

Skin Microbiome Biodiversity of Healthy Western Humans: A New Benchmark and the Effect of Age and Sex

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Abstract

A catastrophic loss of microbial biodiversity on the skin has led to alarming increase in the prevalence of allergies and long-term damage to the skin, which could also have damaging knock on effects to overall health. This study uses 50 human participants, to obtain an average (benchmark) value for the biodiversity of 'healthy' western skin, which is crucial in updating our 2017 skin health measuring mechanism to use standardised methodology. Previous work with a larger sample size was unsatisfactory for use as a benchmark due to its use of different and outdated diversity indices. We also investigated the effect of age and sex, two known skin microbiome affecting factors. Although no statistical significance is seen for age- and sex-related changes in diversity, there appear to be changes related to age which elaborates on previous work which used larger, more general age ranges. Our study indicates adults age 28-37 have highest diversity, and age 48-57 the lowest. Crucially, because of this study we are now able to update the skin health measuring mechanism from our 2017 work. This will aid diagnostic assessment of susceptibility to cutaneous conditions or diseases, and treatment. Testing any human subject will be rapidly improved by obtaining future benchmark diversity values for any age, sex, body site and area of residence, to which they can be compared. This improvement means we can also more accurately investigate the ultimate question: What factors in the western world are a main cause of the skin allergy epidemic? This could lead to future restriction of certain synthetic chemicals or products found to be particularly harmful to the skin.

Keywords: skin microbiome; skin microbiome biodiversity; biodiversity; skin ecosystem; skin allergy epidemic; benchmark skin health values; skin bacteria; 21st century skin ailments; measure skin health; healthy skin ecosystem; healthy skin bacteria; damaged skin bacteria;

Introduction

For the c. 300,000 years that homo-sapiens have inhabited the earth [1] our bodies have been host to trillions of microorganisms and recent estimates have suggested that, by cell count, we may be more microbe than human [2]. On the skin the majority of these are beneficial or harmless [3–7] and vital for defending us against infections and disease [8,9,18,10–17], immune system regulation and lipid metabolism [19]. The skin is like any other ecosystem across nature: if healthy, the organisms (microbes) within it live in peaceful mutual symbiosis with the host [5,20,21], existing commensally or symbiotically the majority of the time, and only rarely becoming pathogenic when the ecosystem balance is disturbed. Our skin microbiome is made up of viruses, fungi, archaea and bacteria but it is the latter we focus on in this study.

So, what does a healthy microbiome look like? This is complicated by large intrapersonal and interpersonal variation, where every human has a unique ecosystem of microbes inhabiting their body [4,22,23]. As discussed in our previous work [24], changes in skin microbial abundances have been associated with disease states, including acne [25] and psoriasis [26]. However, despite attempts at finding definitive biomarkers for skin health using species or clades, and years of research, we are still very far off [7,27–29], and due to the very nature and complexity of ecosystems which operate using non-linear physics principles, it may not be unreasonable to suggest it could be an academic dead-end for at least the near future. Conversely, biodiversity as a biomarker for healthy or damaged skin is far more conclusive, which led us to our 2017 discovery of what was called ‘the first clear mechanism for measuring skin health’ [30]. We noticed the same phenomenon that occurred across nature, was no different on the skin: an increase in biodiversity equated to a healthier ecosystem [31–36]. Therefore damaged or diseased skin displays a reduced diversity when compared to healthy skin on the same subject and same area of the body [29,37–42], and studies have observed that with higher bacterial diversity, the immune system works more effectively to protect us [10,11,43]. Dysbiosis and decreased skin microbiome biodiversity has been linked with the majority of skin ailments, including eczema [44], psoriasis [45], dermatitis [29], skin cancer [46], and many more [40,42,47–54], but more work would need to be done to properly determine if low skin biodiversity is a cause or a symptom. It is for the above reasons that we focus solely on diversity in this paper, and not analyses of community structure.

Previous work found the highest microbial diversity ever recorded on humans from communities with little or no contact to western civilisation; healthy western humans, in comparison, displayed far lower diversity [38–40]. Western subjects with skin ailments were even further diminished [27,29,41,55–57]. As a result, there has been a rapid increase in skin related problems and allergies in the western world in the last 75 years, labeled a ‘skin allergy epidemic’. Many serious and life-threatening health problems in society have been eliminated by the introduction of 20th century chemicals, drugs, and western, predominantly indoor lifestyle.

But along with that has come a whole host of new problems, because our bodies are being exposed to practices which our ancestors, who can be traced back 6 million years [58,59], or even further back with mammalian evolution [60,61], never experienced or evolved to cope with.

In this study, we collected volar forearm swab samples from 50 human subjects with ‘healthy’ skin (absence of skin ailments) and analysed their bacterial microbiomes using 16S rRNA sequencing. Firstly, we wanted to establish a benchmark value of diversity for healthy western skin using primarily Chao1 diversity. We note that although previous work, such as The Human Microbiome Project with 242 human subjects (<https://hmpdacc.org/hmp/>) [62], used larger sample sizes, different methodology meant alpha diversity analysis did not include the Chao1 index [38,48] rendering them unsatisfactory as a benchmark and incompatible with our research. Therefore, this study uses the largest sample size for Chao1 biodiversity analysis. It is crucial to mention that we are strict on using Chao1 because it offers the most complete evaluation of diversity of an ecosystem. Other methods, such as the entropy-based Shannon and Simpson [63] metrics, can lead researchers to misinterpret the diversity of ecosystems. The former doesn’t account for the fact that some microbes will be rarer than others; and the latter gives too much weight to common or dominant organisms, meaning the presence of rare types with only a few representatives will not affect diversity. Chao1 is the other end of the spectrum and uses the existence of fragile ‘rare’ species as a sign that biodiversity is high. This concept is backed up in other areas of ecology, where it is common knowledge that rare species become extinct first as ecosystems are disrupted, weakened and lowered in biodiversity [64,65].

The main reason for this study, however, was to give us the ability to transfer the remaining benchmarks from our 2017 work, such as ‘Unhealthy Western Skin’ or ‘Perfect Skin’, to the current skin health measuring mechanism which uses updated standardised methodology. Secondly, we acknowledge that the microbiome has been found to change with factors such as age and sex, therefore we investigate how they affect bacterial diversity. This is critical to improving our skin health measuring mechanism for use on any human subject regardless of age or sex, and to aid studies testing what environmental factors are causing of long-term damage to the skin.

Materials & Methods

Study conditions and sampling procedure

A total of 50 human subjects in five different age groups were recruited from the town of Graz in Austria, and split into 5 groups:

- Group 1: 18-27 years
- Group 2: 28-37 years
- Group 3: 38-47 years
- Group 4: 48-57 years
- Group 5: 58-70 years

There were five female and five male study subjects in each group, meaning overall there were 25 females and 25 males. All participants were recruited on the criteria they were without chronic skin diseases (no serious skin conditions such as acne, eczema, psoriasis and skin cancer among others) and had not used antibiotics within the last three months. All participants did not shower on the day of sampling and all of them had their skin sampled once.

The skin microbiome samples were taken from the volar forearm of the non-dominant arm, with sterile BBL™ CultureSwab™ EZ (Becton Dickinson), which were pre-moistened with sampling buffer (50mM Tris (pH 7.2), 1mM EDTA, 0.5% Tween20). The swab was placed on the forearm and the whole area was swabbed with pressure under rotation in three directions (horizontal, vertical and diagonal). After sampling the swabs were directly transferred into DNA-free 1.5ml reaction tubes (Eppendorf AG) and stored at -80°C till further processing. Swabbing the skin has widely been used as a suitable sampling method when analysing the skin microbiome [7]. Sampling buffer controls were taken to control the sterility of the used buffer. We also used negative controls, including PCR and sequencing controls, DNA extraction controls and swab controls to exclude the effect of possible contamination from laboratory reagents.

The entire project was carried out in collaboration with The Medical University of Graz in Austria, and run independently, supervised by Kaisa Koskinen at the Department of Internal Medicine. All study participants were recruited by The Medical University of Graz. Human skin samples were taken non-invasively and handled with approval by and in accordance with the Ethic Commission at the Medical University of Graz. This study was approved by the Ethic Commission at the Medical University of Graz (*Ethikkommission der Medizinischen Universität Graz*: approval number EK – 31-110ex18/19). Approval allowed the use of human subjects and the following procedures in this methodology. All participants provided written informed consent prior to enrolment in this study. Samples were treated anonymously, and human material was not the focus point of this study. Microbial samples or data derived cannot be linked to a certain individual. The process of the experimentation was agreed upon by The Medical University of Graz, and ACIB (The Austrian Centre of Biotechnology), a not-for-profit research organisation through whom the funding application was made.

DNA Extraction and 16S rRNA gene amplification

The swabs were thawed on ice and transferred into Matrix E tubes (MP Biomedicals) with flamed tweezers. The DNA extraction was performed using FastDNA Spin Kit (MP Biomedicals) according the manufacturer's protocol with following derivations: The bead beating step was done with MagNA Lyser (Roche Diagnostic GmbH) at 6400rpm for twice 30 seconds and the first centrifugation step was performed with 10 minutes. For every DNA extraction run, one extraction control (kit control) was processed with the samples to control the sterility of the used kit system. The concentration of the DNA was determined using Qubit ds-

DNA HS Assay Kit (Invitrogen AG) according the manufacturer's protocol. DNA concentrations of all samples were under detection limit. The extracted DNA was stored at -20°C till further downstream applications.

To amplify 16S rRNA gene V4 region of healthy skin microbial communities, PCR (polymerase chain reaction) was performed by using 1-2µl of the extracted DNA as template. Forward (GTG YCA GCM GCC GCG GTA) and reverse (GGG ACT ACN VGG GTW TCT ATT) primers [66] were added to a final concentration of 200nM. Following cycling conditions were applied: initial denaturation at 94°C for 3 minutes, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 60 seconds and elongation at 72°C for 90 seconds, followed by final elongation at 72°C for 10 minutes.

To visualise the PCR products, agarose gel electrophoresis was performed with 1.5% agarose gel for 35 minutes at 70V. We applied Roti Gel Stain (Carl Roth GmbH + Co. KG) as DNA intercalate to dye the DNA, and Fastruler Low Range (Thermo Fisher Scientific Inc.) ladder to assess the size of the PCR products. PCR products were stored at -20°C till sequencing. Library construction and Illumina sequencing (MiSeq) was performed by the Core Facility Molecular Biology at the Center for Medical Research (Graz, Austria). Samples were barcoded during library preparation, but the barcodes are not relevant anymore as the data was used as sample specific forward and reverse fastq-files. Library preparation followed the procedure of previous work, particularly the chapter "Total DNA Isolation, 16S Library Preparation and Sequencing" [67].

Data Analysis

The obtained 16S rRNA sequences were processed using QIIME 2 (Version 2018.11.0). The data were processed and all required files were formatted as described by the QIIME developers (<https://docs.qiime2.org/>): First the fastq data were imported and the sequences were filtered and denoised with DADA 2. After the quality check, the sequences were trimmed to a minimum length of 300bp and a maximum of 350bp. The taxonomy was assigned using 16S rRNA gene reference sequences of the Silva database (version 132).

From the OTUs, alpha and beta diversity analyses were performed using Calypso (<http://cgenome.net/calypso/>) [68]. For Calypso, the data was normalised using Total Sum Normalisation (TSS) and transformed using SquareRoot transformation, no samples removed, no taxa removed and no cut off for rare taxa. Chloroplast and Cyanobacteria were removed. On healthy skin, cyanobacteria have been found to contaminate samples due to their similarity, sequence-wise, to the DNA sequences of chloroplast which can be present after application and long term use of herbal skin care products [69]. We did not ask the participants to complete questionnaires on their skin care regime, so do not know if this would have been applicable. The number of raw reads per sample can be seen in the raw sequence read files (fastq).

For alpha diversity analysis, samples with less than 6600 sequence reads were filtered out to calculate the diversity indices reliably. The removed samples included controls (buffer -, kit - and PCR controls) and one sample of a female study subject. We analysed alpha diversity using both Chao1 and Shannon diversity metrics. For an explanation of the Chao1 index, and how it is different to Shannon, see our first paper [24] or previous work [70,71]. We use Shannon in this paper to evaluate the ‘even-ness’ or spread of organisms distributed in a system. Chao1 is a part of the ‘standardised’ methodology we use for our skin health measuring mechanism. Filtering was performed, because Chao1 and Shannon analyses rarify the number of reads to the lowest number of reads in a sample, and with too few sequences these analyses are not reliable.

Beta diversity between the samples, the overall structural similarity and variation between the microbiomes using the variables under investigation, was also examined. For beta diversity analysis no samples and no taxa were removed in order to visualize the differences between skin microbiome samples and controls. Control samples typically carry very few sequence-reads. Rarefaction analyses were done to assess how well the data represents the diversity of the microbial communities. Here the limit for statistical significance was set to a p-value of $p=0.001$, due to multiple testing. PCoA plots were included, which is a statistical technique that uses clusters of samples which have similar biological communities to assess differences in them in a simple graphical form. Additionally, sequence data from a previous study [30] were compared with the new data set regarding their alpha and beta diversity.

Results

Benchmark for Healthy Western Skin

The alpha diversity of the human participants ($n = 50$; male = 25 & female = 25) in this study (fig. 1.a & 1.b) is compared to all participants from our previous work, which used 32 western human participants from the town of Graz in Austria, all of whom were female, measured at T1 before product use started [30]. This meant they also resembled a normal western person’s skin. The studies were very similar, with average diversity slightly higher in the previous study. All samples (old and new study) were processed using identical procedures from sampling and sample processing to sequencing, including analysing the previous data with QIIME 2. In beta diversity (fig. 1.c & 1.d), the studies show very separate groupings, which suggest ‘batch effect’ - there is no specific factor or reason, which could be controlled, for this difference. Another possible reason for the difference could be processing the data with the QIIME 2 pipeline in this study, compared with the previous version, QIIME, in the previous study. This may imply that the studies should not be compared because their data was obtained from different sequencing runs. However, we only included this comparison as a point of interest; the main aim was to establish the most accurate benchmark value for skin microbiome diversity of healthy ‘western’

skin, which has been achieved, and the value can now update our skin-health measuring mechanism.

Age

In this section, we split the alpha and beta diversity results into age groups: group 1 (18-27 years), group 2 (28-37 years), group 3 (38-47 years), group 4 (48-57 years) and group 5 (58-70 years). There were five males and five females in each group. In alpha diversity, Chao1 index of different age groups shows a loose general trend of decreasing alpha diversity with increasing age, but the observation is not statistically significant (fig. 2.a). The Shannon Index of the age groups shows less of this trend (fig. 2.b). The highest average diversity was observed in Group 2 in both Chao1 and Shannon index, while group 4 displayed the lowest in both. There was an increase in average diversity from group 1 to 2, and from Group 4 to 5. Following the above indication of age-related alterations in alpha diversity, we again further explored the relationship between the microbiome and age, which was also applied in Sections 3.3. and 3.4. In beta diversity (fig. 2.c & fig. 2.d), PCoA plots, which are used to explore and visualize similarities and differences between microbial communities, showed no significant clustering of the age groups. Although there were no significant alpha diversity changes, age-related alterations in community structure and membership, and their effect on the diversification linked to age would need to be investigated in more detail. Redundancy analysis (RDA -plots) showed no significant differences between the age groups (fig. 3). The axes of the RDA plots show a small percentage, indicating that the variance of the data is not substantial.

Sex

In this section we split the alpha and beta diversity results by sex. Of the 50 human participants, 25 were male and 25 were female. There were no statistically significant differences in alpha diversity comparing sex (fig. 4.a & fig. 4.b), however the average diversity was slightly higher in males using Shannon diversity, but slightly lower in Chao1. No clustering of sex (fig. 4.c & 4.d) in beta diversity could be observed, but again the controls were grouped separately. Redundancy analysis (RDA -plots) showed significant differences between female and male study subjects (fig. 5). The axes of the RDA plots show a small percentage, indicating that the variance of the data is not substantial.

Control Testing

Analysing the difference in microbial communities using the PCoA plots revealed the samples (taken from the 50 human participants) were grouped differently to the controls (buffer -, kit - and PCR controls) which indicates that there was no contamination of the samples, and the sampling and manufacturing process was sterile (fig. 6.a & fig. 6.b). Skin microbiome samples, which group near to the control samples in beta diversity plots, carried a low number of reads like the control samples. Rarefaction analysis shows that there was a sufficient number of sequences to represent the diversity of the healthy skin microbial communities (fig. 6.c).

Discussion

The Effect of Age

Age- and sex-related changes vary across body site, between subjects and different diversity evaluating methodologies [72][73], so we take this into account where possible. It is common knowledge that older humans are more susceptible to onset of inflammatory disorders [74], but it is not known why their microbiomes maybe easier to colonise for opportunistic taxa. Our results show humans 28-37 years old have the highest alpha diversity in both Chao1 and Shannon, and 48-57 the lowest, although no statistical significance. Previous work seemed to mainly agree with our results, but differences could be due to differences in methodology and research design. Two studies found higher alpha diversity in older than younger skin across multiple body sites including the volar forearm, both of which used a ‘young’ (23-37; 21-50 years) and ‘old’ (60-76; 51-90 years) group of Japanese women [75] and Chinese adults [76] respectively. Their groupings, however, took in a wider range of ages than ours, which could have impacted the results. In contrast, not only did the two following studies fit in more with our results, but used age ranges a very similar size to ours. The first, a study on Chinese adults, found greater diversity in the skin microbiome across multiple body sites, including the volar forearm, in younger (25-35 years) than older participants (50-60 years) [73]. The second, a more recent study found Chinese females age 25-35 exhibited a higher alpha diversity in Chao1 than females age 50-63 [77]. They analysed the cheek microbiome which is more moist and sebaceous than arms, legs and trunk [78,79], but less sebaceous than forehead and nose [80]. Age and especially sex related changes in diversity and community structure, therefore, may be more prevalent in the cheeks than sebaceous sites, as changes in sebum levels contribute to a larger alteration of the sebaceous nature of moister and dryer sites, meaning a larger impact on the bacterial communities [81].

Our study uses smaller and more regular age groupings compared to previous work, which gives a more accurate insight into how sensitive diversity is to age. It reveals the problem may be more complex than grouping it by broad age ranges categorised as ‘young’ and ‘old’ and that these could give an inaccurate picture of how age affects diversity. A recent study used samples from 495 human subjects split into age groups the same size as ours[72]. They observed the highest diversity on humans age 20-29 but evaluated the diversity differently (relative abundance of ‘important’ *Corynebacterium* OTUs), rendering it very difficult to compare. The lower average diversity in the age range 18-27 in our study compared to 28-37 could be due to some participants still being ‘adolescents’; previous work reported that adolescents displayed significantly lower diversity than adults [73].

We are still unsure why the age-related changes in diversity occur. We know fundamentally that with aging microbial communities evolve by making use of proteins, lipids, minerals and carbohydrates on the skin [82], and the skin’s ability to produce sebum and retain moisture

changes [83], even though short term changes in trans-epidermal water-loss (TEWL) and diversity appear not to be linked [84]. Men are known to produce higher quantities of sebum, a process which stays stable as they grow older [83], which could mean diversity of the skin microbiome progresses differently with increasing age, in men compared to women.

Host physiology, age, sex and body site and ethnicity are all examples of intrinsic factors that affect the skin microbiome, but some of the observed changes could also be due to extrinsic factors, such as habitat, lifestyle, medication use and exposure to western synthetic chemicals. Previous work shows repeated use of modern synthetic cosmetic products can heavily influence aspects of skin chemistry and alter the microbiome [69] [85] and synthetic ingredients commonly found in skin care products can accelerate the skin aging process [86][87]. The microbiome and skin chemistry are not separate, they are closely linked, and changes in one will influence the other. For example, increasing the pH above natural levels accelerates the aging process [88], and skincare products often have a pH of 5.5 or more, which can dry the skin out, strip it of bacteria and allow the onset of premature aging [89][4]. Therefore, age-related changes could be amplified due to certain factors in our western lifestyle, meaning that humans in rural and more isolated communities (tribes-people) may experience a slower decrease in diversity with age, or may not follow the same pattern.

The Effect of Sex

Previous work describes how the microbial community on human skin is heavily influenced by the sex of humans, across all body sites [73,90–92]. This study found no significant differences in alpha diversity between the sexes, but men carried slightly lower average biodiversity using Chao1, and almost identical using Shannon. This finding was echoed in previous work where it was evident on almost all body sites [73], but most distinct on the glabella, which could be caused by cosmetic application [69]. Interestingly, this difference was statistically significant across far more body sites on humans from rural communities than urban ones, where only one site displayed significance, implying that the effect of sex on bacterial diversity grows smaller on western humans. we were not interested in specific bacteria, our RDA findings showed a significant difference in bacterial communities between men and women, which backed up a previous study that also found a significant difference in alpha diversity between men and women one body site, the palm of the hand [92]. As these were western humans this could further point to an environmental factor in the western world forcing this. However, it used phylogenetic diversity, which makes it hard to compare to our results. A further study observed that, on average, the backs of women again possessed higher alpha diversity using Shannon and Chao1 than men, but like our study it was not significant [93]. As different sampling approaches can achieve different results, we compared ours to those taken by swabbing, the methodology used in this study. Finally, recent work found the average total number of bacteria on the feet of women was an order of magnitude higher than on men, but as alpha diversity analysis methods

(Chao1 and Shannon) taking into account more factors than pure amount of bacteria present, this isn't comparable to our study [94].

The fact that men produce more sebum could influence results, especially on high-sebum areas of the skin. On average, sebaceous areas of the skin are less diverse than dry or moist [73,95,96], which could be due to sebum production increasing skin acidity [91] damaged skin promotes resident bacterial flora's dispersal from the skin and leads to pathogenic bacterial and fungal growth [5,6,21]. This could explain why our results did not show significant differences in diversity between the sexes, because we used the 'dry' volar forearm. Differences in facial cosmetics application, far less pronounced on the forearm than the face, could also have diminished the diversity difference. The generally more acidic skin of men [97,98], associated with lower ecosystem diversity [99], along with amount of cosmetic application and hormone production [98,100], wash frequency (women tend to wash more) [92] and perspiration rate could be reasons for differences between the sexes [91,101].

Limitations and Future Work

We note our study took samples from the volar forearm of human participants. Previous work shows body site strongly influences skin microbial diversity and community structure: dry areas such as the volar forearm, the body site found to have highest diversity, characteristically display higher diversity than sebaceous (such as forehead and cheeks) and moist (inguinal crease and popliteal fossa) areas [28,54,105,72,73,75,95,96,102–104]. Due to the body-site-dependent variability of composition and diversity on the human skin microbiome, benchmark values for diversity should be evaluated on all other body parts such as the face and feet, and for different ethnicities and living locations, split into age and sex. Benchmark diversity values for 'unhealthy' or 'diseased' western skin, and 'perfect' or 'caveman' skin (uncontacted tribes people [38]) will be transferred from our 2017 paper in future work, using the multiplication factors found (ratios) between their value and that of healthy western skin. This will be crucial in aiding diagnostic treatments of skin conditions and evaluations of skin health. We want our mechanism to be able to accurately evaluate the skin health of anyone, taking into account age, sex, body site and ethnicity or area of residence [73]. Ethnicity-related differences are most likely due to difference in lifestyle, not driven by inherent physiological variations between ethnicities [76]. The diversity for skin diseases should also be included, as they have a far bigger effect than factors such as age and sex [93].

In follow up work, a larger sample group should be recruited, so that age-related changes in diversity can be investigated independently in men and women. For women, their hormonal status, premenopausal or menopausal, and menstrual cycle status should be included or kept constant. This is because they are known to affect the cutaneous environment and sebum production; the latter's effect not felt on dry sites such as the volar forearm. The inclusion of more detailed bioinformatic analyses focusing on the specific types of microbe present, such as a

LEfSe, which was beyond the scope or remit of this study for the reasons explained in the introduction, should also be investigated. This may help move us closer to being able to use microbiome community structure as a biomarker in skin health and disease. The role of environmental and social or cultural factors should be further investigated, so their effect can be minimised or factored in for studies on this topic. For example, increased urbanization and hyper-cleanliness culture, indoor living, and exposing our skin to soaps, modern cosmetics, steroids and drugs has led to an alteration of the microbiome [3,4,6,96,106–110]. Synthetic additives in modern cosmetics are increasingly being suggested as contributing to an increase in skin ailments in the western world [111–116] stripping the skin of essential oils and bacteria [4,89]. Participants' drug and skin care regimes should be a key part of the recruiting process, to ensure everyone starts from the same base level and that the variables in question are isolated.

The skin (and gut [117]) microbiomes of humans from rural environments with little or no contact to western civilization display far higher levels of microbial diversity compared to humans in urban city environments [38–40]. Humans living in indoor, urban environments host very different microbial communities than agricultural workers in rural communities [43,73,118], who are exposed to a larger variety of microbes from nature. This forms the biodiversity hypothesis [119,120]. Area of residence for participants should be accounted for in the future.

Conclusions

In this study we determined a benchmark value for the microbial diversity, using the Chao1 index, found on healthy western human skin using a sample size of 50 individuals. This allows us, in future work, to transfer all benchmark levels of diversity from our 2017 paper, and to update our skin health measuring mechanism, which will help us determine which environmental factors, such as synthetic chemicals in modern cosmetics, are a major contributor to the skin allergy epidemic in the western world. Previously there was no way of measuring the health of skin, apart from visual observations of obvious ailments, and the use of individual types of clades of microbe as a biomarker is so far unreliable. We also demonstrated that there appear to be changes, albeit non-significant, in skin microbiome diversity related to age, where 'adults' age 28 to 37 harbour more diverse microbial communities than younger and 'old' subjects. There was no significant difference between men and women. A larger sample group should be used in the future to determine whether age-related diversification differs between men and women. We note changes in the microbiome can be due to external factors such as lifestyle, which aren't the intrinsic aging process, or biological sex, which will need to be accounted for in future work. Moreover, to aid dermatological assessments of susceptibility to infections or disease, and associated treatments, diversity benchmarks split into age, sex, ethnicity or area of residence, body site and skin diseases should be added.

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