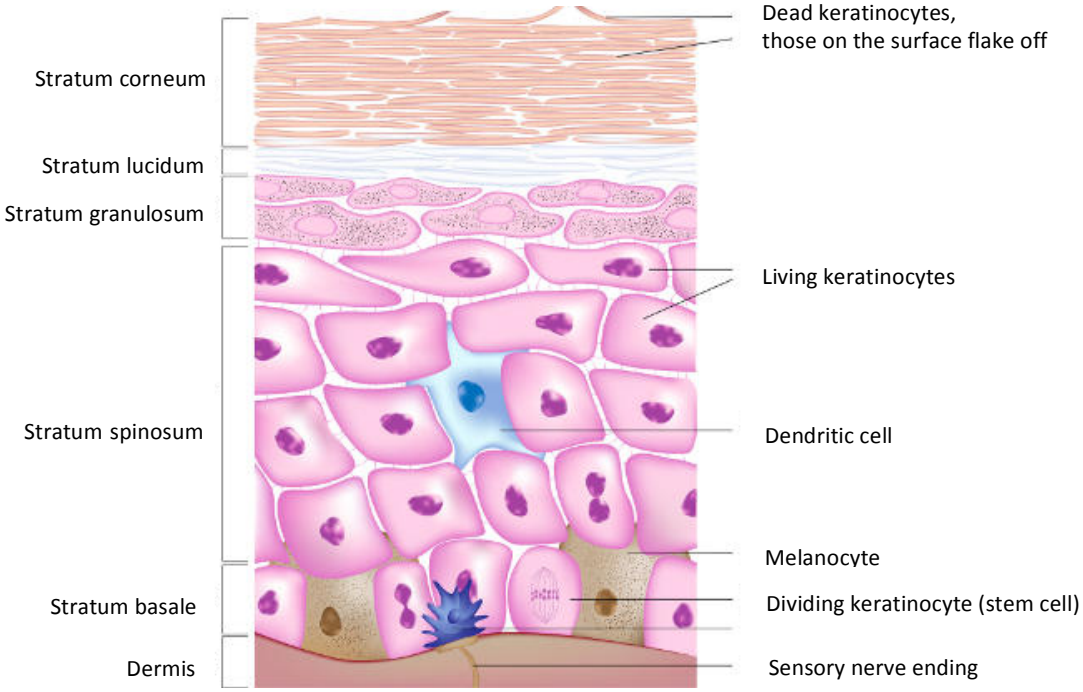


**Figure 2 Skin structure [UkEssayes, 2015]**

## 1.3. Nutrition and skin

Nutrition is associated with skin health and further influences, like possible aging processes and aspects of integrity. Scientific investigations have shown, that dietary interventions and nutritional patterns affect skin health and prevent skin diseases such as, atopic, dermatitis, acne or even aging. Those effects can be attributed to essential nutrients [Pappas et al., 2016].

## 1.4. Aging process

At a fundamental level, aging can be outlined as a progressive decline of the biological functions of human cells, ultimately resulting in senescence. Aging processes, affected

by environmental, nutritional and lifestyle-based factors lead to greater susceptibility of age related diseases. Lopez-Otin et al. categorized nine molecular and cellular hallmarks of aging. Those hallmarks contribute aging process and determine aging phenotype. Hallmarks of aging include several factors like telomere attrition, genomic instability, epigenetic alterations, mitochondrial dysfunction, cellular senescence, deregulated nutrient-sensing, stem cell exhaustion, loss of proteostasis and altered intercellular communication [Lopez-Otin et al., 2013; Kaeberlein M., 2007].

### 1.4.1. Mechanisms of skin aging

Skin aging distinguishes two main mechanisms, which are differenced in intrinsic aging and extrinsic aging. Intrinsic aging, the biological process, is largely genetically determined and extrinsic aging is caused by environmental exposure.

Intrinsic skin aging is comparable with the aging process in most internal organs. Those are defined as slow deterioration in function of the tissue. Whereas the stratum corneum stays relatively unchanged, the epidermis and dermis thin down by flattening of the dermo-epidermal junctions. Fibroblasts reduce biosynthetic capacity and elastic tissue progressive disappear in the papillary dermis. During intrinsic skin aging, collagen content decreases and collagen fibres become increasingly dense and get tightly packed.

Extrinsic skin aging, more commonly called photo-aging, is caused by exposure of ultraviolet light. Photo-damaged skin is marked by loss of elasticity, irregular pigmentation, deep wrinkling and increasing roughness and dryness. It has been suggested that sun exposure cause 80% of facial aging. Photo-damaged skin leads to changes in three components: collagen fibres, glycosaminoglycans and elastic fibre network. (Collagen provides tensile properties to the dermis. Glycosaminoglycan plays an important role in hydration of the skin. Elastic fibres network obliges elasticity of the skin.) [Jenkins G., 2002]

Aging, a tissue-specific process is among other things associated with epigenetic alterations and telomere length [Lopez-Otin et al., 2013].

## 1.5. Scientific background Telomeres and Telomerase

### 1.5.1. Telomeres

Telomeres are nucleoprotein structures at the end of eukaryote chromosomes. Those structures have the ability to protect and stabilize the chromosomes. Telomeres become shorter with each cell division, and are finally reduced to the point of invalid chromosome protection. The unprotect chromosome ends emit signals that ensure that the cell can no longer divide. This status is called “senescence” [Balk et al., 2013].

The DNA structures from the telomeres consist of several thousands of repetitive G-rich sequences. This sequence is in human cells: *5’-TTAGGG-3’*. As mentioned before, telomeres get shorter during every replication. Starting the replication on the 3’-end and binding the strand to the right primer, it is again removed through the 5’-3’-exonuclease, so the DNA polymerase has no free 3’-OH-groups with which it fill the gap. In this way, every round of replication, 50-250 nucleotides get lost. The consequences are the reduction of telomeres and finally it leads to instability of chromosomes. This process is linked to the aging process [Löffler G., 2008].

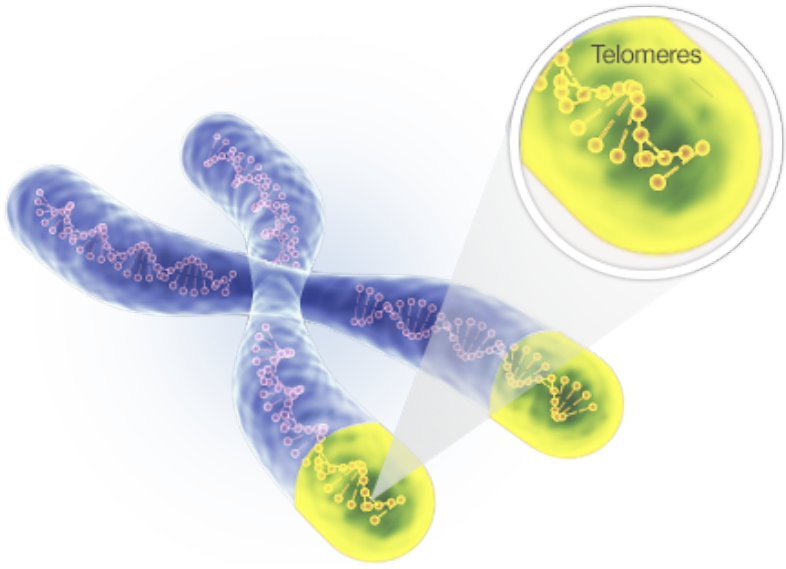


**Figure 3 Chromosome with telomeres [Abbexa, 2016]**

### 1.5.2. Telomerase

Telomerase is an enzyme complex, which maintains telomere length. Telomerase has the ability to repair telomere-damage and eliminate chromosome shortening. The enzyme contains two key components, a reverse transcriptase component named hTERT and a RNA template called hTERC. This Component (hTERC) adds telomere repeats to the end of chromosomes and is utilized by TERT. Other proteins including dyskerin are involved in the telomerase complex. Dyskerin stabilizes small nucleolar RNA's like TERC [Buckingham and Klingelhutz, 2011].

### 1.5.3. Telomerase in human skin

The enzyme complex telomerase was determined in keratinocytes of the epidermis, but not in fibroblasts. There is evidence that telomerase may provide a certain amount of telomere maintenance. Hence, telomerase activity in epidermis depends of its need for repair damages or cell proliferation. Exogenous expression of TERC can lead to telomerase expression. Due the fact, that keratinocytes express low levels of TERT, telomerase can maintain telomeres with a combination of external supply of TERC. This mechanism has only little effect in fibroblast, because they do not express TERT on their own [Buckingham and Klingelhutz, 2011].

### 1.5.4. Telomeres and aging

In 1986 the first observations of associating telomeres directly to aging, were made. Cooke and Smith detected, that the average length of telomeres in sperm cells were longer in adult cells [Aubert and Lansdorp, 2008]. With each cell division, telomeres decrease up to 150 base pairs, so telomere shorting acts like a biologic clock [Buckingham and Klingelhutz, 2011; Kosmadaki and Gilchrest, 2004]. Specialized reverse transcriptase maintains telomere length and is called telomerase. So telomere-shortening results in the telomerase lacks in normal somatic cells [Sugimoto et al., 2005]. As mentioned before, telomerase was detected in keratinocytes but not in skin fibroblasts [Buckingham and Klingelhutz, 2011]. When telomeres get a critically short

length, the cell achieved a status called senescence. Thereby the cell irreversibly stops proliferation.

Various studies showed a decrease of telomere length with age, which varied between individuals and species of the same age. The average reduction rate of telomeres reaches from 19.8 bp/year up to 92 bp/year. Sugimoto et al. showed in epidermis, as well as in dermal age, associated telomere loss with a rate from 9 and 11 bp/year [Sugimoto et al., 2005; Aubert and Lansdorp, 2008].

There is evidence, that estrogen and c-Myc stimulate the expression of TERT [Aubert and Landsdorp, 2008]. Scientists investigated the anti-aging effect of estrogen by stimulation of TERT. Therefore, they examined whether estrogens are capable to prevent senescence of endothelial progenitor cells. 17β-estradiol significantly increased activity of telomerase, which concludes elongation of telomeres [Toshio et al., 2005].

# 2. Objectives

The purpose of this master thesis was to analyse the influence of nutrition, lifestyle and equol intervention on skin structure, absolute telomere length and epigenetic regulation in skin cells.

An important aspect was to establish a method to take skin cells from the forehead. After several methods, we investigated that the stripping method with an adhesive film seemed to be the best procedure. Afterwards, we decided to analyse forehead skin with two scientific certified skin analysis devices.

Soy isoflavones are attributed with various functions like anti-inflammatory properties, antioxidative capacity, reducing risk of cancer and protective effects on coronary heart diseases and menopausal osteoporosis. Furthermore, equol is discussed to have protective properties on skin regulation and health. Therefore, we investigated the effect of the substance on skin aging in our intervention study. We wanted to discover, if treatment with 0.5% equol preparation has a positive impact on structure and molecular parameter. Objective of absolute telomere length was to determine the impact of equol on telomere length of human cells, which were taken from the forehead.

# 3. Material and Methods

## 3.1. Study design

The study was structured by investigating the effect of topically applied equol on the forehead area of women. The exclusion criteria were as follows: no apparent skin disorder (psoriasis, dermatitis, skin cancer), female and at the age of 40-60 years. In the intervention study we used a lecithin-based micro emulsion. It was either with equol or encapsulate-equol offset. The test substance, which contained 0.5% equol-E55 came from System Biology.

Finally 52 female participants were separated randomly in three groups.

- Group one: lecithin emulsion with equol powder (E-55-Equol Skin®)
- Group two: lecithin emulsion with microencapsulated equol (E-55-Equol Skin®)
- Group three: placebo emulsion with lecithin

Lecithin is a natural component of the cells and essential for the correct function of biological mechanisms. Further it is an emulsifying agent. The positive properties of lecithin are due to the phosphatidylcholine. Phosphatidylcholine has a strong effect on the skin regeneration [Dermaviduals, 2016; Waßner D., 2016].

Microencapsulation is a method, in which small portions of solid, liquid or gaseous substances are surrounded with a polymeric or inorganic capsule. A benefit of microencapsulation is the controlled release of substances. Further it may protect sensitive substances against environmental influences [Gouin S., 2004; ChemgaPedia, 2016].

### 3.1.1. Procedure

Group of volunteers were sampled at specific dates and times at university. Participants were asked not to use any other skin care products or cosmetic treatments during the intervention time and they should not wear make up or cosmetic products on days of analysis. We tried to have the same environmental conditions all days of intervention, like humidity and temperature. (Time point 0:

Humidity: 29,5% ± 5,8% and temperature: 25,1°C ± 1,0°; Time point 1: Humidity: 47,4% ± 5,6% and temperature 25,3°C ± 1,3°C). First of all, the participants filled out our prepared Food and Lifestyle questionnaire. As our next step, we started the analysis. The subjects were sitting on a comfortable chair, where they were allowed to relax and support their head on the backrest. We made the measurement in the following order: skin structure parameter by means of UV-light video camera (Visioscan® VC 98), sebum production (Sebufix® F 16) and parameters about elasticity and firmness (Cutometer® dual MPA 580). After those measurements, we removed skin scales of the forehead with a stripping method. At last they got the preparation in the form of a roll-on stick, which ensured hygienic and easy application. Samples were taken again after 10 weeks of intervention (time point 1). This time, instead of a food and lifestyle survey, the subjects received a self-assessment questionnaire.

For molecular analysis we obtained skin cells from our participants, which we were able to purify and investigate on the absolute telomere length and the DNA methylation with LINE-1. We had six strips per subject and consequently six DNA samples. But for analysis, only the first and the second strip/DNA-sample were used.

### 3.1.2. Food and lifestyle questionnaire

Food and lifestyle affect our health and is positively associated with epigenetic mechanisms and telomere length. The questionnaire, used in the intervention study, included parts about anthropometry (BMI, age, weight, height), state of health (diseases, medication, physical activity, sport etc.), eating habits (preferred food and portion size, liquid intake, coffee and alcohol consumption) and cosmetic products (skin cleansing and care).

### 3.1.3. Self-assessment questionnaire

Subjective perception was assessed by means of the self-assessment questionnaire. In this form we asked about their improvement and difference of skin texture. It included questions about the following categories: skin smoothness, moisture, pleasant feeling, freshness, oiliness and general differences.

### 3.1.4. Sample collection

After several non-invasive methods were investigated, we decided to establish our own procedure with sterile adhesive strips. Therefore we dabbed carefully the forehead of the participants, to remove excess sebum. (The women were asked to use no make up and skin care product on the days of analysis.) Afterwards we removed the protective film, under hygienic conditions, of the strips and affixed it gently on the middle of the forehead. Then we pulled the strip off quickly and stuck it back on the protective film. Finally we put the whole strip in a sterile (disinfection with ethanol and irradiation with UV-light) plastic bowl with a lockable cover and stored it at -4 °C.

## 3.2. Cutometer® dual MPA 580

For elasticity and firmness measurement we used the skin analyse device Cutometer® dual MPA 580 from the company Courage & Khazaka electronic GmbH. It is a standardized elasticity measurement method and scientifically proven. The measuring principle is based on suction. Thereby it produces negative pressure on the skin, which primes the skin mechanically into the probe, and after a specified time, is released again. The measuring system depends on a non-contact optical principle. The probe contains a light source, a receiver diode and two opposing mirrors prisms, which guides the light beam from transmitter to receiver. The light intensity depends on the depth of penetration on the skin in the probe.

### 3.2.1. Procedure

For the measurement, the subjects were sitting on a comfortable chair, where they were allowed to relax and support their head on the backrest. We measured the forehead of each subject with a tape measure. This was important for finding the middle and to have the same place for the second time point of intervention. Further we put the probe on the forehead and started the measurement via Cutometer®. Live curves on the screen represented the measurement, and thereof structure parameters were calculated via Cutometer® software.

In the following graph the curves from an example measurement are shown:

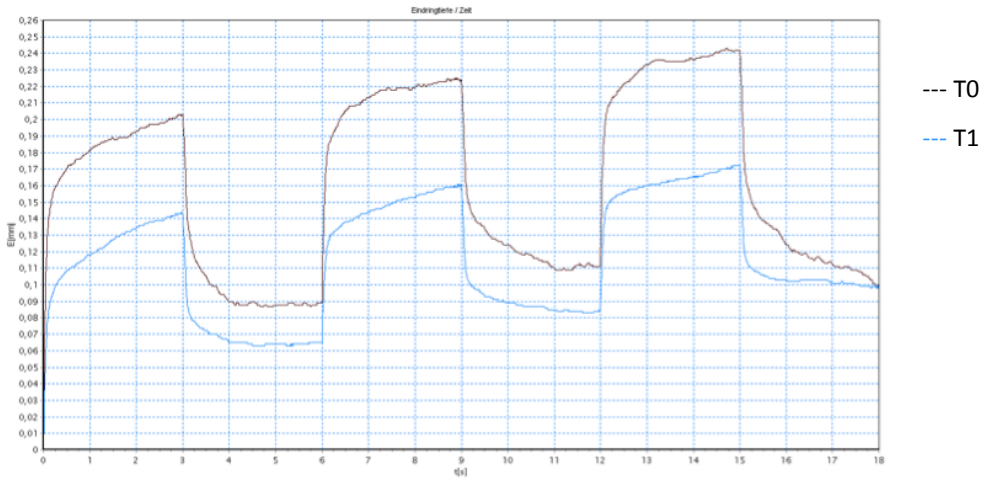


**Figure 4 Cutometer: example curves**

The upper peaks from the curves show the penetration depth into the probe (mm/time) and the lower peaks show the return into the original condition. The measurement principle is thus provides information on the elastic and viscoelastic properties of the skin and detects the biological skin age [Courage and Khazaka, 2016]

## 3.3. Visioscan® VC 98

Visioscan® VC 98, from Courage & Khazaka electronic GmbH as well, is a skin analysis device, with which the topography from the skin is measured. It is a UV-light video camera, which shows high quality pictures of the skin structure. The camera includes a high-resolution black/white video sensor and an annular UVA light pipe. It illuminates the skin equally and provides impressive images, which can directly be seen on the connected screen. Thereof structure parameter were calculated by means of Visioscan® VC 98 software. Apart from the topography analysis, Visioscan® VC 98 can also measure the sebum. Therefore we used special films Sebufix® F 16.

### 3.3.1. Procedure

For the measurement we had the same conditions as for the Cutometer® measurement. We also put the camera on the middle of the forehead and made the picture of the skin surface. Afterwards we stuck a Sebufix® F 16 film on the camera, put them on the forehead and let them stay for 30 seconds on the surface. This is important for seeing the oily spots on the foil. The greater the sports are, the oilier the skin is [Courage and Khazaka, 2016].

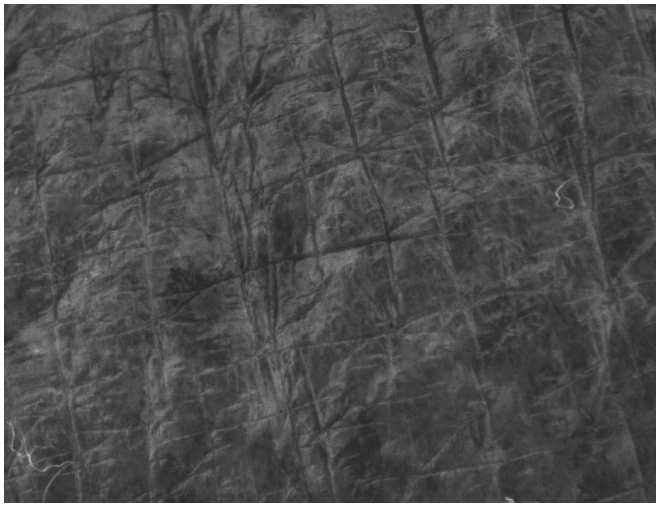

**Figure 5 Structure of the skin surface (example image)**

**Figure 6 Example image of sebum analysis**

## 3.4. DNA extraction

### 3.4.1. Equipment

- Ethanol (96%)
- Phosphate-buffered saline (PBS)
- Buffer AL
- Proteinase K
- Equilibrate Buffer AE
- Wash buffer AW1 and AW2
- Pipettes and tips with aerosol barrier
- Heating block (56°C)
- Micro centrifuge
- Vortex
- 1,5 ml micro centrifuge tubes
- Mini spin columns and collection tubes

### 3.4.2. Procedure

For purification of total DNA we used the QIAamp® DNA Mini Kit from QIAGEN. This method is based on the selectively DNA binding under certain buffer conditions to a silica membrane. This membrane is embedded in the bottom of a spin column, whereby the isolation of DNA is enabled.

First of all we prepared all reagents for the implementation. 125 ml 96% ethanol was added to AW1 buffer and 160 ml ethanol was added to AW2 buffer. If there is precipitate in buffer AL, it has to be heated up shortly at 56°C to solve the precipitate.

After sample collection via stripping with PCR films, (Thermo Scientific ASF 6020/100) we prepared them for purification. Therefore we cut them in small pieces. A 1.5ml micro centrifuge tube was filled with PBS (400µl) and the pieces of the strip were added. After 15 seconds vortexing, 400µl buffer AL and 20µl proteinase K were subjoined. It was followed by mixing (15 sec.) via vortex. Afterwards the tubes were

incubated at 56°C in the heating block for 10 minutes. 400µl ethanol addition followed and 15 sec. vortexing were applied again.

700µl of the extraction liquid was carefully applied to the spin columns and then centrifuged in the micro centrifuge at 8000 RPM for 1 minute. We repeated the later step twice. Afterwards, the spin column was transferred into a new collection tube. For our next step, washing buffer AW1 was added and centrifuged at 8000 RPM 1 another minute. We repeated this step with AW2 at 14000 RPM for 3 minutes instead of AW1. Between the washing steps we emptied the tubes. After the spin column was transferred into a new collection tube again, it was centrifuged "empty" at 14000 RPM for 1 minute. At last the elution step was followed. Therefore we placed the spin column in a 1.5ml micro centrifuge tube and added 150µl buffer AE. After incubation (room temperature, 1 min.) the tube was centrifuged at 8000 RPM for another minute.

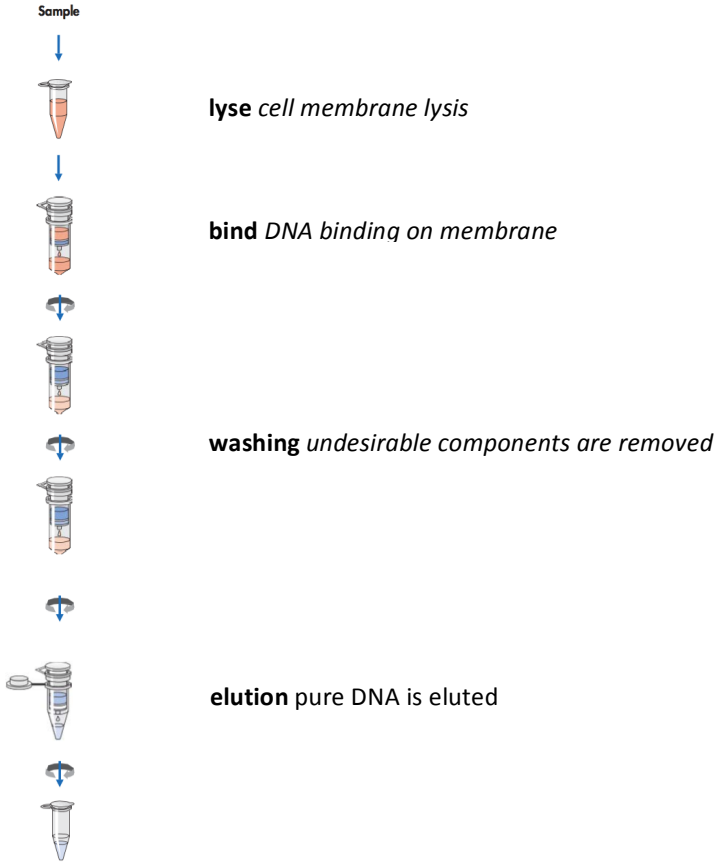


**Figure 7 Steps for DNA extraction [modified from QIAGEN, 2016]**

The samples were stored at the freezer at -15°C [QIAGEN, 2016; Franke N., 2010].

The original protocol is attached in appendix.

## 3.5. Telomere methodology

### 3.5.1. Equipment

- Primer forward and reverse for telomere and single copy gene 36B4
- Master mix (LightCycler master mix with SYBR Green Dye from Roche)
- 96-well plate
- Pipettes and tips with aerosol barrier
- AB StepOnePlus™ Real-time PCR system (Applied Biosystems)
- StepOnePlus™ software (Applied Biosystems)

### 3.5.2. Procedure

Absolute telomere length was being measured via quantitative real-time polymerase chain reaction (qPCR) method. This method is based due fluorescent signal detection, and is well established for small amounts of DNA from human tissues, like we have it by stripping the forehead. For the longitude of the absolute telomere measurement we used an oligomer standard.

For telomere measurement we used the skin samples after extraction and stored at -15°C. First of all, we prepared the standard curves for telomeres and single copy gene 36B4, by attenuation of known quantities of oligomers. (Important: for every qPCR run we prepared the standard curves fresh, because they were not long durable.)

**Table 1 Standards for aTL measurement [modified from: O'Callaghan and Fench, 2011]**

**Standards**

| *Oligomer standard* | *Oligomer sequence (5'-3')* | *Amplicon size* |
|---|---|---|
| Telomere standard | (TTAGGG)14 | 84 bp |
| 36B4 standard | CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAA GGCCAGGACTCGTTTGTACCCGTTGATGATAGAATGGG | 75 bp |

With the above-mentioned standards, we made a standard dilution series. The scheme is shown in the following table:

**Table 2 Standard dilution series for telomere and 36B4 standards**

**Standard dilution series**

| *Telomere standards* | | *36B4 standards* | |
|---|---|---|---|
| TX1 | 1:20 | BX1 | 1:46,5 |
| TX2 | 1:10 | BX2 | 1:10 |
| TX3 | 1:10 | BX3 | 1:10 |
| **T1** | 1:10 (50 pg/µl) | BX4 | 1:10 |
| **T2** | 1:10 | BX5 | 1:10 |
| **T3** | 1:10 | BX6 | 1:10 |
| **T4** | 1:10 | **B1** | 1:10 (50 fg/µl) |
| **T5** | 1:10 | **B1** | 1:10 |
| | | **B3** | 1:10 |
| | | **B4** | 1:10 |
| | | **B5** | 1:10 |

Further we prepared primer mix and master mix for usage. We dilated the primers from the stock, with a concentration of 100 pmol, to 2 pmol and mixed them up. The sequences for the telomere and 36B4 primers are shown in the following tables:

**Table 3 PCR primers forward and reverse [modified from O'Callaghan and Fench, 2011]**

**PCR Primers**

| *Oligomer Name* | *Oligomer sequence (5'-3')* | *Amplicon size* |
|---|---|---|
| Telomere f. | CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT | > 76 bp |
| Telomere r. | GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT | > 76 bp |
| 36B4 f. | ACTGGTCTAGGACCCGAGAAG | 78 bp |
| 36B4 r. | TCAATGGTGCCTCTGGAGATT | 78 bp |

We mixed DNA-elution after purification from strip one and strip two for better measurement. So we took 6µl elution from each tube (Strip one + strip two) and vortex it very well. Subsequently we calculated the master mix (LightCycler Master with SYBR Green Dye from Roche) solution for the samples and standards. Next, we prepared two master mix solutions, one for the telomere and the other for the single copy gene. For each reaction we needed the following ingredients:

**Table 4 Master mix solution for telomere and 36B4**

**Master mix solution**

| *Ingredient* | *Amount for 1 reaction* | |
|---|---|---|
| MM Master mix | 5 µl | |
| PM Primer mix | 0,5 µl | PM: 1 µl primer forward + 1µl primer reverse + 48µl NFW |
| NFW nuclease free water | 2,5 µl | |

So in each well from the 96-well PCR plate we pipetted 8µl master mix solution and added either 2µl of DNA sample or 2µl of telomere or 36B4 standard. (A pipetting

scheme example is shown in appendix.) In addition, we also detected no template control with nuclease free water and a positive control, which was a buccal sample we used in all runs for better comparability. After finishing, we sealed the plate with a PCR clear film and put it in the AB StepOnePlus™. So then the PCR run was started and was taken around two hours [O'Callaghan and Fenech, 2011].

**Table 5 Cycling conditions for telomere and 36B4**

**Cycling conditions**

| *Temperature* | *Time* | |
|---|---|---|
| 60°C | 30 sec | |
| 95°C | 10 min | Initial heating |
| 95°C | 15 sec | ***x 40 cycles*** |
| 60°C | 1 min | |
| 60°C | 30 sec | Holding stage |

## 3.6. Statistics

For statistic analysis we used the statistic software IBM SPSS Statistics. The significance was analysed by ANOVA, t-Test, pearson's and spearman's correlation, Kolmogorow-Smirnow-test and general liner model. For statistically significance a level of $p<0.05$ was considered. Statistic figures were made via boxplot or default bar charts.

# 4. Results

## 4.1. Structure analyses

With equol intervention Cutometer® and Visioscan® have shown significant improved skin parameters. Parameters of firmness, elasticity, elastic regression, signs of fatigue, sebum content, roughness, smoothness, wrinkles and capability of regression were determined.

We investigated parameters at two time points.

- Time point 0 (To): before intervention
- Time point 1 (T1): after 10 weeks of intervention

The significant effects were evaluated by means of t-test ($p<0.05$) with paired random samples.

### 4.1.1. Cutometer® dual MPA 580

First of all, measured parameter of Cutometer® are explained. We performed three recurrences of the measurement cycle by the following conditions: suction period and relaxation period of three seconds and 400mbar of negative pressure [Ohshima et al., 2013]. Our used parameters were:

> "Ua (the difference between the maximum deformation of the first vacuum period and the deformation after 1 s of normal pressure)
>
> Ue (immediate distension of the skin within the first 0.1 s of the first vacuum period)
>
> Uv (the difference between the deformation after 0.1 s and the maximal deformation of the first vacuum period)
>
> Uf (the final distension at the end of the first vacuum period)
>
> Ur (the immediate relaxation within the first 0.1 s after the end of the first vacuum period)" [Ryu et al., 2008]

Cutometer® specific parameters were calculated and analysed by the instrumental software of Courage & Khazaka electronic GmbH.

***R-parameter:***

***R0***, the highest point of the first curve provides insight into firmness of the skin. (The lower the amplitude, the firmer the skin.)

***R1*** consists of the lowest point of the first amplitude minus the highest point of the amplitude and describes the regression capability (ability to go back into initial state). (The lower the parameter, the better the capability of regression.)

***R2*** describes ratio between maximal amplitude and the capability of regression. (The closer the value is at 1, the more elastic the skin.)

***R3*** shows a parameter for signs of fatigue. Therefore the highest point of the last curve is compared with the maximal amplitude of the first curve. The lower the value the smaller the signs of fatigue, because with each curve the amplitude gets higher.

***R4*** is the last point of measurement, which is compared with the minimal amplitude of the first curve (R4-R1). R4 demonstrates signs of fatigue, because the capability of regression decreases with each repetition.

***R5*** is the net elasticity and consists of proportion of the elastic component when aspirating and the elastic component while relaxation. (The closer the value is at 1 (100%), the more elastic the curve.)

***R6*** is a parameter for elasticity. It is calculated through the proportion of the visco-elasticity on the elastic part in the suction period. (The smaller the value, the higher the elasticity.)

***R7*** characterizes the ratio between the proportion of elastic regression and the total curve. (The closer the value is at 1 (100%), the more elastic the skin.)

***R8*** describes the total regression. (The closer the value is at 1 (100%), the more elastic the skin.)

***R9*** reflects the fatigue phenomena of the skin after repeated suction. (The smaller the value, the smaller the signs of fatigue.)

***Surface-parameter:***

***F0:*** Area within the rectangle formed from Uf x suction time, above the curve. This area is considered in a relation to the total area, or deducted. A fully elastic material does not show any surface, but a rectangular curve. The closer the value is to 0, the more elastic the skin.

***F1:*** Area within the rectangle formed from Uf x relaxation, under the curve. This area is considered in a relation to the total area, or deducted. A fully elastic model forms without delay to the initial state: F1 and total surface parameter are identical. The closer the value of F1 is at 0, the more elastic the skin [Courage & Khazaka, 2016]

The results of treatment with emulsion containing only equol are shown in the following table.

**Table 6 Important R- and surface parameters- equol (p<0.05)**

| *parameter* | *improved values* | *importance* | *significance* |
|---|---|---|---|
| R0 | 0.23 → 0.18 | firmness | 0.001 |
| R1 | 0.09 → 0.07 | capability of regression | 0.003 |
| R3 | 0.27 → 0.21 | signs of fatigue | 0.001 |
| R4 | -0.79 → 0.25 | signs of fatigue | 0.000 |
| R5 | 0.49 → 0.55 | elasticity | 0.007 |
| F0 | 0.68 → 0.60 | elasticity | 0.002 |
| F1 | 0.47 → 0.34 | elasticity | 0.048 |

Table 7 represents the significant parameters of treatment with emulsion containing microencapsulated equol.

**Table 7 Important R- and surface parameters- microencapsulated equol (p<0.05)**

| *parameter* | *improved values* | *importance* | *significance* |
|---|---|---|---|
| R0 | 0.20 → 0.17 | firmness | 0.016 |
| R1 | 0.88 → 0.69 | capability of regression | 0.005 |
| R3 | 0.24 → 0.20 | signs of fatigue | 0.015 |
| R4 | -0.77 → 0.26 | signs of fatigue | 0.006 |
| R5 | 0.49 → 0.56 | elasticity | 0.039 |
| R7 | 0.31 → 0.34 | elastic regression | 0.011 |
| F0 | 0.64 → 0.56 | elasticity | 0.006 |

Emulsion (placebo), containing only lecithin, shows significant results at three parameters.

**Table 8 Important R- and surface parameters- lecithin (p<0.05)**

| *parameter* | *improved values* | *importance* | *significance* |
|---|---|---|---|
| R4 | -0.7 → 0.25 | signs of fatigue | 0.032 |
| R9 | 0.035 → 0.03 | signs of fatigue | 0.022 |
| F0 | 0.07 → 0.06 | elasticity | 0.031 |

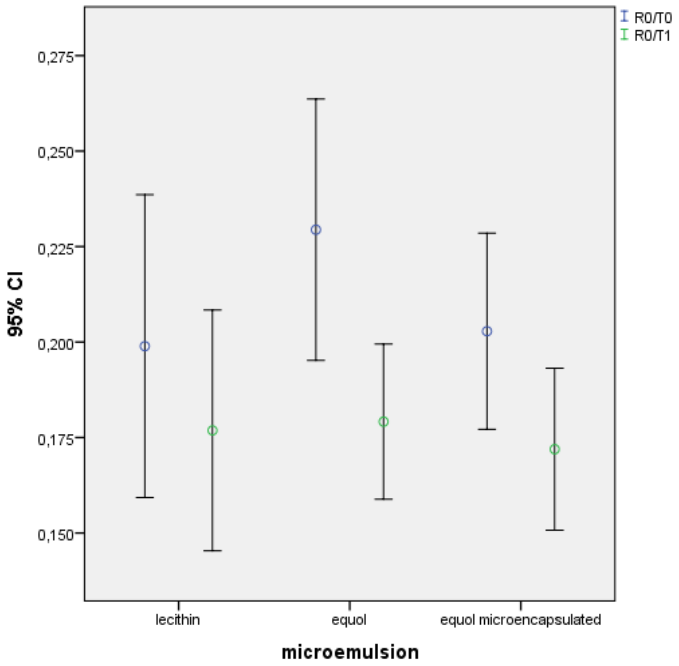


**Figure 8 Parameter R0: firmness, separated after emulsion**

Parameter R0 demonstrates the highest point of the amplitude and reveals the firmness of the skin. The lower the amplitude, the tighter is the skin.

Firmness parameter R0 is displayed, before (first bar) and after (second bar) intervention, via default bar charts. A significant effect could be established with equol and microencapsulated equol emulsion. Lecithin shows no significance in firmness.

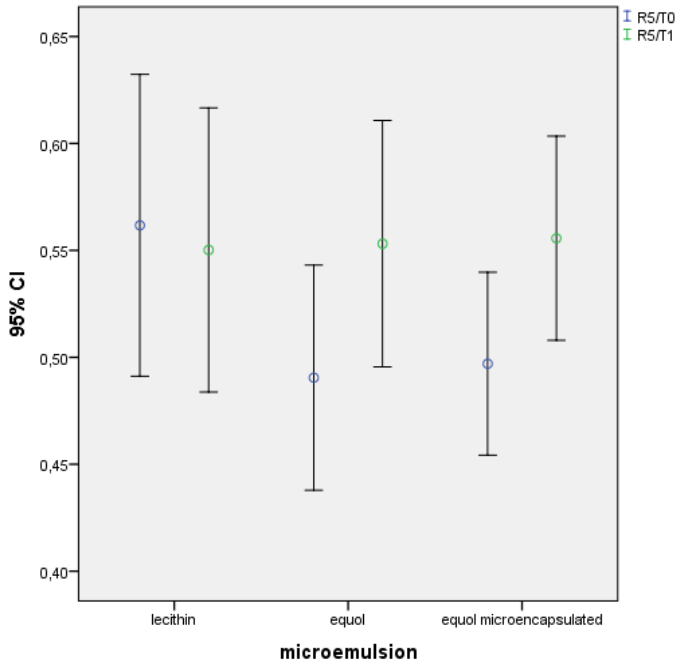


**Figure 9 Parameter R5: elasticity, separated after emulsion (p<0.05)**

Further elasticity improvement is shown at parameter R5. The more the parameter is at 1 (100%), the more elastic the curve.

Emulsion with equol and microencapsulated equol increase elasticity, but lecithin has no significant change.

Emulsion with equol indicates a significant betterment of elasticity at parameter F1, compared to microencapsulated equol and lecithin. But all preparations indicate an improvement in elasticity by Parameter F0 measurement. However, parameter R6 shows a significant deterioration of viscoelasticity on the elastic part in the suction phase.

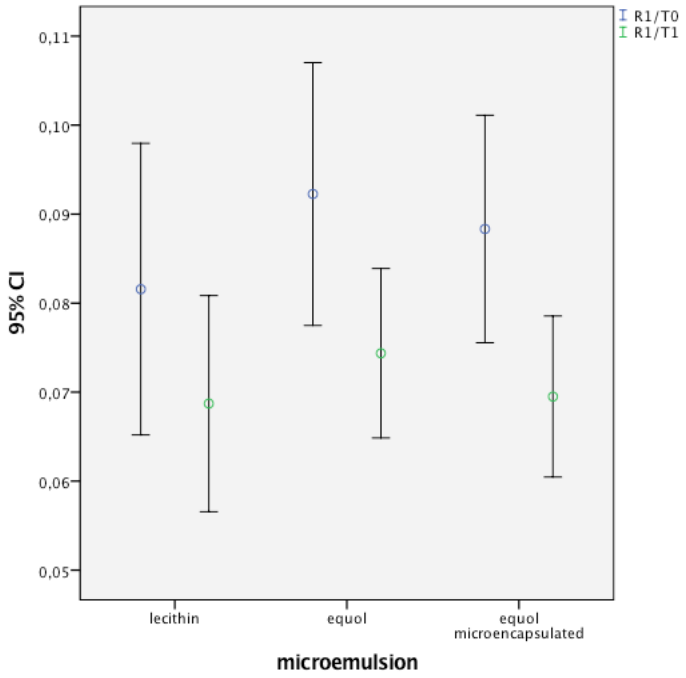


**Figure 10 Parameter R1: capability of regression, separated after emulsion (p<0.05)**

Figure 10 displays a significant decrease in capability of regression of emulsion with equol and microencapsulated equol compared to lecithin.

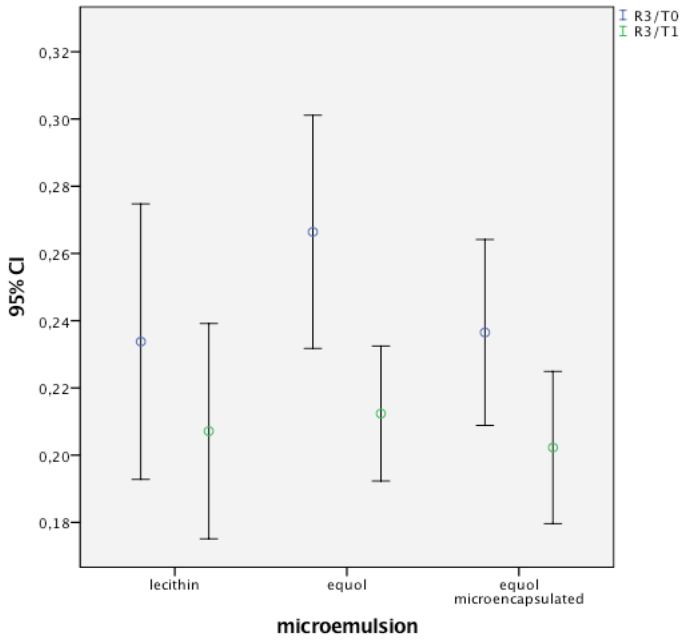


**Figure 11 Parameter R3: signs of fatigue, separated after emulsion (p<0.05)**

Signs of fatigue decrease significantly with equol and microencapsulated equol treatment. The same improving effect was shown at parameter R4.

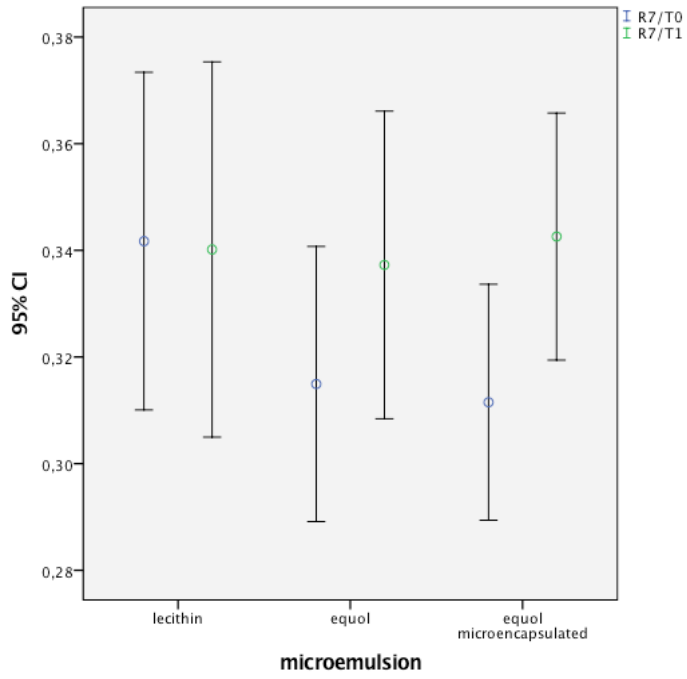


**Figure 12 Parameter R7: elastic regression, separated after emulsion (p<0.05)**

Parameter of elastic regression indicates only with encapsulated equol a significant enhancement. Both, lecithin and equol don't affect elastic regression.

### 4.1.2. Visioscan® VC 98

Further we measured roughness parameter, SELS-parameter, texture and surface parameter, anisotropy and corner-density with the non-invasive method of Visioscan® VC 98 from Courage & Khazaka electronic GmbH.

Visioscan® specific parameters were calculated and analysed by the instrumental software of Courage & Khazaka electronic GmbH.

***Roughness parameter:***

***R1:*** Skin roughness is the distance between the highest peak and the lowest valley of the profile.

***R2:*** For this parameter the profile is separated in 5 sections. With R2 the largest individual roughness is calculated.

***R3:*** For this parameter the arithmetic average of the 5 sections is calculated. Thus, possible artefacts of the lines are not considered as strong. This parameter has particular application in the evaluation of the surface profile.

***R4*** describes the smoothness depth. For the smoothness depth, the software calculates the area above the profile of the line and a line drawn through the highest peak of the profile. This surface is divided through the length of the profile; therefore R4 turns into a stretch.

***R5*** describes the arithmetic average roughness. At this parameter, an area between the actual profile and average profile is calculated. Then each of the upper and lower divergent surface is calculated for the middle profile and the centreline of the deviation. R5 describes the distance between the mean divergent and the mean profile [Courage & Khazak, 2005].

In the following tables, all significant improvements of Visioscan® parameters are listed.

**Table 9 Important roughness parameters (p<0.05)**

| *parameter* | *improved values* | | *importance* | *significance* | |
|---|---|---|---|---|---|
| | *equol* | *enc.equol* | | *equol* | *enc. equol* |
| R1 | 63.11 → 53.00 | 64.11 → 55.00 | skin roughness | 0.001 | 0.001 |
| R2 | 53.74 → 45.63 | 53.94 → 45.39 | maximum roughness | 0.002 | 0.000 |
| R3 | 42.00 → 35.11 | 42.22 → 35.94 | average roughness | 0.000 | 0.000 |
| R4 | 8.53 → 6.84 | 8.50 → 7.17 | smoothness depth | 0.001 | 0.008 |
| R5 | 33.53 → 29.42 | 34.44 → 29.94 | arithmetic average roughness | 0.007 | 0.002 |

It can be seen from the preceding table, that only equol and encapsulated equol show significant improvement regarding to roughness parameter.

***SELS-parameter:***

SELS (Surface Evaluation of Living Skin) parameter comprise five parameters:

***SEsc*** (scaliness) is the percentage of bright pixels. (Detailed description see appendix.) The smaller the value of SEsc, the lower scaliness of the corneal layer and further the higher is skin moisture.

***Rku*** (kurtosis) is related to the shape of the histogram and is therefore a quality parameter. (The closer the value is at three, the smoother the skin is.)

***SEr*** (roughness) calculates the ratio of dark pixels to the roughness factor of the overall picture. (Number of wrinkles) (The smaller Ser, the less roughness.)

***SEw*** (wrinkles) is calculated through the ratio of average width to average number of wrinkles. (The higher Sew, the more wrinkles.)

***SEsm*** (smoothness) delineates the form and width of the wrinkles. (The smaller SEsm, the smoother the skin.) [Courage & Khazaka , 2005]

**Table 10 Important SELS-parameters (p<0.05)**

| *parameter* | *improved values* | | *importance* | *significance* | |
|---|---|---|---|---|---|
| | *equol* | *enc. equol* | | *equol* | *enc. equol* |
| SEr | 2.99 → 2.34 | 3.07 → 2.48 | roughness | 0.022 | 0.020 |
| SEsm | 133.41 → 109.89 | - | smoothness | 0.001 | - |

As can be seen in table 10, only parameter SEr has a significant improvement with both equol emulsions, but no significance with lecithin. A significant betterment is only shown at parameters SEsm in the equol group.

***Texture and surface parameter:***

***Energy:*** This parameter indicates a general overview on skin conditions. (Older skin has a lower energy value compared to younger, elastic, highly hydrated skin.)

***Variance*** is the average of effective variance over a quantity of pixels. (The rougher the skin, the higher the variance.)

***Contrast*** is an indicator for the grey level difference of two pixel neighbours. (The better the skin conditions, the smaller the contrast value.)

***Entropy*** indicates the unstructured grey levels of the image. (Smooth skin shows a smaller entropy value.)

***Homogeneity*** is an indicator for uniformity of a picture. (The more hydrated the skin, the higher homogeneity value.)

***Surface:*** This parameter is evaluated through the divergence between uneven and even surface.

**Table 11 Important texture and surface parameters (p<0.05)**

| *parameter* | *improved values* | | | *importance* | *significance* | | |
|---|---|---|---|---|---|---|---|
| | **lecithin** | **equol** | **enc. equol** | | **lecithin** | **equol** | **enc. equol** |
| energy | 0.05 → 0.06 | 0.05 → 0.07 | 0.05 → 0.06 | skin condition | 0.034 | 0.000 | 0.003 |
| contrast | - | 0.86 → 0.71 | 0.92 → 0.78 | skin condition | - | 0.000 | 0.000 |
| variance | - | 3.55 → 3.13 | 3.69 → 3.30 | roughness | - | 0.000 | 0.000 |
| homogeneity | - | 1.54 → 1.57 | 1.52 → 1.55 | hydration | - | 0.000 | 0.000 |
| surface | - | 397.60 → 286.10 | - | uneven surface/ even surface | - | 0.042 | - |
| entropy | 1.60 → 1.56 | - | - | smoothness | 0.001 | - | - |

As shown in this table, lecithin demonstrates only at energy and entropy a significant improvement. Whereas, equol and encapsulated equol reveal significant improvement at energy, contrast, variance and homogeneity. Further equol has significance at surface parameter.

***Anisotropy and Corner-density:***

With these parameters, the line structure, their orientation, the intersection of the lines and the enclosed cells are considered.

***Anisotropy:*** Young skin shows a low anisotropy, because fine lines hold the skin in all directions. With increasing age, the fine lines disappear and deep wrinkles in certain principal directions are formed instead. The anisotropy increases thereby. Old skin has a higher anisotropy index than younger skin.

***Corner-density:*** Young skin with many fine lines in all directions forms many corners. The corner-density decreases with more disappearance of fine lines.

***Number of cells and number of in-section lines:*** Young skin shows a network of many, fine lines, hence more entrapped cells and lines between the cells (in-section lines).

***Size classes of the cells:*** There are 5 size classes: C0 (0-9 pixel), C1 (10-19 pixel), C2 (20-49 pixel), C3 (50-99 pixel), C4 (100-199 pixel), C5 (>200 pixel). Young skin has a greater number of cells in smaller categories. Older skin has a greater number of cells in higher categories [Courage & Khazaka, 2005].

**Table 12 Important anisotropy and corner-density parameters (p<0.05)**

| *parameter* | *improved values* | | *importance* | *significance* | |
|---|---|---|---|---|---|
| | *equol* | *enc.equol* | | *equol* | *enc. equol* |
| corner-density | 10.62 → 11.55 | - | cell density | 0.000 | - |
| no. of in-section lines | 1773.53 → 1843.37 | - | number of in-section lines | 0.006 | - |
| no. of cells | 644.42 → 681.42 | - | number of cells | 0.001 | - |
| Cellcl3 (50-99 pixel) | 191.21 → 220.05 | - | number of cells in Cellcl3 | 0.000 | - |
| Cellcl4 (100-199 pixel) | 173.79 → 187.95 | 168.50 → 182.00 | number of cells in Cellcl4 | 0.003 | 0.005 |
| Cellcl5 (>200 pixel) | 134.63 → 125.58 | 135.39 → 129.50 | number of cells in Cellcl5 | 0.004 | 0.045 |

It can be seen, that equol improves corner-density, number of in-section line, number of cells, and also cell categories. However, encapsulated equol only shows significance in cell categories.

Structure parameters were also analyzed in connection with lifestyle and nutrition factors. Thereby physical activity showed a correlation with anisotropy. With higher physical activity level, anisotropy values improved significantly ($p<0.05$). (Outputs are attached in appendix.)

Beside of this there were no significant connections with other lifestyle and nutrition factors.

All measured structure parameters are summarized in appendix.

### 4.1.3. Sebum measurement via Visioscan® VC 98

**Table 13 Values of sebum measurement**

| *preparation* | *values* | *significance* |
|---|---|---|
| lecithin | 2,58 → 1,81 | 0.180 |
| equol | 1,91 → 1,41 | 0.134 |
| enc. equol | 1,65 → 2,37 | 0.269 |

As can be seen in table 13, sebum increased with encapsulated equol intervention and decreased with lecithin and equol intervention. However, sebum demonstrated no significant correlation with equol intervention.

## 4.2. Molecular analysis

For this master thesis, absolute telomere length was measured by means of O'Callaghan and Fenech 2011 protocol. Accordingly, a qPCR with single copy gene (acidic ribosomal phosphoprotein 36B4) control was used, with which we standardized the samples. Further relative values were established, while we set T0 to 1 and got a relative factor for F1.

### 4.2.1. Telomere

First of all we tested if there is any improvement of absolute telomere length, after equol treatment. The results are represented in the follow figures:

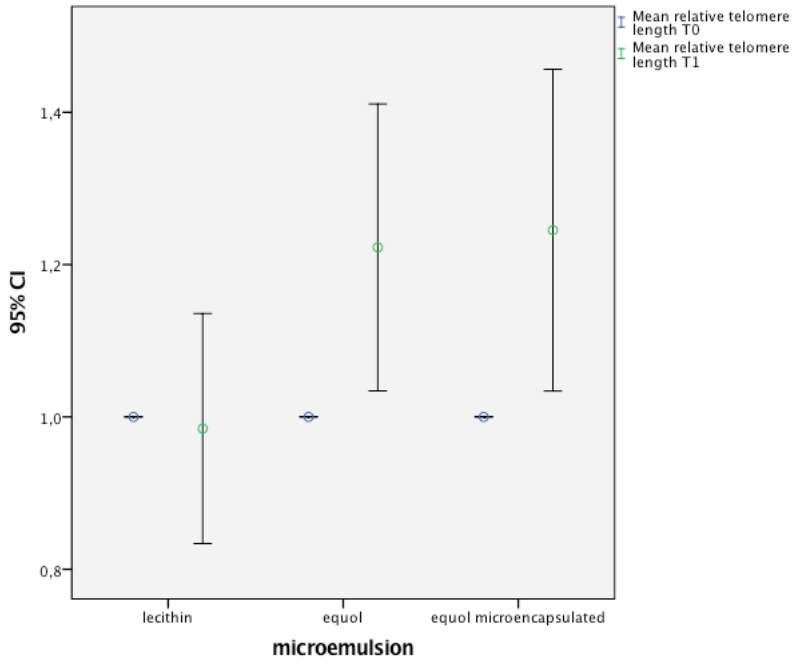


**Figure 13 Mean relative telomere length at T0 and T1 (p<0.05)**

The first bar (green) shows the mean relative telomere length of all participants at time point 0, before treatment (T0) and the second bar (blue) shows the mean relative telomere length of all participants at time point 1, after treatment (T1). It can be seen, that equol and encapsulated equol treatment increase relative telomere length after treatment, compared to lecithin, which shows no change. (p<0.05)

Further we tested the relationship between the longitude of absolute telomere and health, lifestyle and nutrition, which were evaluated. Thereby a correlation between BMI and absolute telomere length displayed.

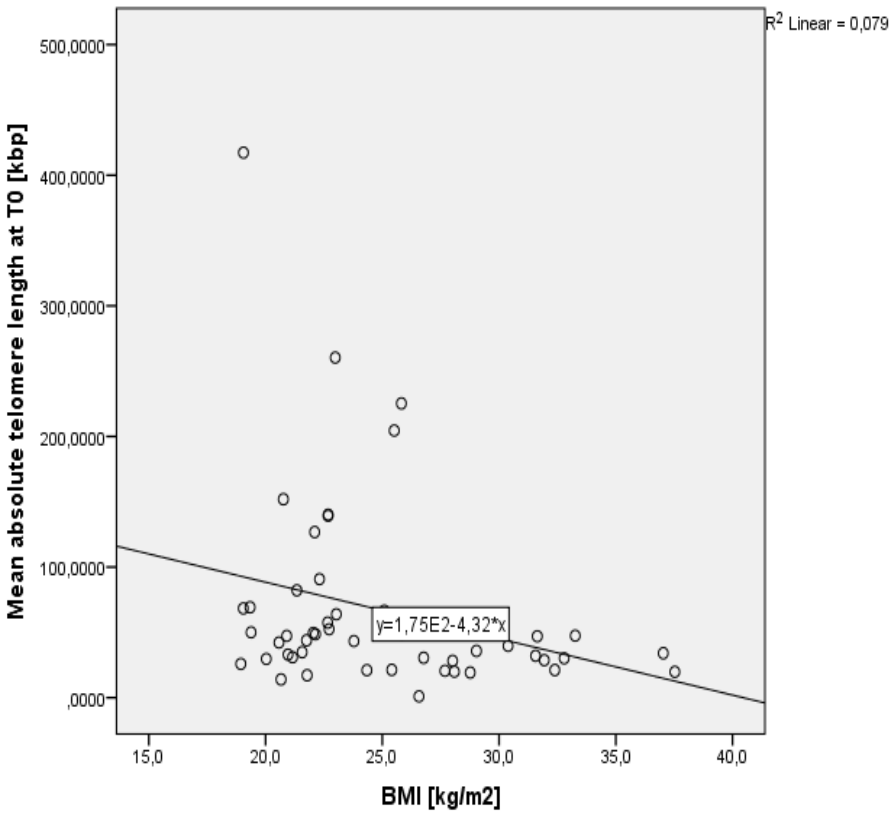


**Figure 14 Correlation between mean aTL and BMI (p<0.05)**

In this scatter plot, the BMI of all participants and their absolute telomere length are presented. As can be seen, that with higher BMI, the mean absolute telomere length is decreasing. (p=0.03)

There were no significances at age, height, weight or any diseases or other health factors (like sport, physical activity etc.).

Further a connection between absolute telomere length and sunbathing were examined.

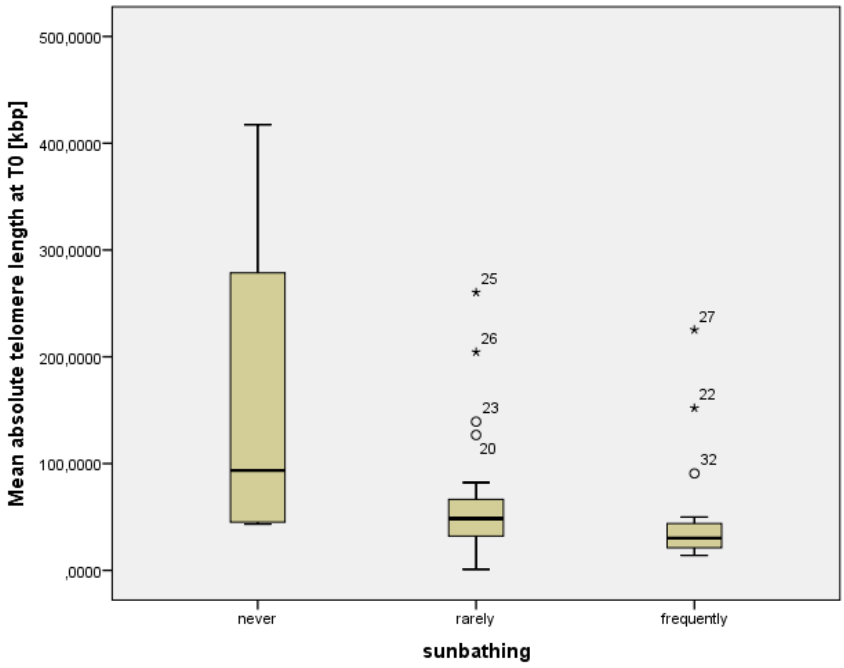


**Figure 15 Correlation between sunbathing and aTL (p<0.05)**

These boxplots show the absolute telomere length of participants divided into three groups. Participants, who never, rarely and frequently take sunbathes. Absolute telomere length was significant lower by those who rarely and frequently sunbathe. (p=0.02)

Statistical evaluation about telomere length and nutrition were investigated. Thereby a significant relation of absolute telomere length and black tea were identified.

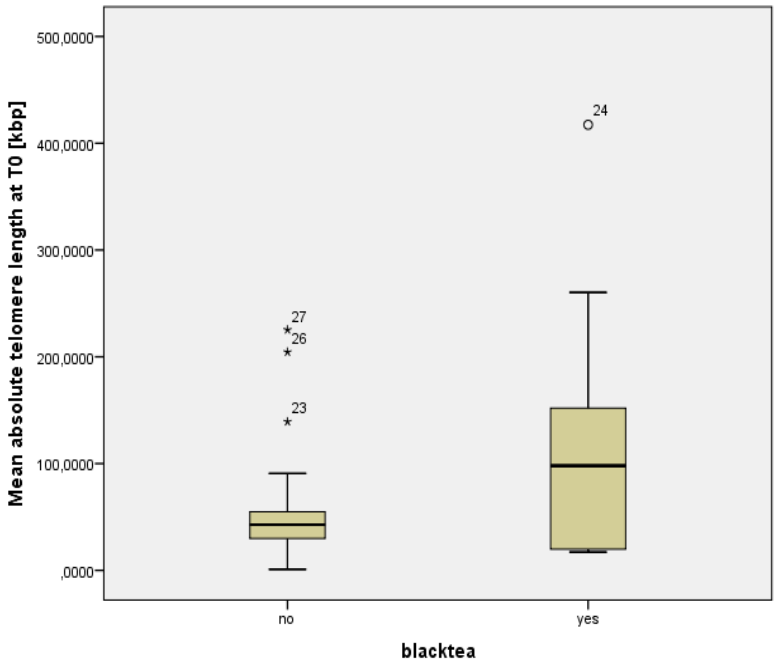


**Figure 16 Correlation between mean aTL and black tea (p<0.05)**

These boxplots show absolute telomere length of participants, which drink (yes) or drink not (no) black tea. The absolute telomere length was significant higher by those subjects, who drink black tea on a day-to-day basis.

There were no other significances with nutritional factors (like liquid intake, alcohol consumption, fruit, vegetable or meat consumption etc.).

## 4.3. Self-assessment frequency

Cosmetic production, peeling or cleansing usage were also investigated, but no significances were founded.

Further we investigated the subjective changes during intervention period. Therefore we consulted a self-assessment frequency, which the participants completed at time point 1. In the following table, the self-evaluations of the participants are shown.

**Table 14 Self-assessment frequency**

***Self-assessment frequency - improvement***

| *skin parameter* | *lecithin* | *equol* | *encapsulated equol* |
|---|---|---|---|
| moisture | 64% | 79% | 67% |
| pleasant feeling | 93% | 84% | 83% |
| texture | 64% | 63% | 67% |
| charisma | 71% | 63% | 72% |
| freshness | 50% | 47% | 56% |
| smoothness | 71% | 63% | 72% |
| oily shine | 57% | 47% | 67% |
| positive difference at all | 86% | 74% | 78% |

In Table 14, self-evaluations of improved skin parameters of the subjects are shown in per cent e.g. 79% of the participants, who got equol preparations had an improved skin moisture feeling (yes or rather yes).

Participants, who received lecithin, had a better pleasant feeling and a positive difference at all compared to equol groups. Whereas subjects with equol intervention indicated only improved skin moisture by contrast with encapsulated equol and lecithin treatment. Volunteers, who received emulsion contained encapsulated equol reported on better skin texture, charisma, freshness, smoothness and fewer oily shine, compared to equol and lecithin groups.

In conclusion there were no significant changes between the preparations in self-assessment.

Self-assessment questionnaire is attached in appendix.

# 5. Discussion

During intrinsic, as well as extrinsic aging, skin changes quantitative and qualitative. Fine lines in skin lead to depth wrinkles during aging, which can lead to a loss of elasticity, reduction of collagen content and epidermal thickness [Ryu et al., 2008; Tobin D.J., 2016].

Non-invasive measurement devices were used for our analysis. We investigated via Cutometer® and Visioscan®, the effects of different skin emulsions (equol, encapsulated equol and lecithin), which were applied on the forehead.

Cutometer® measurement was used to evaluate skin parameters of firmness, elasticity, capability of regression and signs of fatigue. Before and after intervention with equol, encapsulated equol or lecithin emulsion, we measured those skin parameters. After 10 weeks of treatment with equol (N=19), seven skin parameters were improved (R0, R1, R3, R4, R5, F0 and F1). Firmness (R0) was significant ($p<0.05$) decreased from 0.23 to 0.18. This could be due the fact, that equol stimulates collagen gene expression. Further, this effect may also be responsible for the significant betterment of fatigue signs (R3, R4). Equol improved the skin parameters R3 from 0.27 to 0.21 and R4 from -0.79 to 0.25 ($p> 0.05$). Equol preparation improved elasticity in three skin parameters (R5, F0, F1). Elasticity enhancement may due the matter of fact, that equol stimulates elastin expression. This elasticity improvement was likewise underlined through surface parameter F0 and F1. Intervention with encapsulated equol (N=18) displayed, besides significant ($p<0.05$) parameters (R0, R1 R3, R4, R5 and F0) we discussed above, additional skin parameters like R7. R7 represents elastic regression. It may also be based on higher elastin expression through encapsulated equol. In conclusion we can say, considering Cutometer® measurement, equol emulsion, as well as encapsulated equol emulsion, significantly improved skin parameters. It can clearly be seen, that compared to equol and encapsulated equol, lecithin has not such big influence on skin parameters.

Further biomechanical properties were measured by using the digital camera Visioscan®. Roughness parameter, SELS-parameter, texture and surface parameter,

anisotropy and corner-density were screened. Equol and encapsulated equol showed significant ($p<0.05$) changes with all roughness parameters. Roughness is defined as the asperity of the skin, because primary and secondary lines characterize skin surface. Primary lines are characteristic for individuals, their age and body position. Secondary lines are more affected by external influences such as temperature, cosmetics, humidity, UV-radiations and tobacco. With increasing age, the fine lines disappear and deep wrinkles are formed instead [Dermatest, 2016]. Only two SELS-parameters demonstrated significant ($p<0.05$) improvement (SEr roughness and SEsm smoothness) with equol or encapsulated equol treatment. SEr showed significant betterment at both emulsions. Equol decreased roughness from 2.99 to 2.34 and encapsulated equol from 3.07 to 2.48. In conclusion, roughness improved in R-parameter, as well as in SELS- parameter SEr, and equol may enhace roughness through refining wrinkles or refilling wrinkles by equol products. Compatible with this result, the smoothness parameter SEsm also improved significantly with equol emulsion (133.4-109.9 $p<.0.5$), but not with microencapsulated equol. This is maybe due that equol preparation smoothens skin via refilling the wrinkles. Microencapsulated equol does not perhaps own this ability. Equol, as well as encapsulated equol, significantly enhances texture parameter like energy, contrast, variance and homogeneity. Energy indicates the homogeneity of a picture taken by Visioscan® and reflects a general overview on skin conditions. It is important to mention, that after treatment with an anti-aging product, the energy value should be risen up. This means, that equol (0.05-0.07) and encapsulated equol (0.05-0.06) have the ability to improve skin conditions. Unfortunately, lecithin (0.05–0.06) improves energy in the same way as encapsulated equol does. So lecithin, which is contained in all three emulsions, is possibly responsible for those effects and equol merely strengthened it. Contrast, which is a parameter for skin condition too, improved with equol and encapsulated equol intervention. Furthermore, varaiance and homogeneity decreased significantly with equol and encapsulated equol. Those parameters represent roughness and hydration. The ability of equol to bind to the estrogen receptor is the reason, why it replaces estrogen and increases hence epidermal hydration [Thornton M. J., 2013]. Surprisingly,

only lecithin enhanced entropy value from 1.6 to 1.56. Young skin with a network of many fine lines in all direction forms many corners and results in high corner-density. Equol increases corner-density from 10.62 to 11.55 significantly ($p<0.05$). So equol, but not encapsulated equol, refined skin wrinkles, which is also confirmed through numbers of in-section lines, which increased after equol intervention too. Compatible to this, equol enhanced the number of cells from 644.42 to 681.42. Young skin has a greater number of cells in smaller categories. This is proved due to that, equol showed an increase in the small cell class 3 (50-99pixel) and a decrease in higher cell class 5 (>200 pixel). Cell class 4 (100-199 pixel) showed an increase from 173.79 to 187.95. At this point, it is difficult to say, if this category rates among smaller or higher cell classes. Encapsulated equol showed the same result, an increase in cell class 4 and a decrease in cell class 5. With regard to anisotropy and cell parameters, only equol showed good achievements.

Telomeres are shortened with each cell division in skin cells and this is linked with aging. After intervention of 10 weeks, we discovered that treatment with equol and encapsulated equol increased significant ($p<0.05$) relative telomere length compared to lecithin. The reason for this effect is not clear. Therefore maybe telomerase is responsible. Many studies investigated the effect of telomerase on increasing telomere length or other approaches to TERT and TERC expression. Telomerase was detected in keratinocytes of epidermis, which can express low levels of TERT. So with high expression of exogenous TERC, keratinocytes have the ability to activate telomerase and maintain telomeres. Fibroblasts in dermis have no active telomerase and do not express TERT. If this mechanism really would happen, skin aging could be ameliorated [Buckingham and Klingelhutz, 2011]. Therefore more studies are required to investigate the relation between telomere shortening, aging and botanicals like equol. We also ascertained, that absolute telomere length and BMI ($kg/m^2$) correlate significant negatively (correlation coefficient: -0.312). That means, absolute telomere length decreases with higher BMI ($kg/m^2$). The mean BMI in our intervention study was 22,98 $kg/m^2$. In conclusion, metabolic factors especially BMI influences absolute telomere length. Lifestyle factors have possibly indirect effects on absolute telomere

length. However, in our study no more significance was detected with lifestyle or health factors. UV irradiation leads to photo-aging and induces telomere shortening [Olikawa et al., 2001]. Furthermore, UV- radiation induces oxidative stress and pursuing telomere shortening [Sugimoto et al., 2006]. In our intervention study a correlation between absolute telomere length and sunbathing was found. Four participants never sunbathe, 29 rarely and 17 frequently. Absolute telomere length was significant lower by those who frequently sunbathe, compared to those who rarely or never sunbathe. Another importance in our study was the impact of nutrition. Therefore we investigated nutritional factors by means of a food frequency questionnaire. All factors were analysed statistically, but unfortunately, only black tea shows connection with telomere length. We divided participants into black tea consumers or not consumers. Subjects, who drink black tea, have significant higher telomere length compared to not consumers. Beneficial effects of tea are widely known and long-winded reviewed. Thereby, it is important to differentiate the three major types of tea, on their level of fermentation. Black tea is fermented tea, oolong tea is partially fermented tea and green tea is unfermented. The positive effect may due to the antioxidative properties of tea and protection of telomeres from oxidative stress [Chan et al., 2010]. The reason why only black tea showed significance in relation with telomere length is not yet detachable. Further studies, focused on tea consumption and telomere length, are necessary.

## 5.1. Limitations

Limitations of the study were for one thing the small amount of participants for representative subgroup differences and the overly short intervention time. For further researches it could be interesting to investigate the nutritional effects by using dietary intervention. Further an improvement in nutrition survey, through nutritional diary, could lead to better results, because the nutrition and lifestyle questionnaire was often too complex for the subjects (e.g. estimate of portion sizes, frequency of consumption). Another limitation, which explains some possible variations of structure parameters, was the climate, because skin parameter analysis devices are very sensitive to external conditions, like temperature, light and humidity. Analyses were started in April (lower humidity) and finished in June (higher humidity). Another limiting factor was the classification of the group, which was randomly selected. Unfortunately younger participants were allocated in lecithin group and older ones in the equol groups. Therefore parameter R5 from Cutometer® measurement was at time point 0 overall higher than in the equol groups. The last restraining factor was the fact that some participants wear make up at analyses, which maybe distorted results.

# 6. Conclusion

Results of this intervention study showed an effect of equol at several skin parameters, which were measured via Cutometer® and Visioscan®. Thus, equol has the ability to investigate skin characteristics and also the ability to become an important part of cosmetics with anti-aging effect. This became clear due to the investigation of absolute telomere length, which is a biomarker of aging. The length of telomeres was increased through intervention with equol or encapsulated equol. In addition absolute telomere length measurement displayed a correlation with BMI, sunbathing and black tea. Investigation of nutrition and lifestyle factors only showed improved results at physical activity relating to anisotropy. In conclusion it can be said, that there is, due to the results on structure and molecular analyses, an effect of equol, as well as encapsulated equol, in skin.

# 7. Summary

Equol, a degradation product by intestinal bacteria of the isoflavone daizein and which naturally occurs in soy, has versatile biological properties. A major effect of equol is its antioxidant activity, where it acts as a free radical scavenger. Second, it is also a nonsteroidal estrogen, which can bind to the estrogen receptor because of its structurally similarity to estradiol. Furthermore, equol may have an impact on skin regeneration and health due to the fact that it improves skin elasticity and collagen structure. Skin aging process, affected by environmental, nutritional and lifestyle-based factors can be outlined as a progressive decline of the biological function of cells. Due to this fact, we investigated these effects and the intervention with equol on structure and absolute telomere length in skin. Therefore, we examined the impact of topically applied equol substance on the forehead of women. Structure parameters were analysed by the means of scientific certified skin analysis equipment and for molecular analyses, absolute telomere length measurement has been used. Based on skin parameter analyses via Cutometer®, an improvement of firmness, fatigue signs and elasticity was shown with equol, as well as with encapsulated equol intervention. In addition, encapsulated equol displayed improved elastic regression values. By means of Visioscan® analyses, roughness parameter and overall skin conditions were improved at both equol preparations and additional, with only equol, higher smoothness values were detected. Furthermore, equol refined skin wrinkles and may has high effects against environmental and age-related skin changes. Due to the fact, that telomere length is a biomarker of aging, we analysed absolute telomere length of skin samples. After 10 weeks of intervention we determined, that treatment with equol, as well as encapsulated equol, increased telomere length. Nutrition is associated with skin health and further, influences aging processes and telomere length. Subjects, who drink black tea, have significant higher telomere length compared to not consumers. Different food and lifestyle patterns were analysed with a standardized food and lifestyle questionnaire.

# 8. Zusammenfassung

Equol entsteht beim Abbau von Daidzein, einem bekanntem Isoflavon in Soja, durch die Bakterien der Darmflora. Es besitzt vielseitige biologische Wirkungen. Eine der Hauptwirkungen von Equol stellt deren antioxidative Aktivität dar, wobei es als freier Radikalfänger agiert. Weiters kann es durch Stukturgleichheit zum Estradiol, östrogene Wirkungen induzieren, da es an Östrogenrezeptoren binden kann. Außerdem beeinflusst Equol die Hautregeneration und Hautbeschaffenheit positiv. Dies lässt sich durch die Tatsache, dass Equol Elastizität und Collagenstruktur verbessert, beschreiben. Alterungsprozesse der Haut, beeinflusst durch Umwelt, Ernährung und Lifestyle-Faktoren, charakterisieren die zunehmende Abnahme biologischer Funktionen von Zellen. Aufgrund dessen, untersuchten wir die Auswirkungen dieser Faktoren und der Intervention mit Equol auf Struktur und absolute Telomerlänge in der Haut. Im Zuge der Interventionsstudie analysierten wir die Wirkung einer synthetisch hergestellten Equol-Substanz auf Hautzellen von Frauen. Strukturparameter wurden mit Hilfe wissenschaftlich zertifizierter Hautanalysegeräte gemessen und molekulare Parameter durch Quantifizierung absoluter Telomerlänge bestimmt. Hautanalysen mittels Cutometer® zeigten eine Verbesserung der Festigkeit, Ermüdungserscheinungen und Elastizität sowohl bei Equol, als auch bei verkapselter Equol Intervention. Darüber hinaus verbesserte das Präparat mit mikroverkapseltem Equol die elastischen Regressionswerte. Analysen mit Visioscan® zeigten optimierte Rauheitsparameter und Hauterscheinung bei beiden Equol Präparaten. Bei der Behandlung mit Equol zeigte sich zusätzlich eine höhere Ebenmäßigkeit der Haut. Equol besitzt die Fähigkeit, Hautfalten zu verfeinern und außerdem den Effekt, Umwelt- und altersbedingte Hautveränderungen entgegenzuwirken. Telomerlänge ist ein in vielen Studien belegter Biomarker des Alterns. Deshalb analysierten wir die absolute Telomerlänge von Hautproben der Probanden. Nach zehnwöchiger Intervention zeigte sich eine verlängerte Wirkung von Equol und verkapselten Equol auf die Telomerlänge. Mittels einem standardisiertem Fragebogen, evaluierten wir die Nahrungs- und Lebensgewohnheiten.

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# 10. Appendix

## 10.1. Food frequency and lifestyle questionnaire

### Fragebogen

Hautstudie mit Equol

***Bitte ankreuzen:***

□ Ich bin damit einverstanden, dass meine Daten anonym (Material wird nur für wissenschaftliche Fragestellungen genutzt - ohne Bezug zur Person) für wissenschaftliche Zwecke verwendet werden.

**Alter:**

**Geschlecht:**

□weiblich □männlich

**Körpergröße: ..... cm**

**Körpergewicht: ..... kg**

**<u>Fragen zu Ihrem Gesundheitszustand</u>**

1. **Sind Sie Diabetiker?**

□ja □nein

2. **Seit wann leiden Sie schon unter Diabetes?**

3. **Leiden Sie unter anderen chronischen oder akuten Erkrankungen?**

□ja □nein

**Wenn Ja, welche?**

4. **Müssen sie regelmäßig Medikamente einnehmen?**

□ja □nein

**Wenn ja, welche?**

5. **Machen Sie regelmäßig körperliche Bewegung?** *(z.B.: Spazierengehen,....)*

□mehrmals täglich □1 mal täglich
□4-6 mal wöchentlich □1-3 mal wöchentlich
□jede zweite Woche oder seltener □nie

**6. Betreiben Sie regelmäßig Sport?** *(mindestens 30 min. pro Einheit)*

□mehrmals täglich □1 mal täglich
□4-6 mal wöchentlich □1-3 mal wöchentlich
□jede zweite Woche oder seltener □nie

**7. Gehen Sie ins Solarium?**

□ja □nein

**8. Falls ja, wie häufig gehen Sie ins Solarium?**

□< als 1mal monatlich □1-3 mal monatlich
□1 mal wöchentlich □1-2 mal wöchentlich
□> 2 mal wöchentlich

**9. Wie häufig halten sie sich im Freien auf?**

□häufig □selten
□nie

**10. Gehen Sie im Sommer regelmäßig Sonnenbaden?**

□häufig □selten
□nie

***11.* Verwenden Sie beim Sonnenbaden Sonnenschutzmittel?**
*(bitte Lichtschutzfaktor angeben)*

□ja (LSF_____) □gelegentlich (LSF____)
□nein

**12. Rauchen Sie?**

□ja □nein
□nicht mehr

**13. Wie viele Zigaretten rauchen Sie?**

□gelegentlich □täglich bis 10 Stk.
□täglich 11 – 20 Stk. □täglich > 20 Stk.

**14. Wie lange Rauchen Sie schon?**

## Fragen zu Ihren Essgewohnheiten

**1. Sind Sie Vegetarier oder Veganer?**

□ Ja, Vegetarier □ Ja, Veganer
□ Nein, keines von beiden

**2. Seit wann leben Sie schon als Vegetarier oder Veganer?**

**3. Wie viel Flüssigkeit nehmen Sie durchschnittlich täglich zu sich?**
*(außer Kaffee und Alkohol)*

□¼ Liter – ½ Liter
□½ Liter – 1 Liter
□1 Liter – 1 ½ Liter
□1 ½ Liter – 2 Liter
□> 2 Liter

**4. Welche Getränke bevorzugen Sie?**
*(Mehrfachnennungen möglich → bitte nach der Gewichtung nummerieren 1,2,3,..)*

□Leitungswasser/ Mineralwasser
□Obstsäfte 100%
□Obstnektar
□Wellnessgetränke
□Verdünnungssäfte
□Softdrinks (Fanta, Sprite..)
□Kräutertee
□Früchtetee
□Grüner Tee
□Schwarztee

**5. Trinken Sie Kaffee?**

□ja
□nein

**6. Wenn ja, wie viele Tassen Kaffee trinken Sie am Tag?**

□< als 1 Tasse
□1-2 Tassen
□3-5 Tassen
□> als 5 Tassen

**7. Wie oft trinken Sie Alkohol?**

□nie
□nur zu besonderen Anlässen
□1 –2 mal monatlich
□2-5 mal monatlich
□1-2 mal die Woche
□2-5 mal die Woche
□jeden Tag
□mehrmals täglich

***8.* Welchen Alkohol trinken Sie ?** *(Mehrfachnennungen möglich)*

□Rotwein
□Weißwein
□Bier
□Schnäpse
□Mixgetränke
□sonstiges (bitte angeben!)

**9. Welche Menge Alkohol trinken sie durchschnittlich im Monat?**

□< als ¼ Liter
□¼ - ½ Liter
□½ - 1 Liter
□1 – 2 Liter
□2 – 3 Liter
□> 3 Liter

***10.* Wie viel Milch und Milchprodukte konsumieren sie wöchentlich?**
*(Portion: z.B. 250ml Milch, 150g Joghurt, 1EL Sauerrahm, 1 Scheibe Käse..)*

□< als 1 Portion
□1-3 Portionen
□3-6 Portionen
□6-10 Portionen
□> als 10 Portionen

**11. Welche Milchprodukte sind dabei enthalten?**

□Käse
□Joghurt
□Buttermilch
□Sauermilch
□Molke
□Butter
□Sauerrahm
□Schlagobers
□sonstiges (bitte angeben!)

***12.*** **Wie viel Obst konsumieren Sie wöchentlich?**
*(Portionsgröße: geballte Faust; z.B. 1 Apfel, 1 Handvoll Beeren.. )*

□keines
□1-5 Portionen
□6-10 Portionen
□> 10 Portionen

**13. Welche Obstsorten konsumieren Sie bevorzugt?**

□Weintrauben
□Erdbeeren
□Kirschen
□Orangen
□Apfel
□Banane
□sonstige (bitte angeben!)

**14. Wie viel Gemüse konsumieren Sie wöchentlich?** *(Portionsgröße: geballte Faust)*

□keines
□1-5 Portionen
□6- 10 Portionen
□> 10 Portionen

**15. Welches Gemüse konsumieren Sie bevorzugt?**

□Spinat
□Tomaten
□Karotten
□Brokkoli
□Spargel
□Paprika
□Salat
□sonstige (bitte angeben!)

**16. Ernähren Sie sich bevorzugt von Vollkornprodukten oder Weißbrot?**

□Vollkornprodukte
□Weißbrot

**17. Wie oft in der Woche essen Sie Fleisch?**

□Nie
□1-2 mal
□3-5 mal
□5-7 mal
□> als 7 mal

**18. Welche Fleischsorten konsumieren Sie?**

□Rindfleisch
□Schweinefleisch
□Putenfleisch
□Hühnerfleisch
□Lammfleisch
□sonstige (bitte angeben!)

**19. Wie oft in der Woche essen Sie Wurst und Schinken?**

□nie □1-2 mal
□3-5 mal □5-7 mal
□> als 7 mal

**20. Wie oft in der Woche essen Sie durchschnittlich Fisch?**

□nie □1 mal
□2 mal □> 2 mal

**21. Wie oft in der Woche essen Sie Süßwaren?** *(inkl. Kuchen, Torten..)*

□nie □1-2 mal
□3-4 mal □5-6 mal
□täglich

**22. Wie oft in der Woche essen Sie Knabbereien?** *(z.B. Chips, Cracker ..)*

□nie □1-2 mal
□3-4 mal □5-6 mal
□täglich

**23. Nehmen Sie Nahrungsergänzungsmittel/Spurenelemente?**

□ja □nein

**24. Wenn ja, welche?**

□Vitamin C □Vitamin D
□Calcium □Carnitin
□Biotin □Knoblauchpräparate
□Folsäure □Magnesium
□sonstige (bitte angeben!)

## Fragen zu Kosmetikprodukten?

**1. Wie würden Sie Ihre Haut einschätzen?**

□normal □trocken
□ölig

**2. Wie fühlt sich Ihre Haut nach der Reinigung an?**

□meine Haut spannt und ist trocken
□meine Haut fühlt sich ölig an
□keines von beiden (Bitte selber beschreiben!)

***3.* Wie reinigen Sie Ihre Haut?** *(Mehrere Antworten möglich)*

□Reinigungsmilch/-seife /-schaum
□Tonic
□Peeling

**4. Wie oft verwenden Sie ein Peeling?**

□täglich □2-3 mal pro Woche
□1-2 mal pro Monat □alle paar Monate
□nie

**5. Wie oft gehen Sie zur Kosmetikerin?**

□1 mal pro Woche □1-2 mal im Monat
□alle paar Monate □nie

**6. Welche Hautpflege verwenden Sie?**

□Feuchtigkeitspflege
□Anti-Aging pflege
□Mattierende Pflege
□Andere (bitte angeben!)

**7. Was stört Sie an Ihrer Haut am Meisten?**

**8. Welchen Lichtschutzfaktor verwenden Sie? (LSF/SPF)**

□≤ 10 □15- 20
□20- 30 □> 30
□keinen

**9. Verwenden Sie Makeup/BB-Cream/getönte Tagespflege oder Puder?**

□ja □nein

## 10.2. Self-assessment questionnaire

### Fragebogen zur Selbstbewertung Ihrer Haut

### nach Anwendung der Mikroemulsion

□ Ich bin damit einverstanden, dass meine Daten anonym (Material wird nur für wissenschaftliche Fragestellungen genutzt - ohne Bezug zur Person) für wissenschaftliche Zwecke verwendet werden.

**Bitte bewerten Sie folgende Parameter:**

1) **Verbesserung der Hautfeuchtigkeit:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein
2) **Angenehmeres Hautgefühl seit der Anwendung:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein
3) **Verbesserung der Hautbeschaffenheit:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein
4) **Verbesserung der Hautausstrahlung:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein
5) **Verringerung von Müdigkeitserscheinungen:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein
6) **Glattere, vollere Haut:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein
7) **Verringerung von Ölglanz:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein
8) **Positive Veränderung insgesamt:**
   □ Ja □ Nein
   □ eher Ja □ eher Nein

**Welche Veränderungen haben Sie sonst bemerkt?**

**Wie oft haben Sie die Mikroemulsion angewandt?**

□ 2 x/Tag □ 1 x/Tag
□ 3 x/Woche □ seltener

## 10.3. Outputs of structure parameter – Cutometer® dual MPA 580

### 10.3.1. Emulsion with equol

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | R0/T0 | ,22942 | 19 | ,070973 | ,016282 |
| | R0/T1 | ,17916 | 19 | ,042128 | ,009665 |
| Pair 2 | R1/T0 | ,09226 | 19 | ,030634 | ,007028 |
| | R1/T1 | ,07437 | 19 | ,019760 | ,004533 |
| Pair 3 | R2/T0 | ,592763 | 19 | ,0840702 | ,0192870 |
| | R2/T1 | ,583295 | 19 | ,0794923 | ,0182368 |
| Pair 4 | R3/T0 | ,26642 | 19 | ,071950 | ,016506 |
| | R3/T1 | ,21237 | 19 | ,041651 | ,009555 |
| Pair 5 | R4/T0 | ,13258 | 19 | ,040077 | ,009194 |
| | R4/T1 | ,09932 | 19 | ,025274 | ,005798 |
| Pair 6 | R5/T0 | ,490468 | 19 | ,1092125 | ,0250551 |
| | R5/T1 | ,553147 | 19 | ,1194569 | ,0274053 |
| Pair 7 | R6/T0 | ,547789 | 19 | ,1464427 | ,0335962 |
| | R6/T1 | ,635395 | 19 | ,1529601 | ,0350915 |
| Pair 8 | R7/T0 | ,314937 | 19 | ,0534970 | ,0122730 |
| | R7/T1 | ,337268 | 19 | ,0598282 | ,0137255 |
| Pair 9 | R8/T0 | ,13716 | 19 | ,050468 | ,011578 |
| | R8/T1 | ,10479 | 19 | ,031926 | ,007324 |
| Pair 10 | R9/T0 | ,03700 | 19 | ,006888 | ,001580 |
| | R9/T1 | ,03321 | 19 | ,007764 | ,001781 |
| Pair 11 | F0/T0 | ,068611 | 19 | ,0125599 | ,0028814 |
| | F0/T1 | ,060395 | 19 | ,0068187 | ,0015643 |
| Pair 12 | F1/T0 | ,047611 | 19 | ,0278812 | ,0063964 |
| | F1/T1 | ,034126 | 19 | ,0161090 | ,0036957 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | R0/T0 - R0/T1 | ,050263 | ,056811 | ,013033 | ,022881 | ,077645 | 3,856 | 18 | ,001 |
| Pair 2 | R1/T0 - R1/T1 | ,017895 | ,022395 | ,005138 | ,007101 | ,028689 | 3,483 | 18 | ,003 |
| Pair 3 | R2/T0 - R2/T1 | ,0094684 | ,0900035 | ,0206482 | -,0339119 | ,0528487 | ,459 | 18 | ,652 |
| Pair 4 | R3/T0 - R3/T1 | ,054053 | ,056899 | ,013054 | ,026628 | ,081477 | 4,141 | 18 | ,001 |
| Pair 5 | R4/T0 - R4/T1 | ,033263 | ,030887 | ,007086 | ,018376 | ,048150 | 4,694 | 18 | ,000 |
| Pair 6 | R5/T0 - R5/T1 | -,0626789 | ,0888238 | ,0203776 | -,1054906 | -,0198673 | -3,076 | 18 | ,007 |
| Pair 7 | R6/T0 - R6/T1 | -,0876053 | ,1388490 | ,0318542 | -,1545284 | -,0206822 | -2,750 | 18 | ,013 |
| Pair 8 | R7/T0 - R7/T1 | -,0223316 | ,0477996 | ,0109660 | -,0453702 | ,0007071 | -2,036 | 18 | ,057 |
| Pair 9 | R8/T0 - R8/T1 | ,032368 | ,043835 | ,010056 | ,011241 | ,053496 | 3,219 | 18 | ,005 |
| Pair 10 | R9/T0 - R9/T1 | ,003789 | ,009157 | ,002101 | -,000624 | ,008203 | 1,804 | 18 | ,088 |
| Pair 11 | F0/T0 - F0/T1 | ,0082158 | ,0096061 | ,0022038 | ,0035858 | ,0128458 | 3,728 | 18 | ,002 |
| Pair 12 | F1/T0 - F1/T1 | ,0134842 | ,0277252 | ,0063606 | ,0001211 | ,0268473 | 2,120 | 18 | ,048 |

## 10.3.2. Emulsion with encapsulated equol

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | R0/T0 | ,20283 | 18 | ,051648 | ,012174 |
| | R0/T1 | ,17194 | 18 | ,042625 | ,010047 |
| Pair 2 | R1/T0 | ,08833 | 18 | ,025718 | ,006062 |
| | R1/T1 | ,06950 | 18 | ,018193 | ,004288 |
| Pair 3 | R2/T0 | ,561844 | 18 | ,0673904 | ,0158841 |
| | R2/T1 | ,593317 | 18 | ,0681862 | ,0160716 |
| Pair 4 | R3/T0 | ,23650 | 18 | ,055579 | ,013100 |
| | R3/T1 | ,20228 | 18 | ,045551 | ,010737 |
| Pair 5 | R4/T0 | ,11800 | 18 | ,033421 | ,007877 |
| | R4/T1 | ,09567 | 18 | ,024387 | ,005748 |
| Pair 6 | R5/T0 | ,497000 | 18 | ,0860318 | ,0202779 |
| | R5/T1 | ,555667 | 18 | ,0959864 | ,0226242 |
| Pair 7 | R6/T0 | ,595122 | 18 | ,1509171 | ,0355715 |
| | R6/T1 | ,620733 | 18 | ,1677351 | ,0395356 |
| Pair 8 | R7/T0 | ,311506 | 18 | ,0444888 | ,0104861 |
| | R7/T1 | ,342589 | 18 | ,0465937 | ,0109822 |
| Pair 9 | R8/T0 | ,11450 | 18 | ,033696 | ,007942 |
| | R8/T1 | ,10244 | 18 | ,030028 | ,007078 |
| Pair 10 | R9/T0 | ,03367 | 18 | ,007654 | ,001804 |
| | R9/T1 | ,03033 | 18 | ,005678 | ,001338 |
| Pair 11 | F0/T0 | ,063806 | 18 | ,0131652 | ,0031031 |
| | F0/T1 | ,056483 | 18 | ,0079229 | ,0018674 |
| Pair 12 | F1/T0 | ,035356 | 18 | ,0126152 | ,0029734 |
| | F1/T1 | ,030972 | 18 | ,0119401 | ,0028143 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | R0/T0 - R0/T1 | ,030889 | ,048952 | ,011538 | ,006545 | ,055232 | 2,677 | 17 | ,016 |
| Pair 2 | R1/T0 - R1/T1 | ,018833 | ,024675 | ,005816 | ,006563 | ,031104 | 3,238 | 17 | ,005 |
| Pair 3 | R2/T0 - R2/T1 | -,0314722 | ,0699368 | ,0164843 | -,0662510 | ,0033065 | -1,909 | 17 | ,073 |
| Pair 4 | R3/T0 - R3/T1 | ,034222 | ,053675 | ,012651 | ,007530 | ,060914 | 2,705 | 17 | ,015 |
| Pair 5 | R4/T0 - R4/T1 | ,022333 | ,030302 | ,007142 | ,007264 | ,037402 | 3,127 | 17 | ,006 |
| Pair 6 | R5/T0 - R5/T1 | -,0586667 | ,1115499 | ,0262926 | -,1141391 | -,0031942 | -2,231 | 17 | ,039 |
| Pair 7 | R6/T0 - R6/T1 | -,0256111 | ,1741864 | ,0410561 | -,1122320 | ,0610098 | -,624 | 17 | ,541 |
| Pair 8 | R7/T0 - R7/T1 | -,0310833 | ,0461508 | ,0108779 | -,0540336 | -,0081331 | -2,857 | 17 | ,011 |
| Pair 9 | R8/T0 - R8/T1 | ,012056 | ,031831 | ,007503 | -,003774 | ,027885 | 1,607 | 17 | ,127 |
| Pair 10 | R9/T0 - R9/T1 | ,003333 | ,008044 | ,001896 | -,000667 | ,007334 | 1,758 | 17 | ,097 |
| Pair 11 | F0/T0 - F0/T1 | ,0073222 | ,0098535 | ,0023225 | ,0024222 | ,0122222 | 3,153 | 17 | ,006 |
| Pair 12 | F1/T0 - F1/T1 | ,0043833 | ,0120377 | ,0028373 | -,0016029 | ,0103695 | 1,545 | 17 | ,141 |

## 10.3.3. Emulsion with lecithin

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | R0/T0 | ,19893 | 14 | ,068688 | ,018358 |
| | R0/T1 | ,17686 | 14 | ,054613 | ,014596 |
| Pair 2 | R1/T0 | ,08157 | 14 | ,028381 | ,007585 |
| | R1/T1 | ,06871 | 14 | ,021025 | ,005619 |
| Pair 3 | R2/T0 | ,588800 | 14 | ,0505355 | ,0135062 |
| | R2/T1 | ,600757 | 14 | ,0901779 | ,0241011 |
| Pair 4 | R3/T0 | ,23379 | 14 | ,070965 | ,018966 |
| | R3/T1 | ,20714 | 14 | ,055438 | ,014816 |
| Pair 5 | R4/T0 | ,11843 | 14 | ,036409 | ,009731 |
| | R4/T1 | ,09421 | 14 | ,022824 | ,006100 |
| Pair 6 | R5/T0 | ,561700 | 14 | ,1222159 | ,0326636 |
| | R5/T1 | ,550207 | 14 | ,1150056 | ,0307365 |
| Pair 7 | R6/T0 | ,636621 | 14 | ,1834086 | ,0490180 |
| | R6/T1 | ,617464 | 14 | ,1736377 | ,0464066 |
| Pair 8 | R7/T0 | ,341721 | 14 | ,0548306 | ,0146541 |
| | R7/T1 | ,340164 | 14 | ,0609376 | ,0162863 |
| Pair 9 | R8/T0 | ,11736 | 14 | ,043489 | ,011623 |
| | R8/T1 | ,10814 | 14 | ,046250 | ,012361 |
| Pair 10 | R9/T0 | ,03486 | 14 | ,009156 | ,002447 |
| | R9/T1 | ,03029 | 14 | ,005567 | ,001488 |
| Pair 11 | F0/T0 | ,066250 | 14 | ,0159220 | ,0042553 |
| | F0/T1 | ,056286 | 14 | ,0066081 | ,0017661 |
| Pair 12 | F1/T0 | ,035064 | 14 | ,0169559 | ,0045317 |
| | F1/T1 | ,034536 | 14 | ,0177444 | ,0047424 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | R0/T0 - R0/T1 | ,022071 | ,050387 | ,013466 | -,007021 | ,051164 | 1,639 | 13 | ,125 |
| Pair 2 | R1/T0 - R1/T1 | ,012857 | ,029693 | ,007936 | -,004287 | ,030001 | 1,620 | 13 | ,129 |
| Pair 3 | R2/T0 - R2/T1 | -,0119571 | ,0792157 | ,0211713 | -,0576949 | ,0337806 | -,565 | 13 | ,582 |
| Pair 4 | R3/T0 - R3/T1 | ,026643 | ,054826 | ,014653 | -,005013 | ,058299 | 1,818 | 13 | ,092 |
| Pair 5 | R4/T0 - R4/T1 | ,024214 | ,037681 | ,010071 | ,002458 | ,045971 | 2,404 | 13 | ,032 |
| Pair 6 | R5/T0 - R5/T1 | ,0114929 | ,1172934 | ,0313480 | -,0562303 | ,0792160 | ,367 | 13 | ,720 |
| Pair 7 | R6/T0 - R6/T1 | ,0191571 | ,1598293 | ,0427162 | -,0731255 | ,1114398 | ,448 | 13 | ,661 |
| Pair 8 | R7/T0 - R7/T1 | ,0015571 | ,0646455 | ,0172772 | -,0357680 | ,0388823 | ,090 | 13 | ,930 |
| Pair 9 | R8/T0 - R8/T1 | ,009214 | ,031611 | ,008448 | -,009037 | ,027466 | 1,091 | 13 | ,295 |
| Pair 10 | R9/T0 - R9/T1 | ,004571 | ,006607 | ,001766 | ,000757 | ,008386 | 2,589 | 13 | ,022 |
| Pair 11 | F0/T0 - F0/T1 | ,0099643 | ,0153703 | ,0041079 | ,0010897 | ,0188389 | 2,426 | 13 | ,031 |
| Pair 12 | F1/T0 - F1/T1 | ,0005286 | ,0139525 | ,0037290 | -,0075274 | ,0085845 | ,142 | 13 | ,889 |

## 10.4. Outputs of structure parameter- Visioscan® VC 98

### 10.4.1. Emulsion with equol

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | LL_T0 | 8,30[a] | 19 | ,000 | ,000 |
| | LL_T1 | 8,30[a] | 19 | ,000 | ,000 |
| Pair 2 | R1_T0 | 63,11 | 19 | 13,174 | 3,022 |
| | R1_T1 | 53,00 | 19 | 10,949 | 2,512 |
| Pair 3 | R2_T0 | 53,74 | 19 | 10,974 | 2,518 |
| | R2_T1 | 45,63 | 19 | 10,029 | 2,301 |
| Pair 4 | R3_T0 | 42,00 | 19 | 8,117 | 1,862 |
| | R3_T1 | 35,11 | 19 | 7,141 | 1,638 |
| Pair 5 | R4_T0 | 8,53 | 19 | 2,294 | ,526 |
| | R4_T1 | 6,84 | 19 | 2,141 | ,491 |
| Pair 6 | R5_T0 | 33,53 | 19 | 6,794 | 1,559 |
| | R5_T1 | 29,42 | 19 | 4,194 | ,962 |
| Pair 7 | SEr_T0 | 2,99 | 19 | 1,053 | ,241 |
| | SEr_T1 | 2,34 | 19 | 1,368 | ,314 |
| Pair 8 | SEsm_T0 | 133,41 | 19 | 52,077 | 11,947 |
| | SEsm_T1 | 109,89 | 19 | 45,860 | 10,521 |
| Pair 9 | SEsc_T0 | ,58 | 19 | ,132 | ,030 |
| | SEsc_T1 | ,65 | 19 | ,147 | ,034 |
| Pair 10 | SEw_T0 | 75,18 | 19 | 30,559 | 7,011 |
| | SEw_T1 | 72,88 | 19 | 27,111 | 6,220 |
| Pair 11 | Rku_T0 | 3,13 | 19 | ,042 | ,010 |
| | Rku_T1 | 3,15 | 19 | ,030 | ,007 |

a. The correlation and t cannot be computed because the standard error of the difference is 0.

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 2 | R1_T0 - R1_T1 | 10,105 | 10,979 | 2,519 | 4,813 | 15,397 | 4,012 | 18 | ,001 |
| Pair 3 | R2_T0 - R2_T1 | 8,105 | 9,718 | 2,229 | 3,422 | 12,789 | 3,636 | 18 | ,002 |
| Pair 4 | R3_T0 - R3_T1 | 6,895 | 6,765 | 1,552 | 3,634 | 10,155 | 4,442 | 18 | ,000 |
| Pair 5 | R4_T0 - R4_T1 | 1,684 | 1,857 | ,426 | ,789 | 2,579 | 3,952 | 18 | ,001 |
| Pair 6 | R5_T0 - R5_T1 | 4,105 | 5,924 | 1,359 | 1,250 | 6,961 | 3,020 | 18 | ,007 |
| Pair 7 | SEr_T0 - SEr_T1 | ,654 | 1,133 | ,260 | ,107 | 1,200 | 2,514 | 18 | ,022 |
| Pair 8 | SEsm_T0 - SEsm_T1 | 23,521 | 25,012 | 5,738 | 11,465 | 35,576 | 4,099 | 18 | ,001 |
| Pair 9 | SEsc_T0 - SEsc_T1 | -,073 | ,178 | ,041 | -,159 | ,013 | -1,791 | 18 | ,090 |
| Pair 10 | SEw_T0 - SEw_T1 | 2,304 | 17,783 | 4,080 | -6,268 | 10,875 | ,565 | 18 | ,579 |
| Pair 11 | Rku_T0 - Rku_T1 | -,019 | ,031 | ,007 | -,034 | -,004 | -2,658 | 18 | ,016 |

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Energie_T0 | ,05 | 19 | ,017 | ,004 |
| | Energie_T1 | ,07 | 19 | ,019 | ,004 |
| Pair 2 | Contrast_T0 | ,86 | 19 | ,226 | ,052 |
| | Contrast_T1 | ,71 | 19 | ,178 | ,041 |
| Pair 3 | Variance_T0 | 3,55 | 19 | ,564 | ,129 |
| | Variance_T1 | 3,13 | 19 | ,452 | ,104 |
| Pair 4 | Entropie_T0 | 1,59 | 19 | ,049 | ,011 |
| | Entropie_T1 | 1,56 | 19 | ,016 | ,004 |
| Pair 5 | Homogenity_T0 | 1,54 | 19 | ,048 | ,011 |
| | Homogenity_T1 | 1,57 | 19 | ,043 | ,010 |
| Pair 6 | Sebum_T0 | 1,91 | 19 | 1,702 | ,391 |
| | Sebum_T1 | 1,41 | 19 | 1,333 | ,306 |
| Pair 7 | Desquamation_T0 | 53,04 | 19 | 5,699 | 1,307 |
| | Desquamation_T1 | 54,63 | 19 | 1,332 | ,306 |
| Pair 8 | Surface_T0 | 397,60 | 19 | 287,933 | 66,056 |
| | Surface_T1 | 286,10 | 19 | 242,829 | 55,709 |
| Pair 9 | Volume_T0 | 8,29 | 19 | 7,629 | 1,750 |
| | Volume_T1 | 6,07 | 19 | 5,858 | 1,344 |
| Pair 10 | Cornerdensity_T0 | 10,62 | 19 | 1,305 | ,299 |
| | Cornerdensity_T1 | 11,55 | 19 | 1,395 | ,320 |
| Pair 11 | Anisotropyldx_T0 | 14,47 | 19 | 11,530 | 2,645 |
| | Anisotropyldx_T1 | 15,47 | 19 | 12,851 | 2,948 |
| Pair 12 | No. ofinsectionlines_T0 | 1773,53 | 19 | 131,427 | 30,151 |
| | No. ofinsectionlines_T1 | 1843,37 | 19 | 144,922 | 33,247 |
| Pair 13 | No.ofcells_T0 | 644,42 | 19 | 56,166 | 12,885 |
| | No.ofcells_T1 | 681,42 | 19 | 63,049 | 14,464 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | Energie_T0 - Energie_T1 | -,016 | ,012 | ,003 | -,022 | -,010 | -5,486 | 18 | ,000 |
| Pair 2 | Contrast_T0 - Contrast_T1 | ,156 | ,108 | ,025 | ,104 | ,208 | 6,303 | 18 | ,000 |
| Pair 3 | Variance_T0 - Variance_T1 | ,421 | ,312 | ,072 | ,271 | ,572 | 5,888 | 18 | ,000 |
| Pair 4 | Entropie_T0 - Entropie_T1 | ,024 | ,054 | ,012 | -,002 | ,050 | 1,937 | 18 | ,069 |
| Pair 5 | Homogenity_T0 - Homogenity_T1 | -,034 | ,023 | ,005 | -,045 | -,022 | -6,299 | 18 | ,000 |
| Pair 6 | Sebum_T0 - Sebum_T1 | ,503 | 1,395 | ,320 | -,170 | 1,175 | 1,571 | 18 | ,134 |
| Pair 7 | Desquamation_T0 - Desquamation_T1 | -1,592 | 5,340 | 1,225 | -4,165 | ,982 | -1,299 | 18 | ,210 |
| Pair 8 | Surface_T0 - Surface_T1 | 111,503 | 221,890 | 50,905 | 4,556 | 218,451 | 2,190 | 18 | ,042 |
| Pair 9 | Volume_T0 - Volume_T1 | 2,215 | 6,267 | 1,438 | -,806 | 5,235 | 1,541 | 18 | ,141 |
| Pair 10 | Cornerdensity_T0 - Cornerdensity_T1 | -,931 | ,793 | ,182 | -1,314 | -,549 | -5,121 | 18 | ,000 |
| Pair 11 | Anisotropyldx_T0 - Anisotropyldx_T1 | -1,000 | 17,920 | 4,111 | -9,637 | 7,637 | -,243 | 18 | ,811 |
| Pair 12 | No. ofinsectionlines_T0 - No. ofinsectionlines_T1 | -69,842 | 97,044 | 22,263 | -116,616 | -23,068 | -3,137 | 18 | ,006 |
| Pair 13 | No.ofcells_T0 - No.ofcells_T1 | -37,000 | 41,214 | 9,455 | -56,864 | -17,136 | -3,913 | 18 | ,001 |

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | CellcI0_T0 | 13,58 | 19 | 5,601 | 1,285 |
| | CellcI0_T1 | 12,05 | 19 | 4,196 | ,963 |
| Pair 2 | CellcI1_T0 | 11,58 | 19 | 4,325 | ,992 |
| | CellcI1_T1 | 10,58 | 19 | 4,312 | ,989 |
| Pair 3 | CellcI2_T0 | 119,63 | 19 | 22,719 | 5,212 |
| | CellcI2_T1 | 125,21 | 19 | 25,924 | 5,947 |
| Pair 4 | CellcI3_T0 | 191,21 | 19 | 33,681 | 7,727 |
| | CellcI3_T1 | 220,05 | 19 | 42,056 | 9,648 |
| Pair 5 | CellcI4_T0 | 173,79 | 19 | 19,447 | 4,461 |
| | CellcI4_T1 | 187,95 | 19 | 26,879 | 6,167 |
| Pair 6 | CellcI5_T0 | 134,63 | 19 | 13,965 | 3,204 |
| | CellcI5_T1 | 125,58 | 19 | 16,008 | 3,672 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | CellcI0_T0 - CellcI0_T1 | 1,526 | 6,611 | 1,517 | -1,660 | 4,713 | 1,006 | 18 | ,328 |
| Pair 2 | CellcI1_T0 - CellcI1_T1 | 1,000 | 5,477 | 1,257 | -1,640 | 3,640 | ,796 | 18 | ,437 |
| Pair 3 | CellcI2_T0 - CellcI2_T1 | -5,579 | 23,035 | 5,284 | -16,681 | 5,523 | -1,056 | 18 | ,305 |
| Pair 4 | CellcI3_T0 - CellcI3_T1 | -28,842 | 28,943 | 6,640 | -42,792 | -14,892 | -4,344 | 18 | ,000 |
| Pair 5 | CellcI4_T0 - CellcI4_T1 | -14,158 | 17,790 | 4,081 | -22,732 | -5,584 | -3,469 | 18 | ,003 |
| Pair 6 | CellcI5_T0 - CellcI5_T1 | 9,053 | 11,778 | 2,702 | 3,376 | 14,729 | 3,350 | 18 | ,004 |

## 10.4.2. Emulsion with encapsulated equol

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | LL_T0 | 8,30[a] | 18 | ,000 | ,000 |
| | LL_T1 | 8,30[a] | 18 | ,000 | ,000 |
| Pair 2 | R1_T0 | 64,11 | 18 | 10,335 | 2,436 |
| | R1_T1 | 55,00 | 18 | 5,358 | 1,263 |
| Pair 3 | R2_T0 | 53,94 | 18 | 6,310 | 1,487 |
| | R2_T1 | 45,39 | 18 | 4,146 | ,977 |
| Pair 4 | R3_T0 | 42,22 | 18 | 5,786 | 1,364 |
| | R3_T1 | 35,94 | 18 | 3,857 | ,909 |
| Pair 5 | R4_T0 | 8,50 | 18 | 2,149 | ,506 |
| | R4_T1 | 7,17 | 18 | 1,249 | ,294 |
| Pair 6 | R5_T0 | 34,44 | 18 | 5,843 | 1,377 |
| | R5_T1 | 29,94 | 18 | 3,316 | ,782 |
| Pair 7 | SEr_T0 | 3,07 | 18 | 1,345 | ,317 |
| | SEr_T1 | 2,48 | 18 | 1,153 | ,272 |
| Pair 8 | SEsm_T0 | 137,71 | 18 | 56,367 | 13,286 |
| | SEsm_T1 | 122,00 | 18 | 36,272 | 8,549 |
| Pair 9 | SEsc_T0 | ,61 | 18 | ,150 | ,035 |
| | SEsc_T1 | ,61 | 18 | ,152 | ,036 |
| Pair 10 | SEw_T0 | 78,32 | 18 | 32,666 | 7,699 |
| | SEw_T1 | 77,04 | 18 | 23,042 | 5,431 |
| Pair 11 | Rku_T0 | 3,13 | 18 | ,047 | ,011 |
| | Rku_T1 | 3,14 | 18 | ,035 | ,008 |

a. The correlation and t cannot be computed because the standard error of the difference is 0.

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 2 | R1_T0 - R1_T1 | 9,111 | 9,042 | 2,131 | 4,615 | 13,607 | 4,275 | 17 | ,001 |
| Pair 3 | R2_T0 - R2_T1 | 8,556 | 5,823 | 1,373 | 5,660 | 11,451 | 6,233 | 17 | ,000 |
| Pair 4 | R3_T0 - R3_T1 | 6,278 | 4,800 | 1,131 | 3,891 | 8,665 | 5,549 | 17 | ,000 |
| Pair 5 | R4_T0 - R4_T1 | 1,333 | 1,879 | ,443 | ,399 | 2,268 | 3,011 | 17 | ,008 |
| Pair 6 | R5_T0 - R5_T1 | 4,500 | 5,294 | 1,248 | 1,867 | 7,133 | 3,606 | 17 | ,002 |
| Pair 7 | SEr_T0 - SEr_T1 | ,582 | ,964 | ,227 | ,102 | 1,061 | 2,560 | 17 | ,020 |
| Pair 8 | SEsm_T0 - SEsm_T1 | 15,709 | 47,283 | 11,145 | -7,804 | 39,222 | 1,410 | 17 | ,177 |
| Pair 9 | SEsc_T0 - SEsc_T1 | ,002 | ,224 | ,053 | -,109 | ,114 | ,042 | 17 | ,967 |
| Pair 10 | SEw_T0 - SEw_T1 | 1,275 | 25,644 | 6,044 | -11,478 | 14,027 | ,211 | 17 | ,835 |
| Pair 11 | Rku_T0 - Rku_T1 | -,005 | ,050 | ,012 | -,030 | ,020 | -,425 | 17 | ,677 |

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Energie_T0 | ,05 | 18 | ,013 | ,003 |
| | Energie_T1 | ,06 | 18 | ,012 | ,003 |
| Pair 2 | Contrast_T0 | ,92 | 18 | ,172 | ,040 |
| | Contrast_T1 | ,78 | 18 | ,146 | ,034 |
| Pair 3 | Variance_T0 | 3,69 | 18 | ,413 | ,097 |
| | Variance_T1 | 3,30 | 18 | ,381 | ,090 |
| Pair 4 | Entropie_T0 | 1,58 | 18 | ,043 | ,010 |
| | Entropie_T1 | 1,56 | 18 | ,015 | ,004 |
| Pair 5 | Homogenity_T0 | 1,52 | 18 | ,034 | ,008 |
| | Homogenity_T1 | 1,55 | 18 | ,036 | ,008 |
| Pair 6 | Sebum_T0 | 1,65 | 18 | 1,193 | ,281 |
| | Sebum_T1 | 2,37 | 18 | 2,976 | ,701 |
| Pair 7 | Desquamation_T0 | 53,13 | 18 | 6,104 | 1,439 |
| | Desquamation_T1 | 47,16 | 18 | 17,513 | 4,128 |
| Pair 8 | Surface_T0 | 372,12 | 18 | 239,195 | 56,379 |
| | Surface_T1 | 442,60 | 18 | 448,140 | 105,628 |
| Pair 9 | Volume_T0 | 7,09 | 18 | 5,362 | 1,264 |
| | Volume_T1 | 10,44 | 18 | 13,517 | 3,186 |
| Pair 10 | Cornerdensity_T0 | 10,69 | 18 | 2,072 | ,488 |
| | Cornerdensity_T1 | 11,03 | 18 | 1,698 | ,400 |
| Pair 11 | Anisotropyldx_T0 | 17,22 | 18 | 10,653 | 2,511 |
| | Anisotropyldx_T1 | 19,06 | 18 | 15,195 | 3,581 |
| Pair 12 | No. ofinsectionlines_T0 | 1776,67 | 18 | 212,084 | 49,989 |
| | No. ofinsectionlines_T1 | 1817,44 | 18 | 187,019 | 44,081 |
| Pair 13 | No.ofcells_T0 | 649,28 | 18 | 90,409 | 21,310 |
| | No.ofcells_T1 | 663,00 | 18 | 72,932 | 17,190 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | Energie_T0 - Energie_T1 | -,008 | ,010 | ,002 | -,013 | -,003 | -3,396 | 17 | ,003 |
| Pair 2 | Contrast_T0 - Contrast_T1 | ,142 | ,114 | ,027 | ,085 | ,199 | 5,274 | 17 | ,000 |
| Pair 3 | Variance_T0 - Variance_T1 | ,389 | ,312 | ,073 | ,234 | ,544 | 5,288 | 17 | ,000 |
| Pair 4 | Entropie_T0 - Entropie_T1 | ,017 | ,049 | ,012 | -,007 | ,042 | 1,488 | 17 | ,155 |
| Pair 5 | Homogenity_T0 - Homogenity_T1 | -,029 | ,024 | ,006 | -,041 | -,017 | -5,204 | 17 | ,000 |
| Pair 6 | Sebum_T0 - Sebum_T1 | -,723 | 2,684 | ,633 | -2,057 | ,612 | -1,143 | 17 | ,269 |
| Pair 7 | Desquamation_T0 - Desquamation_T1 | 5,973 | 18,767 | 4,423 | -3,359 | 15,306 | 1,350 | 17 | ,195 |
| Pair 8 | Surface_T0 - Surface_T1 | -70,478 | 444,655 | 104,806 | -291,600 | 150,644 | -,672 | 17 | ,510 |
| Pair 9 | Volume_T0 - Volume_T1 | -3,350 | 12,110 | 2,854 | -9,372 | 2,672 | -1,174 | 17 | ,257 |
| Pair 10 | Cornerdensity_T0 - Cornerdensity_T1 | -,339 | 1,539 | ,363 | -1,104 | ,426 | -,935 | 17 | ,363 |
| Pair 11 | Anisotropyldx_T0 - Anisotropyldx_T1 | -1,833 | 15,745 | 3,711 | -9,663 | 5,997 | -,494 | 17 | ,628 |
| Pair 12 | No. ofinsectionlines_T0 - No. ofinsectionlines_T1 | -40,778 | 168,568 | 39,732 | -124,605 | 43,049 | -1,026 | 17 | ,319 |
| Pair 13 | No.ofcells_T0 - No.ofcells_T1 | -13,722 | 68,732 | 16,200 | -47,902 | 20,457 | -,847 | 17 | ,409 |

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | CellcI0_T0 | 13,61 | 18 | 6,757 | 1,593 |
| | CellcI0_T1 | 13,50 | 18 | 5,993 | 1,412 |
| Pair 2 | CellcI1_T0 | 11,94 | 18 | 3,977 | ,937 |
| | CellcI1_T1 | 12,06 | 18 | 5,139 | 1,211 |
| Pair 3 | CellcI2_T0 | 125,78 | 18 | 40,851 | 9,629 |
| | CellcI2_T1 | 119,56 | 18 | 26,376 | 6,217 |
| Pair 4 | CellcI3_T0 | 194,06 | 18 | 49,232 | 11,604 |
| | CellcI3_T1 | 206,39 | 18 | 43,911 | 10,350 |
| Pair 5 | CellcI4_T0 | 168,50 | 18 | 23,920 | 5,638 |
| | CellcI4_T1 | 182,00 | 18 | 23,065 | 5,437 |
| Pair 6 | CellcI5_T0 | 135,39 | 18 | 15,538 | 3,662 |
| | CellcI5_T1 | 129,50 | 18 | 14,234 | 3,355 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | CellcI0_T0 - CellcI0_T1 | ,111 | 6,379 | 1,504 | -3,061 | 3,283 | ,074 | 17 | ,942 |
| Pair 2 | CellcI1_T0 - CellcI1_T1 | -,111 | 6,498 | 1,532 | -3,342 | 3,120 | -,073 | 17 | ,943 |
| Pair 3 | CellcI2_T0 - CellcI2_T1 | 6,222 | 34,678 | 8,174 | -11,023 | 23,467 | ,761 | 17 | ,457 |
| Pair 4 | CellcI3_T0 - CellcI3_T1 | -12,333 | 37,358 | 8,805 | -30,911 | 6,245 | -1,401 | 17 | ,179 |
| Pair 5 | CellcI4_T0 - CellcI4_T1 | -13,500 | 17,810 | 4,198 | -22,357 | -4,643 | -3,216 | 17 | ,005 |
| Pair 6 | CellcI5_T0 - CellcI5_T1 | 5,889 | 11,565 | 2,726 | ,138 | 11,640 | 2,160 | 17 | ,045 |

## 10.4.3. Emulsion with lecithin

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | LL_T0 | 8,30[a] | 14 | ,000 | ,000 |
| | LL_T1 | 8,30[a] | 14 | ,000 | ,000 |
| Pair 2 | R1_T0 | 56,00 | 14 | 8,115 | 2,169 |
| | R1_T1 | 54,93 | 14 | 10,307 | 2,755 |
| Pair 3 | R2_T0 | 46,07 | 14 | 7,849 | 2,098 |
| | R2_T1 | 45,57 | 14 | 8,197 | 2,191 |
| Pair 4 | R3_T0 | 36,00 | 14 | 5,936 | 1,586 |
| | R3_T1 | 35,64 | 14 | 6,428 | 1,718 |
| Pair 5 | R4_T0 | 7,50 | 14 | 1,225 | ,327 |
| | R4_T1 | 7,29 | 14 | 1,490 | ,398 |
| Pair 6 | R5_T0 | 30,29 | 14 | 4,065 | 1,087 |
| | R5_T1 | 29,93 | 14 | 4,922 | 1,315 |
| Pair 7 | SEr_T0 | 3,25 | 14 | 1,975 | ,528 |
| | SEr_T1 | 3,01 | 14 | 1,712 | ,458 |
| Pair 8 | SEsm_T0 | 130,77 | 14 | 36,480 | 9,750 |
| | SEsm_T1 | 134,80 | 14 | 62,873 | 16,803 |
| Pair 9 | SEsc_T0 | ,53 | 14 | ,121 | ,032 |
| | SEsc_T1 | ,63 | 14 | ,186 | ,050 |
| Pair 10 | SEw_T0 | 81,54 | 14 | 23,971 | 6,406 |
| | SEw_T1 | 87,40 | 14 | 27,587 | 7,373 |
| Pair 11 | Rku_T0 | 3,14 | 14 | ,027 | ,007 |
| | Rku_T1 | 3,15 | 14 | ,028 | ,008 |

a. The correlation and t cannot be computed because the standard error of the difference is 0.

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 2 | R1_T0 - R1_T1 | 1,071 | 8,251 | 2,205 | -3,692 | 5,835 | ,486 | 13 | ,635 |
| Pair 3 | R2_T0 - R2_T1 | ,500 | 7,633 | 2,040 | -3,907 | 4,907 | ,245 | 13 | ,810 |
| Pair 4 | R3_T0 - R3_T1 | ,357 | 5,943 | 1,588 | -3,074 | 3,789 | ,225 | 13 | ,826 |
| Pair 5 | R4_T0 - R4_T1 | ,214 | 1,424 | ,381 | -,608 | 1,036 | ,563 | 13 | ,583 |
| Pair 6 | R5_T0 - R5_T1 | ,357 | 4,749 | 1,269 | -2,385 | 3,099 | ,281 | 13 | ,783 |
| Pair 7 | SEr_T0 - SEr_T1 | ,238 | 1,379 | ,368 | -,558 | 1,034 | ,646 | 13 | ,530 |
| Pair 8 | SEsm_T0 - SEsm_T1 | -4,028 | 51,535 | 13,773 | -33,783 | 25,728 | -,292 | 13 | ,775 |
| Pair 9 | SEsc_T0 - SEsc_T1 | -,098 | ,215 | ,057 | -,222 | ,026 | -1,703 | 13 | ,112 |
| Pair 10 | SEw_T0 - SEw_T1 | -5,859 | 21,476 | 5,740 | -18,259 | 6,541 | -1,021 | 13 | ,326 |
| Pair 11 | Rku_T0 - Rku_T1 | -,005 | ,030 | ,008 | -,022 | ,012 | -,633 | 13 | ,538 |

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Energie_T0 | ,05 | 14 | ,012 | ,003 |
| | Energie_T1 | ,06 | 14 | ,015 | ,004 |
| Pair 2 | Contrast_T0 | ,80 | 14 | ,193 | ,052 |
| | Contrast_T1 | ,73 | 14 | ,215 | ,057 |
| Pair 3 | Variance_T0 | 3,33 | 14 | ,503 | ,134 |
| | Variance_T1 | 3,14 | 14 | ,531 | ,142 |
| Pair 4 | Entropie_T0 | 1,60 | 14 | ,034 | ,009 |
| | Entropie_T1 | 1,56 | 14 | ,016 | ,004 |
| Pair 5 | Homogenity_T0 | 1,55 | 14 | ,040 | ,011 |
| | Homogenity_T1 | 1,57 | 14 | ,051 | ,014 |
| Pair 6 | Sebum_T0 | 2,58 | 14 | 2,518 | ,673 |
| | Sebum_T1 | 1,81 | 14 | 2,505 | ,670 |
| Pair 7 | Desquamation_T0 | 52,08 | 14 | 7,299 | 1,951 |
| | Desquamation_T1 | 50,02 | 14 | 14,658 | 3,917 |
| Pair 8 | Surface_T0 | 452,92 | 14 | 323,036 | 86,335 |
| | Surface_T1 | 329,27 | 14 | 317,158 | 84,764 |
| Pair 9 | Volume_T0 | 11,21 | 14 | 11,308 | 3,022 |
| | Volume_T1 | 7,96 | 14 | 11,428 | 3,054 |
| Pair 10 | Cornerdensity_T0 | 11,05 | 14 | 1,791 | ,479 |
| | Cornerdensity_T1 | 10,98 | 14 | 1,465 | ,392 |
| Pair 11 | AnisotropyIdx_T0 | 17,07 | 14 | 11,744 | 3,139 |
| | AnisotropyIdx_T1 | 14,36 | 14 | 11,817 | 3,158 |
| Pair 12 | No. ofinsectionlines_T0 | 1807,29 | 14 | 184,949 | 49,430 |
| | No. ofinsectionlines_T1 | 1786,00 | 14 | 133,074 | 35,566 |
| Pair 13 | No.ofcells_T0 | 659,57 | 14 | 77,454 | 20,701 |
| | No.ofcells_T1 | 656,64 | 14 | 61,386 | 16,406 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | Energie_T0 - Energie_T1 | -,007 | ,011 | ,003 | -,014 | -,001 | -2,363 | 13 | ,034 |
| Pair 2 | Contrast_T0 - Contrast_T1 | ,070 | ,170 | ,045 | -,029 | ,168 | 1,531 | 13 | ,150 |
| Pair 3 | Variance_T0 - Variance_T1 | ,188 | ,419 | ,112 | -,053 | ,430 | 1,682 | 13 | ,116 |
| Pair 4 | Entropie_T0 - Entropie_T1 | ,034 | ,032 | ,008 | ,016 | ,052 | 4,016 | 13 | ,001 |
| Pair 5 | Homogenity_T0 - Homogenity_T1 | -,018 | ,034 | ,009 | -,038 | ,001 | -2,017 | 13 | ,065 |
| Pair 6 | Sebum_T0 - Sebum_T1 | ,769 | 2,033 | ,543 | -,405 | 1,943 | 1,416 | 13 | ,180 |
| Pair 7 | Desquamation_T0 - Desquamation_T1 | 2,059 | 13,821 | 3,694 | -5,920 | 10,039 | ,558 | 13 | ,587 |
| Pair 8 | Surface_T0 - Surface_T1 | 123,646 | 368,142 | 98,390 | -88,913 | 336,205 | 1,257 | 13 | ,231 |
| Pair 9 | Volume_T0 - Volume_T1 | 3,254 | 9,253 | 2,473 | -2,088 | 8,597 | 1,316 | 13 | ,211 |
| Pair 10 | Cornerdensity_T0 - Cornerdensity_T1 | ,076 | 1,390 | ,372 | -,727 | ,879 | ,205 | 13 | ,841 |
| Pair 11 | AnisotropyIdx_T0 - AnisotropyIdx_T1 | 2,714 | 16,392 | 4,381 | -6,750 | 12,178 | ,620 | 13 | ,546 |
| Pair 12 | No. ofinsectionlines_T0 - No. ofinsectionlines_T1 | 21,286 | 158,382 | 42,329 | -70,161 | 112,733 | ,503 | 13 | ,623 |
| Pair 13 | No.ofcells_T0 - No.ofcells_T1 | 2,929 | 62,111 | 16,600 | -32,933 | 38,790 | ,176 | 13 | ,863 |

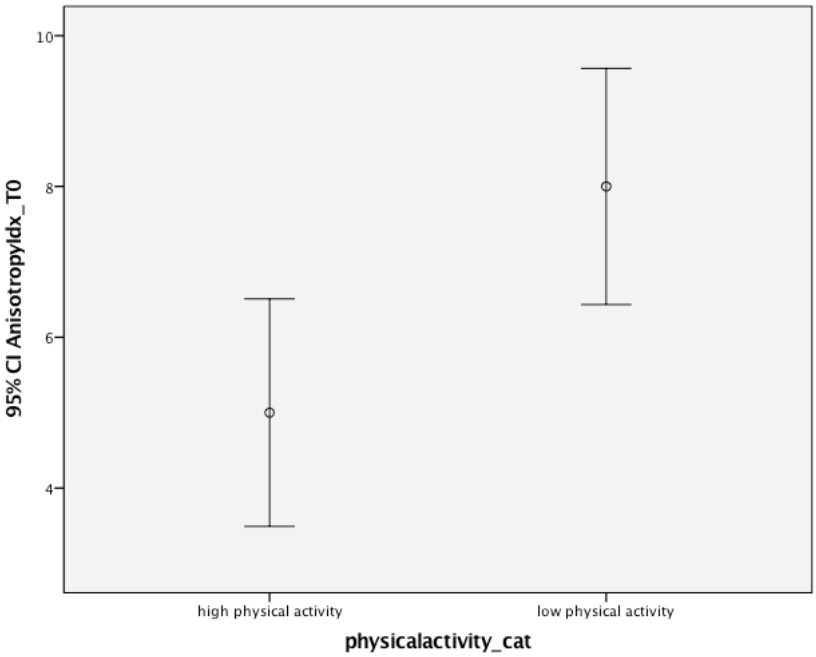
**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | CellcI0_T0 | 8,57 | 14 | 3,502 | ,936 |
| | CellcI0_T1 | 11,00 | 14 | 6,668 | 1,782 |
| Pair 2 | CellcI1_T0 | 11,29 | 14 | 6,318 | 1,688 |
| | CellcI1_T1 | 9,57 | 14 | 3,546 | ,948 |
| Pair 3 | CellcI2_T0 | 121,79 | 14 | 32,688 | 8,736 |
| | CellcI2_T1 | 116,21 | 14 | 20,310 | 5,428 |
| Pair 4 | CellcI3_T0 | 210,14 | 14 | 41,906 | 11,200 |
| | CellcI3_T1 | 204,79 | 14 | 34,852 | 9,315 |
| Pair 5 | CellcI4_T0 | 179,71 | 14 | 30,246 | 8,084 |
| | CellcI4_T1 | 185,29 | 14 | 26,389 | 7,053 |
| Pair 6 | CellcI5_T0 | 128,07 | 14 | 14,673 | 3,922 |
| | CellcI5_T1 | 129,79 | 14 | 12,546 | 3,353 |

**Paired Samples Test**

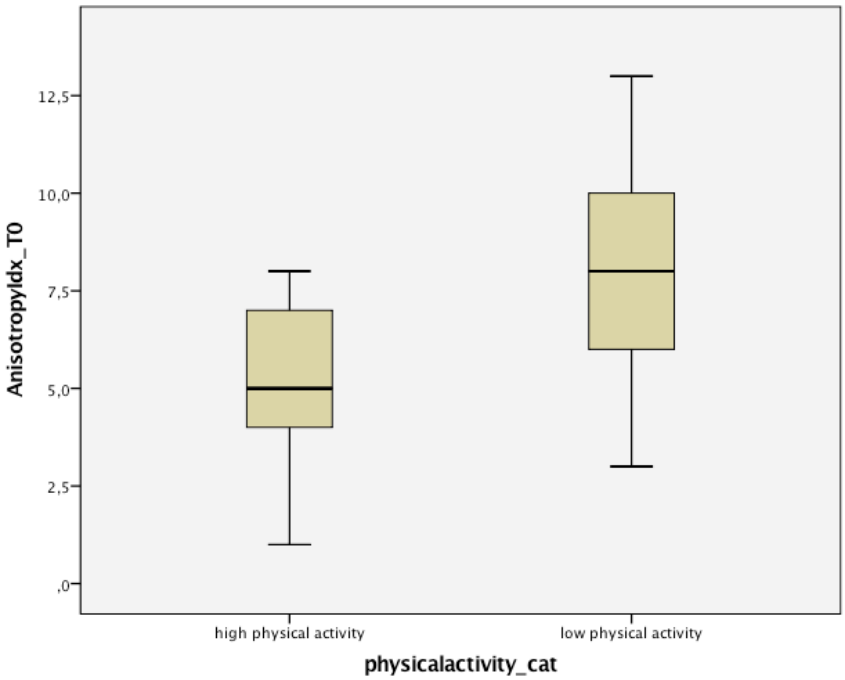
| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | CellcI0_T0 - CellcI0_T1 | -2,429 | 5,273 | 1,409 | -5,473 | ,616 | -1,723 | 13 | ,109 |
| Pair 2 | CellcI1_T0 - CellcI1_T1 | 1,714 | 7,226 | 1,931 | -2,458 | 5,887 | ,888 | 13 | ,391 |
| Pair 3 | CellcI2_T0 - CellcI2_T1 | 5,571 | 28,565 | 7,634 | -10,921 | 22,064 | ,730 | 13 | ,478 |
| Pair 4 | CellcI3_T0 - CellcI3_T1 | 5,357 | 36,390 | 9,726 | -15,654 | 26,368 | ,551 | 13 | ,591 |
| Pair 5 | CellcI4_T0 - CellcI4_T1 | -5,571 | 26,978 | 7,210 | -21,148 | 10,005 | -,773 | 13 | ,454 |
| Pair 6 | CellcI5_T0 - CellcI5_T1 | -1,714 | 15,857 | 4,238 | -10,870 | 7,441 | -,405 | 13 | ,692 |

## 10.4.4. Anisotropy and physical activity




**Case Processing Summary**

| | physicalactivity_cat | Cases | | | | | |
|---|---|---|---|---|---|---|---|
| | | Valid | | Missing | | Total | |
| | | N | Percent | N | Percent | N | Percent |
| Anisotropyldx_T0 | high physical activity | 10 | 100,0% | 0 | 0,0% | 10 | 100,0% |
| | low physical activity | 15 | 100,0% | 0 | 0,0% | 15 | 100,0% |



**Between-Subjects Factors**

| | | Value Label | N |
|---|---|---|---|
| physicalactivity | 1 | more than once a day | 4 |
| | 2 | once a day | 4 |
| | 3 | 4-6 times a week | 2 |
| | 4 | 1-3 times a week | 12 |
| | 5 | every second week or less | 3 |
| sport | 0 | never | 1 |
| | 1 | more than once a day | 1 |
| | 2 | once a day | 1 |
| | 3 | 4-6 times a week | 2 |
| | 4 | 1-3 times a week | 12 |
| | 5 | every second week or less | 8 |
| outdoortime | 1 | rarely | 1 |
| | 2 | frequently | 24 |
| sunbathing | 0 | never | 3 |
| | 1 | rarely | 10 |
| | 2 | frequently | 12 |
| smoker | 0 | no | 18 |
| | 1 | no more | 5 |
| | 2 | yes | 2 |

**Tests of Between-Subjects Effects**

Dependent Variable: AnisotropyIdx_T0

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 164,222[a] | 13 | 12,632 | 3,326 | ,027 |
| Intercept | 13,983 | 1 | 13,983 | 3,682 | ,081 |
| physicalactivity | 123,768 | 4 | 30,942 | 8,147 | ,003 |
| sport | 81,284 | 4 | 20,321 | 5,351 | ,012 |
| outdoortime | 23,335 | 1 | 23,335 | 6,144 | ,031 |
| sunbathing | 18,440 | 2 | 9,220 | 2,428 | ,134 |
| smoker | 13,075 | 2 | 6,538 | 1,721 | ,224 |
| Error | 41,778 | 11 | 3,798 | | |
| Total | 1362,000 | 25 | | | |
| Corrected Total | 206,000 | 24 | | | |

a. R Squared = ,797 (Adjusted R Squared = ,558)

**Parameter Estimates**

Dependent Variable: AnisotropyIdx_T0

| Parameter | B | Std. Error | t | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Intercept | 6,209 | 2,835 | 2,190 | ,051 | -,031 | 12,449 |
| [physicalactivity=1] | -8,070 | 2,741 | -2,944 | ,013 | -14,104 | -2,037 |
| [physicalactivity=2] | -8,063 | 3,065 | -2,631 | ,023 | -14,810 | -1,317 |
| [physicalactivity=3] | -9,642 | 3,397 | -2,838 | ,016 | -17,120 | -2,165 |
| [physicalactivity=4] | -2,688 | 2,867 | -,937 | ,369 | -8,999 | 3,623 |
| [physicalactivity=5] | 0[a] | . | . | . | . | . |
| [sport=0] | 3,845 | 2,062 | 1,865 | ,089 | -,694 | 8,383 |
| [sport=1] | 9,861 | 3,081 | 3,201 | ,008 | 3,081 | 16,642 |
| [sport=3] | -4,470 | 2,686 | -1,664 | ,124 | -10,381 | 1,441 |
| [sport=4] | 3,485 | 1,729 | 2,015 | ,069 | -,321 | 7,290 |
| [sport=5] | 0[a] | . | . | . | . | . |
| [outdoortime=1] | -4,555 | 1,838 | -2,479 | ,031 | -8,600 | -,510 |
| [outdoortime=2] | 0[a] | . | . | . | . | . |
| [sunbathing=0] | -1,856 | 1,896 | -,979 | ,349 | -6,028 | 2,317 |
| [sunbathing=1] | 2,011 | 1,243 | 1,619 | ,134 | -,724 | 4,746 |
| [sunbathing=2] | 0[a] | . | . | . | . | . |
| [smoker=0] | 1,938 | 1,587 | 1,221 | ,248 | -1,555 | 5,430 |
| [smoker=1] | 3,791 | 2,059 | 1,841 | ,093 | -,741 | 8,323 |
| [smoker=2] | 0[a] | . | . | . | . | . |

a. This parameter is set to zero because it is redundant.

# 10.5. Outputs of molecular parameter: telomere analysis

## *Statistical analysis of lecithin and equol:*

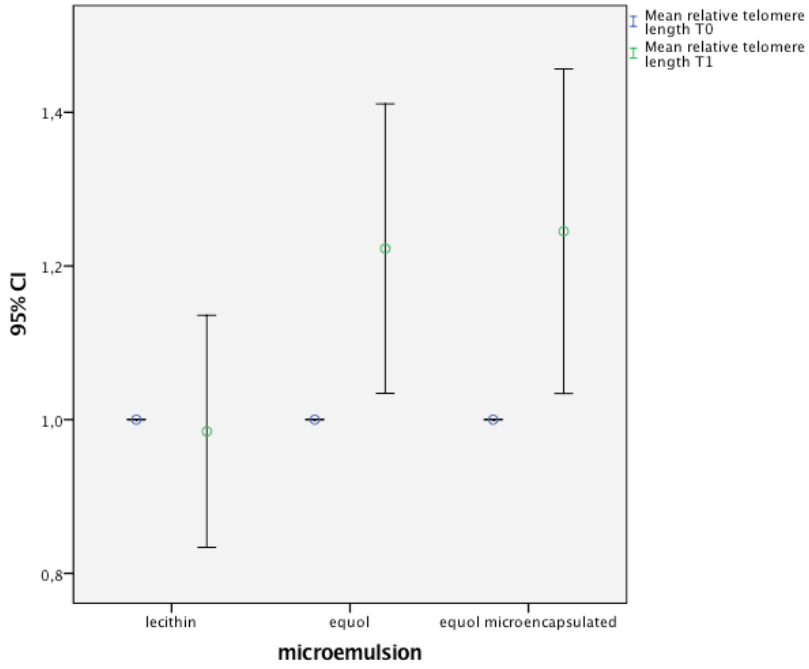
**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Mean relative telomere length T1 | Equal variances assumed | 2,647 | ,114 | -2,001 | 32 | ,054 | -,23779 | ,11887 | -,47991 | ,00433 |
| | Equal variances not assumed | | | -2,086 | 31,594 | ,045 | -,23779 | ,11399 | -,47010 | -,00549 |

## *Statistical analysis of lecithin and encapsulated equol:*

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Mean relative telomere length T1 | Equal variances assumed | 2,568 | ,119 | -2,047 | 31 | ,049 | -,26041 | ,12723 | -,51990 | -,00092 |
| | Equal variances not assumed | | | -2,128 | 29,277 | ,042 | -,26041 | ,12236 | -,51056 | -,01026 |

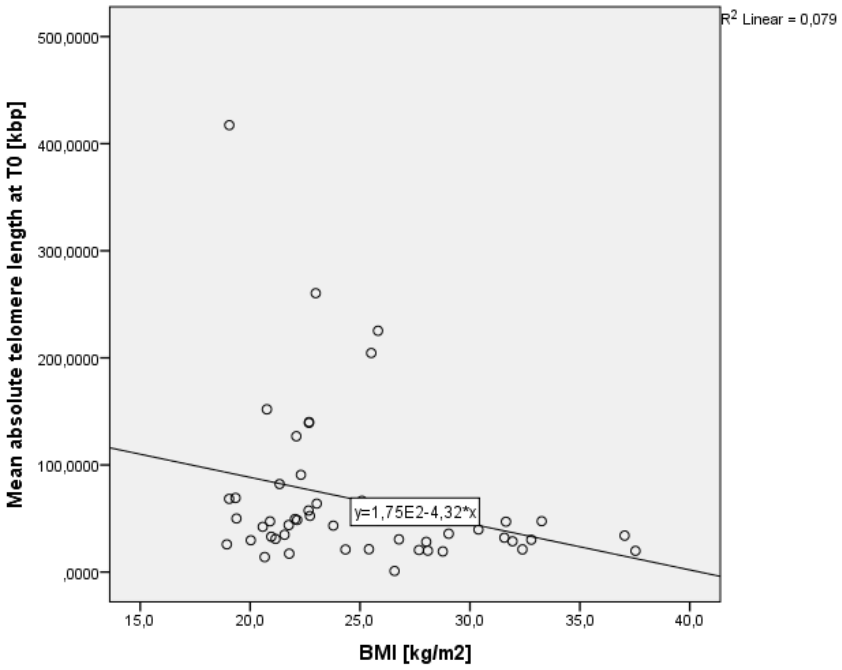


***Statistical analysis of BMI and absolute telomere length:***

**Correlations**

| | | | BMI [kg/m2] | Mean absolute telomere length at T0 [kbp] |
|---|---|---|---|---|
| Spearman's rho | BMI [kg/m2] | Correlation Coefficient | 1,000 | -,312* |
| | | Sig. (2-tailed) | . | ,027 |
| | | N | 50 | 50 |
| | Mean absolute telomere length at T0 [kbp] | Correlation Coefficient | -,312* | 1,000 |
| | | Sig. (2-tailed) | ,027 | . |
| | | N | 50 | 50 |

*. Correlation is significant at the 0.05 level (2-tailed).



***Statistical analysis of sunbathing and absolute telomere length:***

**Between-Subjects Factors**

| | | Value Label | N |
|---|---|---|---|
| sunbathing | 0 | never | 4 |
| | 1 | rarely | 29 |
| | 2 | frequently | 17 |

**Tests of Between-Subjects Effects**

Dependent Variable: Mean absolute telomere length at T0 [kbp]

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 40840,920[a] | 2 | 20420,460 | 4,155 | ,022 |
| Intercept | 221426,497 | 1 | 221426,497 | 45,058 | ,000 |
| sunbathing | 40840,920 | 2 | 20420,460 | 4,155 | ,022 |
| Error | 230970,989 | 47 | 4914,276 | | |
| Total | 493953,832 | 50 | | | |
| Corrected Total | 271811,909 | 49 | | | |

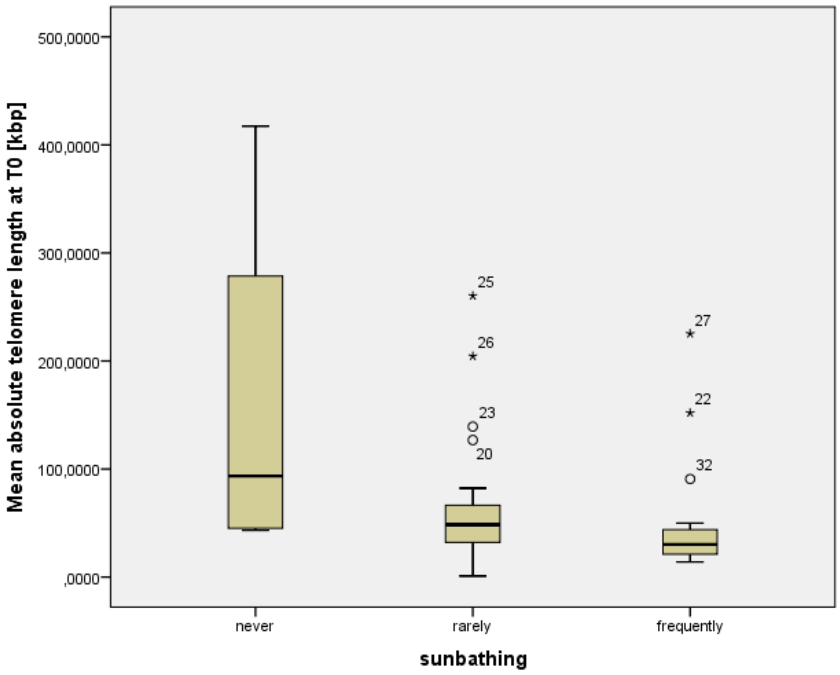
a. R Squared = ,150 (Adjusted R Squared = ,114)

**Parameter Estimates**

Dependent Variable: Mean absolute telomere length at T0 [kbp]

| Parameter | B | Std. Error | t | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Intercept | 51,227 | 17,002 | 3,013 | ,004 | 17,023 | 85,431 |
| [sunbathing=0] | 110,701 | 38,957 | 2,842 | ,007 | 32,329 | 189,072 |
| [sunbathing=1] | 11,330 | 21,413 | ,529 | ,599 | -31,748 | 54,408 |
| [sunbathing=2] | 0[a] | . | . | . | . | . |

a. This parameter is set to zero because it is redundant.


Mean absolute telomere length at T0 [kbp]
500,0000
400,0000
300,0000
200,0000
100,0000
,0000
25
26
23
20
27
22
32
never
rarely
frequently
sunbathing

***Statistical analysis of tea and absolute telomere length:***

**Between-Subjects Factors**

| | | Value Label | N |
|---|---|---|---|
| herbaltea | 0 | no | 45 |
| | 1 | yes | 5 |
| fruittea | 0 | no | 30 |
| | 1 | yes | 20 |
| greentea | 0 | no | 38 |
| | 1 | yes | 12 |
| blacktea | 0 | no | 40 |
| | 1 | yes | 10 |

**Tests of Between-Subjects Effects**

Dependent Variable: Mean absolute telomere length at T0 [kbp]

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 66034,819[a] | 4 | 16508,705 | 3,709 | ,011 |
| Intercept | 61177,761 | 1 | 61177,761 | 13,744 | ,001 |
| herbaltea | 17911,412 | 1 | 17911,412 | 4,024 | ,051 |
| fruittea | 22632,272 | 1 | 22632,272 | 5,085 | ,029 |
| greentea | 1079,489 | 1 | 1079,489 | ,243 | ,625 |
| blacktea | 39768,995 | 1 | 39768,995 | 8,934 | ,005 |
| Error | 200304,768 | 45 | 4451,217 | | |
| Total | 518543,400 | 50 | | | |
| Corrected Total | 266339,587 | 49 | | | |

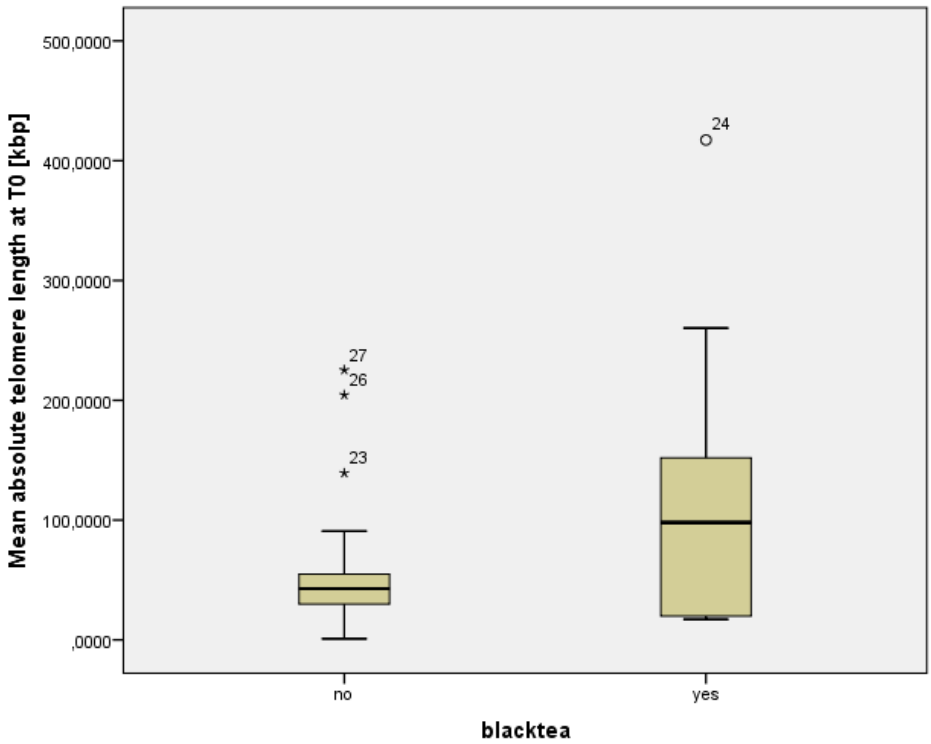
a. R Squared = ,248 (Adjusted R Squared = ,181)

## Parameter Estimates

Dependent Variable: Mean absolute telomere length at T0 [kbp]

| Parameter | B | Std. Error | t | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Intercept | 54,094 | 38,482 | 1,406 | ,167 | -23,413 | 131,601 |
| [herbaltea=0] | 63,589 | 31,700 | 2,006 | ,051 | -,258 | 127,436 |
| [herbaltea=1] | 0[a] | . | . | . | . | . |
| [fruittea=0] | 45,648 | 20,244 | 2,255 | ,029 | 4,874 | 86,422 |
| [fruittea=1] | 0[a] | . | . | . | . | . |
| [greentea=0] | -10,985 | 22,306 | -,492 | ,625 | -55,910 | 33,941 |
| [greentea=1] | 0[a] | . | . | . | . | . |
| [blacktea=0] | -74,179 | 24,817 | -2,989 | ,005 | -124,163 | -24,195 |
| [blacktea=1] | 0[a] | . | . | . | . | . |

a. This parameter is set to zero because it is redundant.



## 10.6. Structure parameter- definition: Cutometer® dual MPA 580

**R-Parameter**

Die nach der Messung abgebildeten Kurven ermöglichen eine Vielzahl von Berechnungen verschiedenster Art. Bei den R-Parametern haben wir uns auf die Resultate je Messmodus beschränkt, die allgemein auch in der Literatur als Berechnungsgrundlage verwendet werden. Sie können diese Berechnungen für die Kurven 1-4 durchführen lassen.

Zur Erklärung:

Die Formeln, die den Parametern R0-R9 zugrunde liegen, hängen vom jeweiligen Modus ab.

Parameter:

a= Ansaugzeit, vom Anwender eingestellt.

b= Relaxationszeit, vom Anwender eingestellt.

p= Unterdruck in mbar, vom Anwender eingestellt.

d= Sondenöffnungsdurchmesser in mm

r= Anzahl der Wiederholungen, vom Anwender eingestellt.

e(x)= Amplitude an der Stelle t=x

f(x)= $\Sigma$e(x) = Fläche unter der Kurve.

*Modus 1 (Erklärung für gesunde Hautstellen)*

R0 = e(a) = Uf = höchster Punkt der ersten Kurve (Amplitude), gibt Aufschluss über die Festigkeit (firmness) der Haut; je niedriger die Amplitude, desto fester die Haut (Beispiel: ein unterschiedlich stark aufgeblasener Ballon: die Elastizität des Material ist zwar immer 100%, aber ein stark aufgeblasener Ballon ist fester als ein weniger stark aufgeblasener). Das Verhalten der Haut auf passive Kraft wird dargestellt.

R1 = e(a+b) = Uf-Ua = niedrigster Punkt der ersten Kurve (Rückbildungsfähigkeit).

R2 = (e(a)-e(a+b))/e(a) = Ua/Uf = Verhältnis zwischen max. Amplitude und Fähigkeit der Rückbildung (Bruttoelastizität), je näher an 1 (100%), desto elastischer die Haut (sehr wichtiger Parameter).

R3 = e((r*a)+((r-1)*b)) = höchster Punkt der letzten Kurve; verglichen mit der max. Amplitude der ersten Kurve lassen sich „Ermüdungserscheinungen“ der Haut durch das Ansaugen feststellen, da die Amplitude mit jeder Wiederholung höher wird (s. auch R9).

R4 = e((a+b)*r) = letzter Messpunkt; verglichen mit der min. Amplitude der ersten Kurve lassen sich „Ermüdungserscheinungen“ der Haut durch das Ansaugen feststellen, da die Fähigkeit zur Rückbildung immer mehr abnimmt (R4-R1).

R5 = (e(a)-e(a+0.1))/e(0.1) = Ur/Ue = Anteil der elastischen Komponente beim Ansaugen zur elastischen Komponente bei der Entspannung. Nettoelastizität, je näher an 1 (100%), desto elastischer die Kurve.

R6 = (e(a)-e(0.1))/e(0.1) = Uv/Ue = Anteil der Visko-Elastizität am elastischen Teil in der Ansaugphase, je kleiner der Wert desto höher die Elastizität.

R7 = (e(a)-e(a+0.1))/e(a) = Uv/Uf = Anteil der elastischen Rückbildung an der Gesamtkurve; je näher der Wert an 1 (100%) desto elastischer die Haut.

R8 = e(b) = Ua der ersten Kurve = Gesamtrückbildung. Je näher R8 an R0 desto besser die Elastizität.

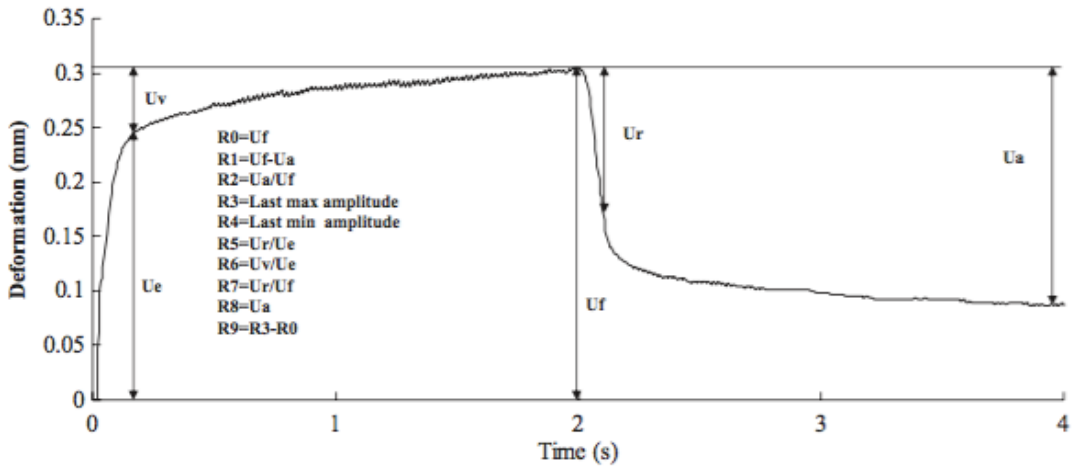
R9 = R3-R0. Gibt die Ermüdungserscheinungen der Haut nach mehrmaligem Ansaugen wieder. Je keiner der Wert, desto kleiner die Ermüdungserscheinungen.

**Flächenparameter**

Da die bisher berechneten Parameter alle sehr stark von der Maximal Amplitude Uf abhängen und diese jedoch stark von der Handhabung der Sonde (gerade halten, mäßiger Aufsatzdruck, Hautfeuchtigkeit, etc.) beeinflusst wird, werden in der neuesten Software erstmalig Flächenparameter berechnet, die diesen Einfluss ausschließen sollen.

F0 = Fläche innerhalb des Rechtecks, gebildet aus Uf x Ansaugzeit, oberhalb der Kurve. Diese Fläche wird ins Verhältnis gesetzt zu der Gesamtfläche (Rechteck aus max. Amplitude x Zeit), bzw. davon abgezogen. Ein vollständig elastisches Modell zeigt keine Fläche, sondern einen rechteckigen Kurvenverlauf, je näher der Wert an 0, desto elastischer die Haut.

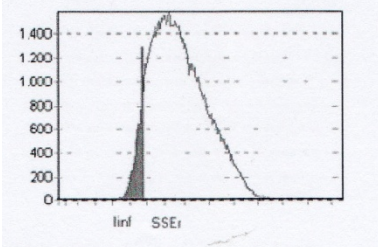
F1 = Fläche innerhalb des Rechtecks, gebildet aus Uf x Relaxationszeit, unterhalb der Kurve. Diese Fläche wird ins Verhältnis gesetzt zu der Gesamtfläche (Rechteck aus max. Amplitude x Zeit), bzw. davon abgezogen. Ein vollständig elastisches Material bildet sich ohne Verzögerung in den Ausgangszustand zurück: F1 und Gesamtfläche sind hier identisch, je näher der wert für F1 an 0 herankommt, desto elastischer die Haut.



## 10.7. Structure parameter- definition: Visioscan® VC 98

**SEr**

SEr: Rauheit/Roughness berechnet das Verhältnis der dunklen Pixel (jenseits des gesetzten Schwellenwertes) zum Rauheitsfaktors des Gesamtbildes (Anzahl der Falten der rechnerisch gezogenen Linien).




SEr = 10000 x linf/((Fax+ Fay)/2 x b x h)

linf = Anzahl der Pixel, deren Grauwert kleiner ist als der Schwellenwert von SEr (sie befinden sich im dunklen Bereich und repräsentieren die Falten). SEr wird durch den Wendepunkt (Schlaufe) des aufsteigenden Teils des Histogramms bestimmt (Punkt an dem fiktive Tangenten die Richtung ändern würden).

Fa= mittlere Anzahl der Falten horizontal (x) und vertikal (y).

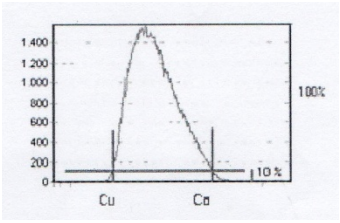
b = Breite des Bildes, h = Höhe des Bildes (in Pixel)

Je größer SEr, desto rauer ist die Haut.

**SEsm**

SEsm: Glätte/Smoothness bezieht die mittlere Breite des Histogramms und der rechnerisch gezogenen Linien/Falten (Fmx und Fmy) in die Berechnung ein.

Co, Cu : Schwellenwerte von SEsm als 10 % Anteil des Maximums des Histogramms.




SEsm = (Co - Cu) * (Fmx + Fmy)/10

Dieser Faktor schneidet den unteren Teil des Histogramms ab, in dem häufig alle möglichen Artefakte mitgemessen werden. Der Gedanke dahinter ist, dass eine glatte Haut nur wenig verschiedene Grauwerte hat und daher die Breite des Histogramms (Co-Cu) klein ist.

Fm : Die mittlere Faltenbreite horizontal und vertikal.

Je kleiner SEsm desto glatter die Haut (desto weniger unterschiedlich die Pixel und desto enger das Histogramm ihrer Verteilung).

**SEw**

SEw: Faltigkeit/Wrinkles ergibt sich aus mittlerer Anzahl und mittlerer Breite der rechnerisch gezogenen Falten/Linien

Je mehr Falten und je breiter diese sind, desto höher der Ergebniswert.

SEw = (Fmx +Fmy) x 100/ (Fax + Fay)

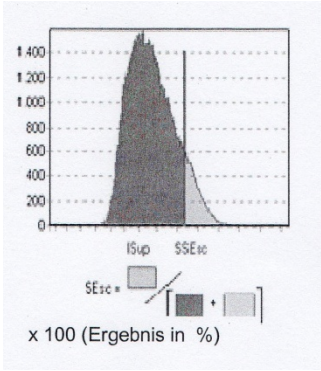
mittlere Faltenbreite/ mittlere Faltenanzahl

**SEsc**

Sesc: Schuppigkeit/Scaliness ist der prozentuale Anteil der hellen Pixel vom Gesamtbild.

Anzahl der Pixel, deren Grauwert höher ist, als der Schwellenwert von SEsc (die also im hellen Bereich des Histogramms liegen). SEsc wird durch den Wendepunkt (Schlaufe) des absteigenden Teils des Histogramms bestimmt (Punkt an dem fiktive Tangenten

die Richtung ändern würden).

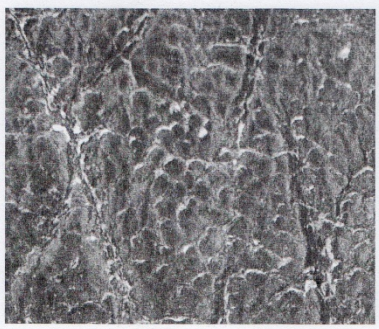



**Je kleiner SEsc, desto geringer die Schuppigkeit der Hornschicht (deutet auf höhere Hautfeuchtigkeit hin).**

**Rku**

Kurtosis bezieht sich auf die Form des Histogramms und gibt seine Qualität wieder. Je näher dieser Wert an 3 liegt desto idealer ist die Histogrammkurve (desto glatter die Haut). Der Kurtosisparameter ist ein statistischer Wert und erlaubt den Vergleich der Breite der Grauwertverteilung mit der Gausschen Kurve. Das Programm setzt vor dem Vergleich einige Filter zum Glätten auf die Histogrammkurve. Der Kurtosis-Parameter hat bisher in der Literatur für die Beschreibung der Haut kaum Anwendung gefunden.

Beispiele für SELS Parameter vor und nach einer Behandlung mit Feuchtigkeits-creme:



SEr : 1,48 SEsm : 46,49
SEsc : 2,42 % SEw : 16,979
Rku : 3,38

SEr : 0,49 SEsm : 32,69
SEsc : 0,74 % SEw : 15,724
Rku : 3,21

**Der Oberflächen-Parameter**

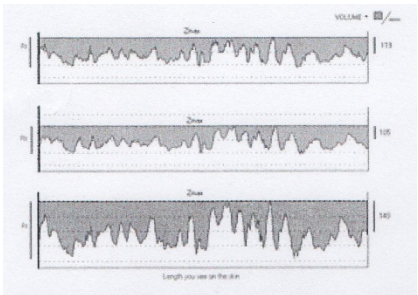
Dieser virtuelle Parameter berechnet die Größe der welligen Oberfläche des Hautbildes, die zu der flachen ("geplätteten") Ebene im Verhältnis x : 1 steht. Je glatter eine Fläche vor dem

"Bügeln" war, desto näher liegen beide Werte aneinander. Das Ergebnis wird im Ergebnisfeld *Oberfläche* in % angezeigt (z. B. 113 = die gestreckte Fläche ist 13 % größer als die originale).

**Der Volumen-Parameter**

Mit dem Befehl *Volumen* wird im Berechnungsfeld die virtuelle Menge von Flüssigkeit berechnet die benötigt würde, um das Rechteck bis zur gemittelten Höhe aller Berge zu füllen. Je glatter eine Fläche vor dem "Auffüllen" war, desto weniger Flüssigkeit wird gebraucht.

Das Ergebnis wird im *Ergebnis-Fenster* 3 im Feld *Volumen* in mm angezeigt.



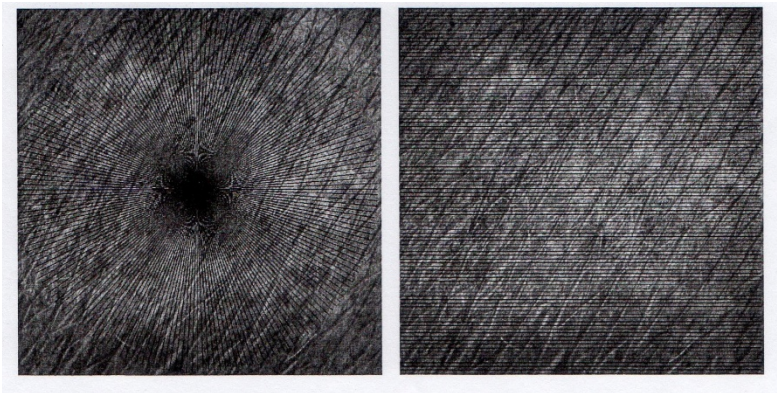
Bitte achten Sie darauf, dass das unter *Oberfläche* und *Volumen* gewonnene Ergebnisse stark von der Größe des gewählten Rechtecks abhängen. Wählen Sie daher für Vergleichsmessungen immer das gleiche Rechteck.

**Rauheitsparameter R1-R5**

Die Rauheitsparameter R1 - R5 kommen ursprünglich aus der Metallindustrie und sind in den Vorschriften DIN 4762-4768 als Ra-Rz festgelegt und in mm ausgedrückt. In der Visioscan USB Software werden diese Ergebnisse jedoch nur als Index (in Graustufen) ausgedrückt und lehnen sich daher nur an die ursprünglichen Ra-Rz Werte an.

Diese Rauheitsparameter werden als Mittelwert für mehrere Linien (Profile) rechts im *Ergebnis-Kasten* angezeigt.

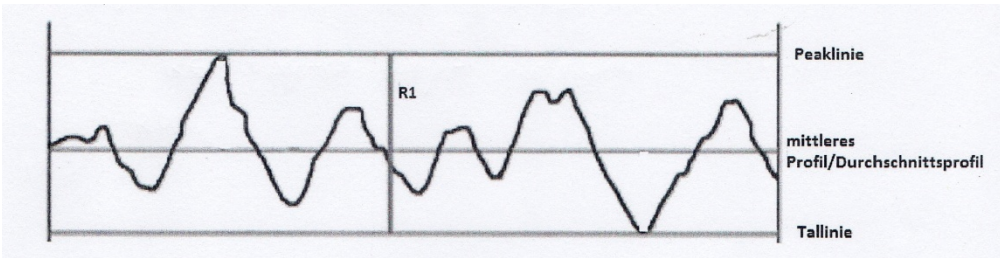
Wählen Sie, ob die Linien horizontal, vertikal oder kreisförmig angeordnet sein sollen und wie viele Linien automatisch gezogen werden sollen (max.180). Die Einstellung der Linienanzahl kann im Menü *Konfiguration Berechnungsparameter* vorgenommen werden.



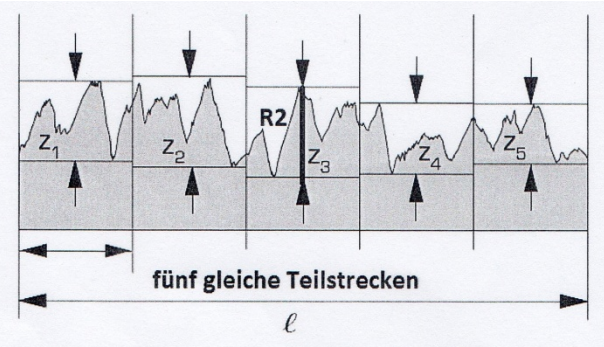
Der Vorteil von kreisförmig angeordneten Linien, liegt darin, dass der Einfluss der Richtung der Falten auf die Messung ausgeglichen wird. Bei dem Report der Ergebnisse in der Multi Image Analyse können sie sich auch den Mittelwert der horizontalen und vertikalen Linien zusammen anzeigen lassen. Bitte achten Sie darauf, dass das unter *Rauheit* gewonnene Ergebnisse stark von der Größe der gewählten Linie, also des Rechtecks abhängen. Wählen Sie daher für Vergleichsmessungen immer das gleiche Berechnungsrechteck.

**Rautiefe R1**

Als Rautiefe bezeichnet man den Abstand zwischen dem höchsten Berg und dem tiefsten Tal des Profils. In der DIN-Norm wird dieser Parameter als Rt bezeichnet.



**Maximale Rautiefe R2**




Für diesen Parameter teilt man das Profil in 5 gleiche Teilstrecken. Mit R2 wird die größte Einzelrautiefe der 5 Teilstrecken berechnet. In der DIN-Norm wird dieser Parameter als Rm oder Rmax bezeichnet.

R3 = Z1 + Z2 + Z3 + Z4 + Z5 / 5

**Gemittelte Rautiefe R3**

Die gemittelte Rautiefe ist das arithmetische Mittel von 5 Einzelrautiefen, die durch Teilen der Linie in fünf gleiche Strecken entstehen. Im Gegensatz zu R1 werden bei R3 durch das Mitteln, mögliche Artefakte in der Linie nicht so stark berücksichtigt. Dieser Parameter findet bei der Evaluierung der Oberflächenprofils besondere Anwendung. In der DIN-Norm wird dieser Parameter als Rz bezeichnet.

Quantitativ besteht zwischen den Parametern folgender Zusammenhang:

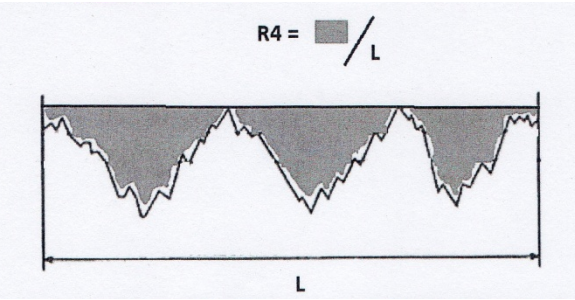
R1 ≥ R2 ≥R3.

**Glättungstiefe R4**

Für die Glättungstiefe berechnet die Software die Fläche über dem Profil der

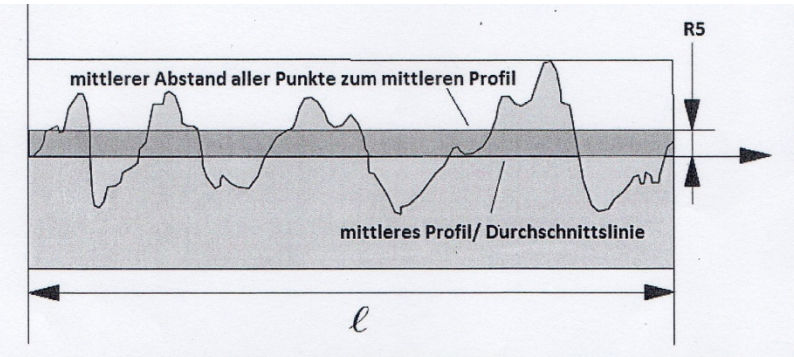
Linie (Ist-Profil) und einer Linie gezogen über dem höchsten Berg des Profils

(Maximalprofil). Diese Fläche wird geteilt durch die Länge des Profils, daher ist R4 wiederum eine Strecke (mittlerer Abstand zwischen Maximalprofil und Ist-Profil). In der DIN-Norm wird dieser Parameter als Rp bezeichnet.




**Arithmetischer Mittenrauwert R5**

Hier wird eine Fläche berechnet zwischen Ist-Profil und mittlerem Profil (Durchschnittslinie des Ist-Profils). Dann wird jeweils die obere und untere abweichende Fläche zum mittleren Profil errechnet und auch Mittellinie der Abweichungen (mittlerer Abstand aller Punkte zum mittleren Profil). R5 beschreibt die Distanz zwischen der mittleren Abweichungen und dem mittleren Profil. In der DIN-Norm wird dieser Parameter als Ra bezeichnet.




Da diese Parameter aus der Metallverarbeitung stammen, und sehr stark von der Länge der gewählten Linie abhängen, sollten für die Haut zusätzlich immer auch die anderen Parameter, z. B. SELS oder Flächenparameter wie *Oberfläche* und *Volumen* betrachtet werden.

**Anisotropie und Corner Density**

Mit diesen Parametern wird die Linienstruktur, ihre Ausrichtung, die Kreuzungspunkte der Linien und die von ihnen umschlossenen Zellen betrachtet.

Durch die hohe Auflösung der Bilder nimmt die Berechnung einige Zeit in Anspruch.

Die einzelnen Parameter:

**Corner Density (Anzahl der Kreuzungspunkte):**

Je mehr feine Linien man hat, desto mehr ihrer Kreuzungspunkte können von Programm gezählt werden. Sie werden in % ausgedrückt (CD = N/cm² ). Eine jüngere Haut zeigt daher höhere Zellendichte-Werte, da mehr, feine Linien einander kreuzen.

**Anisotropie** kommt aus dem Griechischen und bezeichnet die Richtungsabhängigkeit einer Eigenschaft oder eines Materials (Gegenteil von Isotropie). Für die Haut bedeutet dies: junge Haut zeigt eine niedrige Anisotropie, da feine Linien die Haut in alle Richtungen festhalten (isotrop). Mit zunehmendem Alter verschwinden die feinen Linien und es bilden sich tiefere Falten in gewisse Hauptrichtungen. Die Anisotropie, also die Richtungsabhängigkeit der Haut nimmt zu. Bei einer jungen Haut mit vielen feinen Linien in alle Richtungen bilden sich auch viele Kreuzungspunkte der Linien (hohe *Corner Density).* Je richtungsabhängiger die Linien werden und je mehr der feinen Linien verschwinden, desto mehr nimmt die Dichte der Kreuzungspunkte *(Corner Density/Zellendichte)* ab. Beide Parameter beschreiben also sehr gut den Hautalterungsprozess.

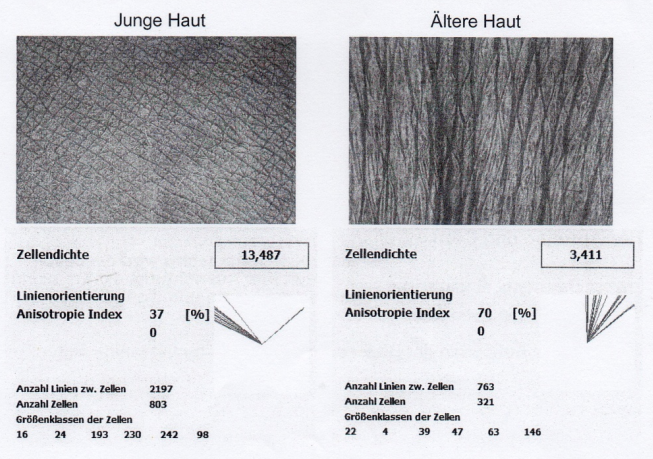
**Anisotropie Index:** je höher dieser Index, desto richtungsabhängiger sind die Linien in der Haut. Junge Haut hat niedrigere Anisotropie als ältere.

**Anzahl der Zellen und Linien zwischen den Zellen:** junge Haut zeigt ein Netz von vielen, feinen Linien, daher auch mehr eingeschlossenen Zellen und Linien zwischen den Zellen.

**Größenklassen der Zellen:** Die von den Linien eingeschlossene Zellen werden in fünf verschiedene Größenklassen eingeteilt. Von links nachts rechst: Anzahl der Zellen pro Größenklasse: C0 0-9 Pixel, C1 10-19 Pixel, C2 20-49 Pixel, C3 50-99 Pixel, C4 100-199 Pixel, C5 über 200 Pixel

Junge Haut wird eine größere Anzahl Zellen und den kleineren Kategorien aufweisen.

Bei älterer Haut zeigen sich immer mehr Zellen in den größeren Klassen.




**Texturparameter**

Diese Parameter beziehen sich auf Farbunterschiede zwischen Nachbarpixeln. Es werden folgende Parameter berechnet: Energie, Varianz, Kontrast, Entropie und Homogenität.

**Energie**

Energie wird abgekürzt NRJ und ist ein Indikator für die Einheitlichkeit eines Bildes.

Der Parameter prüft die Wiederholung einer Farbkombination (a, b). Dabei kann der Farbunterschied zwischen zwei benachbarten Pixeln sehr groß sein, ohne dass der Wert beeinflusst wird. Der Energiewert ist jedoch umso höher, je öfter diese Farbkombination wiederholt wird. Auf die Haut bezogen, gibt der Parameter *Energie* einen generellen Überblick über den Hautzustand.

Bei Behandlungen der Haut mit Feuchtigkeits- oder Anti-Aging Produkten, sollte der Energiewert nach der Behandlung angestiegen sein. Eine ältere, sehr trockene Haut mit vielen Falten hat einen kleinen Energiewert, wohingegen junge, elastische, ausreichend feuchte Haut einen hohen Energiewert besitzt.

**Varianz**

Varianz wird abgekürzt: VAR und ist der Mittelwert der lokalen Varianz über eine Anzahl von Pixel. Es wird hierbei der tatsächliche Wert des Pixels mit der mit dem mittleren Wert verglichen. Je rauer die Haut, desto höher der Varianzwert.

**Kontrast**

Kontrast wird abgekürzt: CONT und ist ein Indikator für den Grauwertunterschied zwischen zwei Nachbarpixeln. Der Kontrast ist umso größer, je größer der Farbunterschied zwischen zwei Nachbarpixeln. Je besser der Hautzustand, desto kleiner ist der Kontrastwert.

**Entropie**

Entropie wird abgekürzt: ENT und ist ein Indikator für die Unordnung/ Unstrukturiertheit von Grauwerten eines Bildes. Er ist umso höher je mehr verschiedene Grauwertkombinationen im Bild enthalten sind (max.; alle Grauwertkombinationen sind gleichverteilt, es gibt keine bevorzugte Kombination). Glatte Haut hat daher einen niedrigeren Entropiewert als raue Haut.

**Homogenität**

Homogenität wird abgekürzt: HOM und ist ein Indikator für die Gleichförmigkeit eines Bildes. Je größer der Unterschied in den Grauwerten, desto kleiner ist der Homogenitätswert.

Eine ausreichend feuchte Haut hat einen höheren Homogenitätswert als eine sehr trockene.

## 10.8. DNA extraction protocol

### *Protocol: DNA Purification from Buccal Swabs (Spin Protocol)*

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from buccal swabs using a microcentrifuge. For total DNA purification using a vacuum manifold, see “Protocol: DNA Purification from Buccal Swabs (Vacuum Protocol)” on page 39.

*Important points before starting*

- Due to the increased volume of Buffer AL that is required for the buccal swab protocol, fewer preparations can be performed. Additional Buffer AL can be purchased separately (see ordering information on page 70).
- This protocol is recommended for the following swab types: C.E.P. (Omni Swabs from Whatman Bioscience, www.whatman.com), cotton, and DACRON (Daigger, Puritan® applicators with plastic stick and cotton or DACRON tip from Hardwood Products Company, www.hwppuritan.com, or from Hain Diagnostika, www.hain-diagnostika.de).
- To collect a sample, scrape the swab firmly against the inside of each cheek 6 times. Air-dry the swab for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.
- All centrifugation steps are carried out at room temperature (15–25°C).

*Things to do before starting*

- Prepare a 56°C water bath for use in step 3.
- Equilibrate Buffer AE or distilled water to room temperature (15–25°C) for elution in step 10.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on page 16.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

*Procedure*

1. Place buccal swab in a 2 ml microcentrifuge tube. Add 400 µl (cotton and DACRON swab) or 600 µl (Omni Swab) PBS to the sample.

   The Omni Swab is ejected into the microcentrifuge tube by pressing the stem end towards the swab. Cotton or DACRON swabs are separated from the stick by hand or with scissors.

   QIAamp Mini spin columns copurify RNA and DNA in parallel when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock

solution (100 mg/ml) should be added to the sample prior to the addition of Buffer AL.

2. Add 20 µl QIAGEN Protease stock solution (or proteinase K) and 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) Buffer AL to the sample. Mix immediately by vortexing for 15 s.

   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

   Note: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.

3. Incubate at 56°C for 10 min. Briefly centrifuge to remove drops from inside the lid.

4. Add 400 µl (cotton or DACRON swab) or 600µl (Omni Swab) ethanol (96–100%) to the sample, and mix again by vortexing. Briefly centrifuge to remove drops from inside the lid.

5. Carefully apply 700 µl of the mixture from step 4 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.

   Close each spin column to avoid aerosol formation during centrifugation.

6. Repeat step 5 by applying up to 700 µl of the remaining mixture from step 4 to

   the QIAamp Mini spin column.

7. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.

8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

9. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

   This step helps to eliminate the chance of possible Buffer AW2 carryover.

10 Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 150 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

Elution with 100 µl buffer increases the final DNA concentration in the eluate significantly, but may slightly reduce the overall DNA yield. Elution with volumes of less than 100 µl is not recommended as the overall DNA yield decreases dramatically.

A second elution step with the same 150 µl eluate containing the DNA will increase yield significantly. However this is not recommended when using the eluate for PCR.

For long-term storage of DNA, eluting in Buffer AE and placing at –30 to –15°C is recommended.

One buccal swab typically yields 0.5–3.5 µg of DNA in 150 µl of buffer (3–23 ng/µl), with A260/A280 ratios of 1.7–1.9 (measured in water).

## 10.9. Telomere: Pipetting scheme

96- well plate

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | T1 | T1 | 207 | 207 | 211 | 211 | B1 | B1 | 207 | 207 | 211 | 211 |
| B | T2 | T2 | 108 | 108 | 112 | 112 | B2 | B2 | 108 | 108 | 112 | 112 |
| C | T3 | T3 | 208 | 208 | 212 | 212 | B3 | B3 | 208 | 208 | 212 | 212 |
| D | T4 | T4 | 109 | 109 | 113 | 113 | B4 | B4 | 109 | 109 | 113 | 113 |
| E | T5 | T5 | 209 | 209 | 213 | 213 | B5 | B5 | 209 | 209 | 213 | 213 |
| F | 106 | 106 | 110 | 110 | pos | pos | 106 | 106 | 110 | 110 | pos | pos |
| G | 206 | 207 | 210 | 210 | neg | neg | 206 | 206 | 210 | 210 | neg | neg |
| H | 107 | 107 | 111 | 111 | | | 107 | 107 | 111 | 111 | | |

T = telomere standards of different dilutions

B = single copy gene 36B4 standards of different dilutions

nr. = Samples

pos. = positive control: buccal sample

neg. = negative control: nuclease free water