Similar Skin Barrier Function in Persons with Type 1 Diabetes Compared with Healthy Controls

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Contact dermatitis because of use of diabetes devices is frequent in individuals with type 1 diabetes (TD1), especially in the pediatric age group, but the putative role of a constitutional impaired skin barrier in persons with TD1 is unclear. This study examined the skin barrier function by the measurement of natural moisturizing factor and free cytokines collected through skin tape strips, as well as biophysical markers and the skin microbiome, in persons with TD1 than to age- and sex-matched healthy controls. All measurements were done in nonlesional skin. We found that the skin barrier function was similar in children and adolescents with TD1 than to controls but found that the beta-diversity of skin microbiome at the buttock differed between the two groups. We conclude that individuals with TD1 have normal skin barrier function, and that the increased occurrence of contact dermatitis following pump and sensor use is explained by exogenous factors.

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INTRODUCTION

In persons with type 1 diabetes (T1D) using diabetes devices such as insulin pumps and/or glucose sensors, irritant and allergic contact dermatitis (CD) may commonly occur, especially in children; however, the reasons for this is unknown (Berg et al., 2018; Kamann et al., 2021). Interestingly, the risk of developing contact sensitization and allergic CD is significantly decreased in individuals with T1D than in controls (Bangsgaard et al., 2011; Engkilde et al., 2006), whereas the risk of developing irritant CD is currently unknown. T1D is a disease with many comorbidities and possible complications in a whole range of organ systems, including certain skin disorders such as vitiligo (Fröhlich-Reiterer et al., 2022), but little is known about the skin barrier function in persons with T1D.

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Abbreviations: CD, contact dermatitis; NMF, natural moisturizing factors; T1D, type 1 diabetes; T2D, type 2 diabetes; TEWL, transepidermal water loss

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Hyperglycemia impairs the skin barrier in the Streptozotocin induced T1D-model as indicated by increased transepidermal water loss (TEWL) (Okano et al., 2016). Where hyperglycemia is present in both type 2 diabetes (T2D) and T1D, FLG sequence variant appear to be less common in T1D, but more frequent in T2D, which may be due to an inverse immune reactivity in T1D and atopic dermatitis (Thyssen et al., 2011). The possible clinical impact is that corticosteroids applied topically may be easier absorbed into the impaired skin barrier and lead to T2D (Thyssen et al., 2018). A recent study found that T1D-prone mice (Streptozotocin induced) had increased TEWL, whereas T2D-mice (both N-Streptozotocin and KK-Ay/TaJcl) had normal TEWL, but reduced skin hydration, which is usually associated with increased TEWL (Horikawa et al., 2021). These different findings in T1D and T2D highlight the need for selective studies in T1D-populations alone.

An altered skin barrier function including changes in cytokine function or skin microbiome (Lee and Kim, 2022) may affect the risk of developing allergic and irritant CD caused by wearing diabetes devices by persons with T1D, because an impaired skin barrier increases the risk of CD (Borok et al., 2019), but previous studies have not adequately examined the skin barrier in T1D (Han and Park, 2017; Han et al., 2020; Lai and Nor, 2021; Mackiewicz-Wysocka et al., 2015; Sakai et al., 2005; Seirafi et al., 2009). We, therefore, investigated changes in the skin including skin microbiome, skin barrier function, and cytokine levels in pediatric and adult persons with T1D, which might facilitate the risk assessment and prevention of skin problems toward the use of diabetes devices.

RESULTS

Basic demographics for all participants are shown in Table 1, divided into the two age groups. In those with T1D, the

Table 1. Basic Demographics of T1D-Cases and Controls in the Two Age Groups

	Pediatric Age Group		Adult Age Group	
Baseline Variables	**Case (n = 36)**	**Control (n = 36)**	**Case (n = 9)**	**Control (n = 9)**
Age in years as median [min, max]	11.8 [4.21, 18.2]	12.4 [4.69, 19.3]	60.0 [39.2, 75.3]	63.1 [37.7, 75.9]
Sex (females)	17 (47.2%)	17 (47.2%)	5 (55.6%)	5 (55.6%)
BMI in kg/m^2 as median [min, max]	19.2 [15.2, 26.4]	17.7 [13.5, 22.6]	26.4 [20.3, 38.0]	23.6 [20.2, 40.3]
BMI Z-score[1] as median [min, max]	0.555 [−1.55, 2.06]	0.0500 [−2.58, 1.36]	N/A	N/A
Diabetes duration in years as median [min, max]	4.10 [0.880, 12.0]	N/A	34.7 [30.9, 59.8]	N/A
Plasma glucose at visit in mmol/L as median [min, max]	9.50 [3.80, 22.6]	5.20 [4.20, 7.20]	9.80 [7.70, 10.5]	5.90 [5.20, 7.70]
HbA1c in mmol/mol as median [min, max]	58.0 [36.0, 93.0]	34.0 [29.0, 39.0]	53.0 [48.0, 66.0]	37.0 [31.0, 46.0]
Hba1c in target?				
Yes (<53 mmol/mol)	9 (25.0%)	34 (94.4%)	3 (33.3%)	9 (100%)
No (≥53 mmol/mol)	27 (75.0%)	0 (0%)	6 (66.7%)	0 (0%)
Missing	0 (0%)	2 (5.6%)	0 (0%)	0 (0%)
FLG status				
FLG mutation	5 (13.9%)	3 (8.3%)	1 (11.1%)	1 (11.1%)
FLG wildtype	31 (86.1%)	32 (88.9%)	8 (88.9%)	8 (88.9%)
Missing	0 (0%)	1 (2.8%)	0 (0%)	0 (0%)

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; max, maximum; min, minimum; N/A, not applicable.
[1]BMI Z-score is based on WHO Growth Reference Values from R-package Zscorer and is only available for 0–20-year-olds, explaining the N/A in adult age group.

median age was 11.8 (range = 4–18) years in the pediatric group and 60.0 (range = 39–75) years in the adult group. There was an equal distribution of sexes in both children (47.2% females) and adult (55.6% females) groups. Healthy controls were age- and sex- matched. Body mass index and Z-scores for body mass index were higher for those with T1D than healthy controls. In persons with T1D, the median diabetes duration was 4.10 (range = 0.8–12) years for the pediatric age group and 34.7 (range = 31–60) years for adults. Less than 34% of persons with T1D achieved the hemoglobin A1c target of <53 mmol/mol (de Bock et al., 2022). FLG sequence variant were found in 10/89 (11%) of all study participants, but with no statistically significant difference between patients with T1D and healthy controls ($P = 0.7$).

Table 2 shows similar levels of TEWL, pH, hydration, sebum, and skin cytokine levels in persons with T1D than to healthy controls. TEWL, our primary outcome, was non-normally distributed by both test and plots, and values from the forearm in cases as a median (quartile 1–3) were 12.00 (10.20–15.99) than to 13.25 (11.80–17.35) in healthy controls and from the buttock for cases 13.80 (10.84–23.38) than to 15.77 (10.78–20.04) in healthy controls. The multivariable analyses of TEWL did not show any significant differences between cases and controls when adjusting for potentially influencing covariates in measurement conditions. Tables 3 and 4 show the results stratified by age group in the pediatric and adult age groups, respectively. Boxplots for important skin barrier measures are shown in Figure 1. Levels of natural moisturizing factors (NMFs) (and its individual component 2-pyrrolidone-5-carboxylic acid) were significantly lower in adult persons with T1D than to healthy controls on the buttock, but not on the forearm. All other indices of skin barrier function and cytokine levels were similar between persons with T1D and healthy controls in both age groups, and no relation to the duration of diabetes was found in persons with T1D.

Alpha diversity as observed, Shannon and phylogenic diversity of both skin and nasal microbiome as the diversity measure for each sample (Li et al., 2022) were similar among persons with T1D and healthy controls, which is shown in boxplots in Figure 2. However, the beta-diversity, describing differences between the two groups, showed a significant difference in permutational multivariable ANOVA analysis from the buttock skin ($P = 0.033$), but not from the nasal cavity among persons with T1D than to controls ($P = 0.983$). This is shown by the principal coordinate analysis plots in Figure 3, where the circles are overlapping in Figure 3a but are more separated in Figure 3b.

A post hoc analysis was made to examine the effect of FLG sequence variant on skin barrier measures. Total levels of NMF and the levels of histidine and 2-pyrrolidone-5-carboxylic acid were all significantly lower in skin tape strips collected at the forearm and the buttock skin from persons with FLG sequence variant, whereas TEWL, cytokine levels, and the skin microbiome did not differ significantly in the two groups.

DISCUSSION

Previous studies on the skin barrier function and diabetes have pooled different ages and types of diabetes, despite reported differences between T1D and T2D (Horikawa et al., 2021). Earlier work has shown no difference in TEWL, sebum, and skin hydration at the forearm between those with diabetes versus healthy controls (Han and Park, 2017; Lai and Nor, 2021; Seirafi et al., 2009), but one of the studies did find decreased TEWL in other skin sites (Han and Park, 2017) and another found a trend for increased TEWL (Lai and Nor, 2021). However, the measurement of TEWL displays experimental variability, for example in different environmental conditions, which can influence conclusions (Peer et al., 2022). We tried to overcome the environmental variability, but this did not change the conclusions. Similar to our findings, others

Table 2. Skin Barrier Function and Cytokine Levels in Skin of Forearm and Buttock

	Forearm			Buttock		
Skin Barrier Variables	**Cases (n = 45)**	**Controls (n = 45)**	***P*-value[1]**	**Cases (n = 45)**	**Controls (n = 45)**	***P*-value[1]**
TEWL[2]	12.00 (10.20–15.99)	13.25 (11.80–17.35)	0.561	13.80 (10.84–23.38)	15.77 (10.78–20.04)	0.992
Sebum[2]	0.00 (0.00–1.00)	0.00 (0.00–1.00)	1.000	0.00 (0.00–1.00)	0.00 (0.00–1.00)	0.925
Ph[3]	5.36 [5.17–5.55]	5.71 [5.50–5.92]	0.093.	5.58 [5.40–5.76]	5.75 [5.56–5.94]	0.624
Hydration[3]	37.89 [34.49–41.30]	36.17 [33.27–39.08]	0.916	33.33 [30.42–36.25]	35.63 [32.15–39.12]	0.916
NMFs in nmol/μg protein[4]						
NMF total[3]	0.75 [0.66–0.83]	0.78 [0.71–0.85]	0.916	0.65 [0.58–0.72]	0.72 [0.62–0.83]	0.793
HIS[3]	0.14 [0.12–0.16]	0.15 [0.13–0.17]	0.916	0.15 [0.13–0.17]	0.16 [0.14–0.19]	0.916
PCA[3]	0.44 [0.39–0.50]	0.46 [0.41–0.51]	0.965	0.37 [0.33–0.41]	0.41 [0.34–0.47]	0.912
UCA total[3]	0.16 [0.14–0.19]	0.17 [0.15–0.19]	0.947	0.14 [0.12–0.16]	0.15 [0.13–0.18]	0.561
trans-UCA[3]	0.11 [0.09–0.13]	0.12 [0.10–0.14]	0.990	0.13 [0.11–0.15]	0.15 [0.13–0.18]	0.561
cis-UCA[2]	0.04 (0.00–0.08)	0.02 (0.00–0.08)	1.000	0.00 (0.00–0.00)	0.00 (0.00–0.00)	1.000
Cytokine levels in pg/μg protein						
IFN-γ[3]	0.21 [0.18–0.23]	0.19 [0.16–0.21]	0.882	0.27 [0.23–0.31]	0.27 [0.23–0.32]	1.000
IL-17[3]	0.15 [0.14–0.17]	0.15 [0.13–0.16]	0.925	0.15 [0.14–0.17]	0.23 [0.20–0.26]	0.916
IL-18[2]	0.09 (0.06–0.17)	0.09 (0.06–0.12)	0.992	0.17 (0.14–0.29)	0.15 (0.10–0.25)	0.916
IL-1β[2]	0.07 (0.05–0.12)	0.07 (0.04–0.10)	0.916	0.15 (0.10–0.28)	0.20 (0.12–0.30)	0.992
IL-4[3]	0.01 [0.01–0.01]	0.01 [0.01–0.01]	0.916	0.02 [0.02–0.02]	0.02 [0.02–0.02]	0.990
IL-6[3]	0.04 [0.04–0.05]	0.04 [0.04–0.04]	0.992	0.06 [0.05–0.06]	0.06 [0.05–0.07]	0.916
IL-8[2]	0.03 (0.02–0.05)	0.03 (0.02–0.04)	0.793	0.04 (0.03–0.07)	0.04 (0.03–0.05)	0.754
TARC[3]	0.02 [0.02–0.03]	0.02 [0.02–0.03]	0.990	0.03 [0.03–0.04]	0.03 [0.03–0.03]	0.916
TNF- α[3]	0.06 [0.05–0.07]	0.06 [0.05–0.07]	0.990	0.08 [0.07–0.10]	0.08 [0.07–0.10]	0.990
IL-1α[3]	88.40 [71.03–105.78]	81.36 [66.39–96.33]	0.947	96.48 [75.91–117.05]	107.82 [91.82–123.82]	0.916

Abbreviations: HIS, histidine; NMF, natural moisturizing factor; PCA, 2-pyrrolidone-5-carboxylic acid; Q, quartile; TARC, thymus and activation-regulated chemokine; TEWL, transepidermal water loss; UCA, urocanic acid.

[1]*P*-value from statistical paired test, respectively Wilcoxon paired test and paired *t* test for nonparametric and parametric variables *P*-values are shown after Benjamini-Hochberg adjustment for 132 tests.

[2]Nonparametric variables shown as median (Q1–Q3).

[3]Parametric variables shown as mean [95% CI].

[4]NMFs can be separated in different components including HIS, PCA and UCA. UCA can also be separated in a molecular cis- or trans version.

have shown that skin pH was 0.3 lower in persons with T1D than in controls (Mackiewicz-Wysocka et al., 2015). A study in persons with T1D and T2D showed decreased skin hydration and sebum on the forehead skin than to healthy controls, but with no TEWL-difference by levels of hyperglycemia (Sakai et al., 2005). NMF as a biomarker for the skin barrier in persons with T1D has not been investigated before. We found reduced NMF and 2-pyrrolidone-5-carboxylic acid levels in nine adults with longstanding T1D than to healthy controls, but this applied only for the buttock skin. Our finding suggests reduced pro-FLG expression in the buttock skin, but no study has examined this before in persons with T1D. We found similar prevalence of FLG sequence variant, although only in a small number of adults that were examined. There was no significant correlation between NMF and the duration of diabetes. In contrast to NMF, there was no increase in TEWL levels, indicating that it was either a chance finding, or a very small change in barrier function because NMF is more sensitive than TEWL (Soltanipoor et al., 2018). The lack of consistency in the findings from the buttock to the forearm skin also indicates that it may be a random finding, which must be investigated further before clear conclusions can be made. Another explanation of the inconsistency between findings on the forearm and the buttock skin could be different UVR exposure, although the time when the measurement was taken (avoiding summer month) may have reduced this difference (Dąbrowska et al., 2018). For experimentally induced CD and atopic dermatitis, skin barrier impairment has been shown by increased TEWL and decreased NMF, and a clear correlation between the measures (McAleer et al., 2018; Soltanipoor et al., 2018). Therefore, we would have assumed that an important and clinically relevant skin barrier impairment of the normal intact skin would have been found in our results, if such a difference was present. Our clear association between the different NMF-levels and FLG mutation status is clearly expectable, because NMF is a measure of the FLG degradation products (Kezic et al., 2009), but it did not show a relation to other skin barrier measures in our study.

The skin microbiome in T1D has not been studied before, and our results must therefore be replicated. In T2D, only results from individuals with foot ulcers exist on the skin microbiome and shows conflicting results (Han et al., 2020). We did find a difference in beta-diversity between persons with T1D and controls on the buttock skin only and not in the nasal cavity, both being a normal investigational site for skin microbiome. Unfortunately, microbial changes in the forearm skin were not investigated. The implications of changed microbiome on the skin barrier could be altered by the secretion of lipids or a

Table 3. Skin Barrier Function and Cytokine Levels for Pediatric Age Group

	Forearm			Buttock		
Skin Barrier Variables	**Cases (n = 36)**	**Controls (n = 36)**	***P*-value[1]**	**Cases (n = 36)**	**Controls (n = 36)**	***P*-value[1]**
TEWL[2]	12.79 (10.36–16.01)	13.34 (12.24–16.67)	0.742	13.85 (11.09–23.77)	15.92 (11.57–20.54)	0.992
Sebum[2]	0.00 (0.00–1.00)	0.00 (0.00–1.00)	0.916	0.00 (0.00–1.00)	0.00 (0.00–1.00)	0.992
pH[3]	5.29 [5.07–5.52]	5.69 [5.44–5.94]	0.093.	5.45 [5.26–5.64]	5.66 [5.45–5.88]	0.561
Hydration[3]	35.27 [31.88–38.67]	34.26 [31.34–37.19]	0.990	32.96 [29.54–36.38]	34.72 [31.34–38.09]	0.925
NMFs in nmol/μg protein[4]						
NMF total[3]	0.75 [0.65–0.85]	0.76 [0.68–0.84]	0.990	0.65 [0.57–0.72]	0.64 [0.53–0.74]	1.000
HIS[3]	0.15 [0.12–0.17]	0.15 [0.13–0.17]	0.916	0.15 [0.13–0.17]	0.15 [0.12–0.18]	1.000
PCA[3]	0.44 [0.38–0.51]	0.44 [0.39–0.50]	1.000	0.36 [0.32–0.41]	0.35 [0.29–0.42]	1.000
UCA total[3]	0.16 [0.13–0.19]	0.16 [0.14–0.19]	0.992	0.13 [0.11–0.15]	0.13 [0.11–0.15]	0.990
trans-UCA[3]	0.11 [0.08–0.13]	0.11 [0.09–0.13]	1.000	0.13 [0.11–0.15]	0.13 [0.11–0.15]	0.970
cis-UCA[2]	0.05 (0.00–0.08)	0.04 (0.01–0.08)	1.000	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.916
Cytokine activity in pg/μg protein						
IFN-γ[3]	0.19 [0.17–0.22]	0.19 [0.16–0.22]	1.000	0.30 [0.26–0.34]	0.30 [0.25–0.35]	1.000
IL-17[3]	0.15 [0.13–0.17]	0.15 [0.13–0.17]	1.000	0.23 [0.21–0.26]	0.25 [0.21–0.28]	0.916
IL-18[2]	0.08 (0.06–0.12)	0.08 (0.06–0.12)	1.000	0.17 (0.14–0.28)	0.19 (0.13–0.27)	1.000
IL-1β[2]	0.07 (0.04–0.11)	0.07 (0.04–0.09)	1.000	0.13 (0.10–0.27)	0.19 (0.12–0.28)	0.965
IL-4[3]	0.01 [0.01–0.01]	0.01 [0.01–0.01]	0.992	0.02 [0.02–0.03]	0.02 [0.02–0.03]	1.000
IL-6[3]	0.04 [0.03–0.04]	0.04 [0.04–0.05]	0.925	0.06 [0.06–0.07]	0.07 [0.06–0.08]	0.990
IL-8[2]	0.03 (0.02–0.04)	0.03 (0.02–0.04)	1.000	0.04 (0.03–0.07)	0.04 (0.03–0.06)	0.916
TARC[3]	0.02 [0.02–0.03]	0.02 [0.02–0.03]	1.000	0.04 [0.03–0.04]	0.03 [0.03–0.04]	0.916
TNF- α[3]	0.06 [0.05–0.07]	0.06 [0.05–0.07]	0.916	0.09 [0.08–0.11]	0.09 [0.08–0.11]	1.000
IL-1α[3]	71.52 [59.01–84.02]	70.22 [57.54–82.90]	1.000	99.72 [74.78–124.67]	103.43 [87.19–119.67]	1.000

Abbreviations: HIS, histidine; NMF, natural moisturizing factor; PCA, 2-pyrrolidone-5-carboxylic acid; Q, quartile; TARC, thymus and activation-regulated chemokine; TEWL, transepidermal water loss; UCA, urocanic acid.

[1]*P*-value from statistical paired test, respectively Wilcoxon paired test and paired *t* test for nonparametric and parametric variables *P*-values are shown after Benjamini-Hochberg adjustment for 132 tests.

[2]Nonparametric variables shown as median (Q1–Q3).

[3]Parametric variables shown as mean [95% CI].

[4]NMFs can be separated in different components including HIS, PCA and UCA. UCA can also be separated in a molecular cis- or trans version.

keratinization process (Lee and Kim, 2022). Further investigation in a larger population is warranted to shed light on possible anatomical differences in the skin microbiome, although gut microbiome research has shown T1D-related differences (Siljander et al., 2019).

Based on our findings, significant skin barrier impairment cannot be detected or explain the increase in cases of CD due to diabetes devices (Kamann et al., 2021). Study strengths include the relatively well-matched and paired groups, the systematical and standardized investigations, the broad age-span across most of the pediatric age group, and the range of glycemic control, making it representative for many persons with T1D. Although differences were found for adults with long-standing T1D, our study was limited by a small sample size, and these aspects therefore need further investigation for clear conclusions to be made.

We conclude that the skin barrier function in nonlesional skin and skin levels of inflammatory cytokines in persons with T1D are comparable to that of healthy controls, rejecting a constitutional impaired skin barrier as a cause of prevalent CD toward diabetes devices.

MATERIALS AND METHODS

This study was a case-control study, where the case group consisted of 36 persons with T1D aged 4–20 years and 9 adults with a duration of T1D spanning more than 30 years. The control group was age- and sex-matched healthy controls recruited from the Department of Pediatrics and Adolescent Medicine and their relatives, where each case was matched with a participant of the same sex and age within 2 years in the pediatric age group and within 5 years in the adult age group. Persons with skin lesions at the sites of investigation, known skin diseases including former atopic dermatitis or allergic CD, active infections, and users of anti-inflammatory medications were not able to participate. Use of emollients were avoided 3 days before and showering was avoided on the day of the study.

Skin measurements were performed on the volar forearm and the upper buttock. TEWL was measured by Aquaflux AF200 from BIOX Systems (London, United Kingdom) with at least two measurements, and the DermaUnit SSC from Courage-Khazaka (Köln, Germany) was used for three measurements of skin pH and hydration and one measurement of sebum. Ten consecutive circular tape strips (of type D100 with Ø 22 mm from D-Squame, Clinical and Derm, Dallas, TX) were collected from the exact same skin site at both the volar forearm and the upper buttock with a pressure with D-squame pressure instrument for 5 seconds. All tape strip markers were adjusted for total protein count, which was measured by D-Squame Scan 850A. Tape strips were then quickly stored in an 80 °C freezer until measurements. Tape strip number five was analyzed for NMF by high performance liquid chromatography (Dapic et al., 2013). NMF was defined as the sum of 2-

Table 4. Skin Barrier Function and Cytokine Levels for Adult Age Group

	Forearm			Buttock		
Skin Barrier Variables	**Cases (n = 9)**	**Controls (n = 9)**	***P*-value[1]**	**Cases (n = 9)**	**Controls (n = 9)**	***P*-value[1]**
TEWL[2]	10.26 (9.26–11.22)	11.78 (10.04–23.75)	0.916	12.51 (9.46–16.82)	13.48 (8.32–15.28)	1.000
Sebum[2]	1.00 (0.00–4.00)	0.00 (0.00–1.00)	0.742	0.00 (0.00–1.00)	0.00 (0.00–1.00)	0.916
pH[3]	5.61 [5.32–5.91]	5.78 [5.41–6.14]	0.947	6.07 [5.72–6.42]	6.11 [5.77–6.45]	1.000
Hydration[3]	48.38 [41.20–55.55]	43.82 [36.93–50.70]	0.916	34.82 [29.51–40.13]	39.31 [28.08–50.53]	0.938
NMFs in nmol/μg protein[4]						
NMF total[3]	0.75 [0.61–0.88]	0.85 [0.70–1.00]	0.916	0.67 [0.52–0.82]	1.07 [0.87–1.27]	0.012
HIS[3]	0.13 [0.09–0.17]	0.14 [0.11–0.18]	0.947	0.13 [0.09–0.18]	0.20 [0.14–0.25]	0.561
PCA[3]	0.44 [0.36–0.53]	0.51 [0.41–0.61]	0.916	0.38 [0.30–0.47]	0.63 [0.50–0.75]	0.012
UCA total[3]	0.17 [0.13–0.21]	0.20 [0.16–0.24]	0.916	0.16 [0.11–0.20]	0.25 [0.19–0.31]	0.140
trans-UCA[3]	0.14 [0.09–0.19]	0.17 [0.13–0.20]	0.916	0.15 [0.11–0.20]	0.24 [0.18–0.30]	0.216
cis-UCA[2]	0.02 (0.00–0.07)	0.00 (0.00–0.02)	1.000	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.916
Cytokine activity in pg/μg protein						
IFN-γ[3]	0.25 [0.18–0.31]	0.16 [0.11–0.21]	0.635	0.16 [0.09–0.22]	0.18 [0.13–0.23]	0.916
IL-17[3]	0.17 [0.13–0.22]	0.14 [0.10–0.17]	0.916	0.12 [0.09–0.16]	0.15 [0.12–0.19]	0.772
IL-18[2]	0.20 (0.17–0.57)	0.11 (0.09–0.30)	0.965	0.17 (0.10–0.35)	0.08 (0.06–0.09)	0.361
IL-1β[2]	0.16 (0.10–0.28)	0.10 (0.06–0.11)	0.709	0.19 (0.15–0.28)	0.22 (0.07–0.36)	1.000
IL-4[3]	0.02 [0.01–0.02]	0.01 [0.01–0.01]	0.561	0.01 [0.01–0.01]	0.01 [0.01–0.01]	0.362
IL-6[3]	0.05 [0.03–0.07]	0.04 [0.03–0.05]	0.916	0.02 [0.02–0.03]	0.04 [0.03–0.04]	0.362
IL–8[2]	0.04 (0.03–0.06)	0.02 (0.01–0.02)	0.709	0.04 (0.02–0.24)	0.03 (0.01–0.03)	0.772
TARC[3]	0.03 [0.02–0.04]	0.02 [0.02–0.03]	0.916	0.02 [0.01–0.02]	0.02 [0.01–0.02]	0.990
TNF- α[3]	0.06 [0.04–0.07]	0.05 [0.04–0.06]	0.938	0.04 [0.02–0.07]	0.05 [0.04–0.06]	0.990
IL-1α[3]	155.95 [102.77–209.12]	125.92 [79.49–172.34]	0.947	83.50 [58.08–108.93]	125.39 [78.08–172.70]	0.772

Abbreviations: HIS, histidine; NMF, natural moisturizing factor; PCA, 2-pyrrolidone-5-carboxylic acid; Q, quartile; TARC, thymus and activation-regulated chemokine; TEWL, transepidermal water loss; UCA, urocanic acid.

[1]*P*-value from statistical paired test, respectively Wilcoxon paired test and paired *t* test for nonparametric and parametric variables *P*-values are shown after Benjamini-Hochberg adjustment for 132 tests.

[2]Nonparametric variables shown as median (Q1–Q3).

[3]Parametric variables shown as mean [95% CI].

[4]NMFs can be separated in different components including HIS, PCA, and UCA. UCA can also be separated in a molecular cis or trans version.

pyrrolidone-5-carboxylic acid, urocanic acid, and histidine, which are all derived from FLG protein. Tape strip number seven was used to determine the cytokine levels including IFN-γ, IL-13, IL-17A, IL-18, IL-1b, IL-4, IL-6, IL-8, thymus and activation-regulated chemokine, TNF-α, and IL-1a by Merck Shape & Dohme multiplex immunoassay (Mesoscale Diagnostics, Rockville, MD). A buccal swab (from Isohelix 2K-2S, Harrietsham, United Kingdom) was collected and within 24 hours stabilized by adding 500 μl of BLS (Lysis and Stabilisation buffer from Isohelix Buccal-Prep Plus DNA Isolation Kit), then shaken and stored at room temperature for up to one year before the final steps of DNA isolation were performed according to protocol from IsoHelixBuccal-Prep Plus DNA Isolation Kit and afterwards stored at −80 °C freezer until genotyping. DNA samples were genotyped by Fluidigm (San Francisco, CA) SNP Genotyping in a 192.24 chip for the five most common FLG sequence variant, accounting for 96% of FLG sequence variant in North European populations: R501X, 2282del4, S3247X, 3702delG, and R2447X (Irvine et al., 2011), including a total of 11 SNP assays with two replicates of each SNP to check conformity and two control assays with high minor allele frequency to check DNA-validity: rs138726443_GA, rs138726443_GC, rs138726443_GT, rs61816761_GA, rs61816761_GC, rs61816761_GT, rs150597413_GA, rs150597413_GC, rs150597413_GT, rs397507563, rs558269137. Two eSwabs prewetted by Amies medium were scrubbed at minimum 4 cm^2 of skin from the buttock and the nasal cavity, respectively, and stored in -80 °C freezer until the samples were further analyzed for skin microbiome with the first DNA extraction by DNeasy PowerSoil kit (Waltham, MA) and later 16S ribosomal RNA gene amplicon sequencing. Capillary blood from the finger was analyzed for blood glucose with Contour Next One and hemoglobin A1c with TOSOH G8. All examinations were carried out between February 2020 and June 2020 and September 2020 and June 2021. The study was approved by the Research Ethics Committee at Capital Region of Denmark (H-19037160). Oral consent was obtained from all participants irrespectively of age after thorough and age-adjusted information, and all participation was voluntary. Written informed consent was obtained from all participants from ≥18 years, and from all holders of parental authority for participants under 18 years. The observational case-control study was pre-registered at ClinicalTrials.gov (NCT04280315).

Statistics were performed using the statistical software package R version 4.1.0 (The R Foundation for Statistical Computing, Vienna, Austria). All variables were compared between persons with T1D and their matched healthy controls using paired design, with paired *t* test or paired Wilcoxon test depending on distribution of each variable. Differences between groups were assessed as a full group and stratified into adult and pediatric age groups. Sample size was based on the primary outcome TEWL where 5–27 participants in each group were needed according to published results in adults (Han and Park, 2017; Seirafi et al., 2009), and we did therefore include 45 in each group to account for differences followed by age. For TEWL, a multivariable

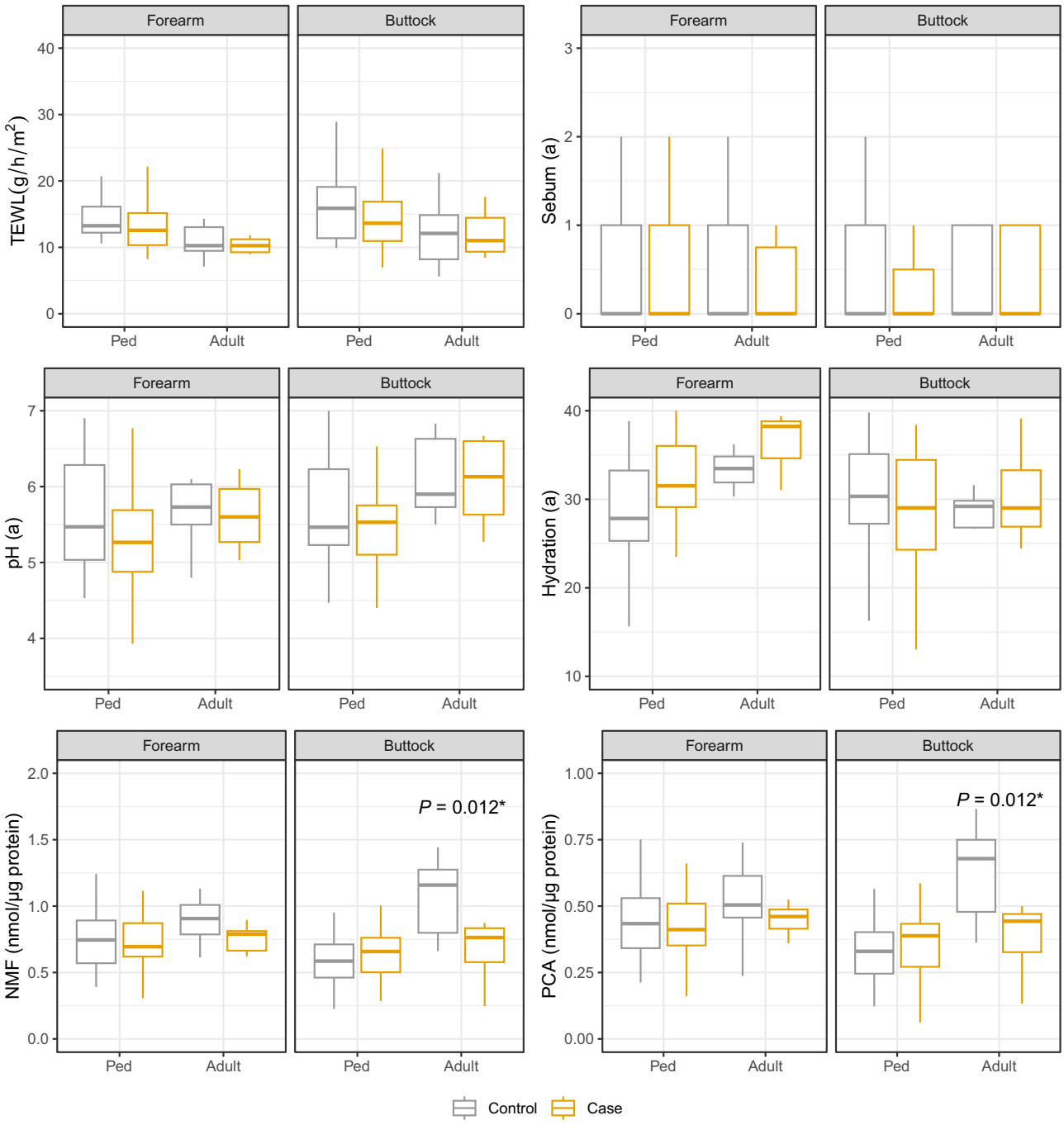

Figure 1. Boxplots of skin barrier measures at the two skin sites separated in age groups and groups of cases and controls. The y-axis represents values of the different variables in the unit described in parentheses, whereas sebum, pH, and hydration are shown in arbitrary units from the Dermaunit SSC (marked with a); x-axis represent the two age groups of children and adolescents represented by "Ped" and adults represented by "Adult." Orange represents persons with diabetes as cases and gray represents healthy controls. The box plots show all values with a dot and the box showing median and first and third quartile values. *P*-values after Benjamini-Hochberg adjustments are shown for significant differences between case- and control group within the age group. NMF, natural moisturizing factor; PCA, 2-pyrrolidone-5-carboxylic acid; TEWL, transepidermal water loss.

linear regression was performed to adjust for the influence of season, humidity, and room temperature. If more than 50% of the samples for one of the cytokines were under the level of detection, no further analysis was performed for that specific cytokine, explaining why no results are shown for IL-13. Microbiome data was investigated using the Phyloseq package (McMurdie and Holmes, 2013) to investigate alpha diversity as number of operational taxonomic units, Shannon diversity, and Phylogenetic diversity with paired Wilcoxon test at both nares and buttock. Beta-diversity was investigated by principal coordinate analysis plots and permutational multivariable ANOVA with rarefication on 5,000 counts and using Bray-Curtis, weighted- and unweighted Unifrac as distance measures. Both alpha- and beta-diversity were also investigated in the two age groups. A two-sided $P < 0.05$ after the Benjamini-Hochberg adjustment was considered statistically significant as defined in our a priori statistical analysis plan.

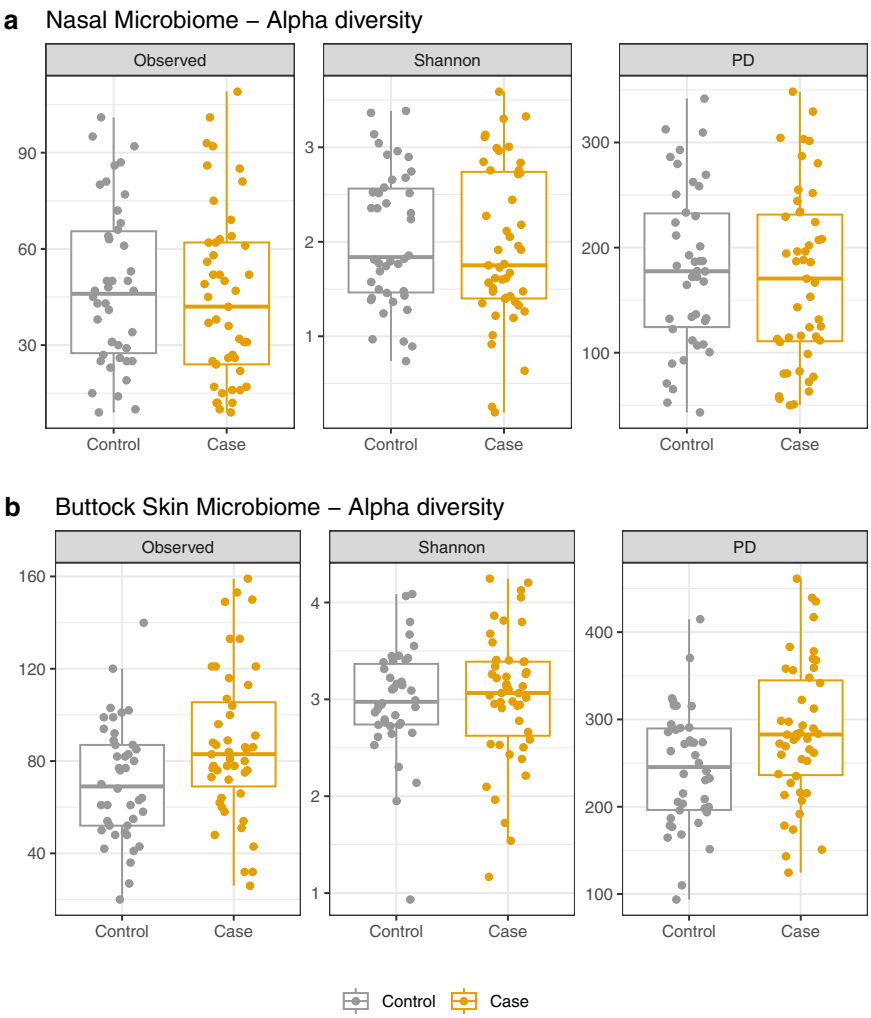

Figure 2. Boxplots of Alpha diversity results. (**a**) Boxplots of Alpha diversity results of the nasal microbiome for three alpha diversity indices: observed, Shannon diversity, and PD. No significant differences were found. (**b**) Boxplots of Alpha diversity results of the buttock skin microbiome for three alpha diversity indices: observed, Shannon diversity, and PD. No significant differences were found. Orange represents persons with diabetes as cases and gray represents healthy controls. PD, phylogenetic diversity.

Data availability statement

The data from this study are not publicly available due to ethical and data protection issues, but an anonymized aggregated data set can be made available on request to the corresponding author according to national law. The study was approved by the Research Ethics Committee at the Capital Region of Denmark (H-19037160). Oral consent was obtained from all participants irrespective of age after thorough information and participation was voluntary. Written informed consent was obtained from the participant if above 18 years and from both parents if the participant was 17 years old or younger. The observational case-control study was preregistered at ClinicalTrials.gov (NCT04280315).

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: AKB, SUT, CZ, JS, JPT; Data Curation: AKB; Formal Analysis: AKB, ASH; Funding Acquisition: AKB; Investigation: AKB, ACG, IJ, SK; Methodology: JPT; Project Administration: AKB, JS; Resources: KN, IJ, SK; Supervision: SUT, CZ, JS, JPT; Validation: ASH; Visualization: AKB; Writing — Original Draft Preparation: AKB; Writing — Review and Editing: ACG, KN, SUT, CZ, ASH, IJ, SK, JS, JPT

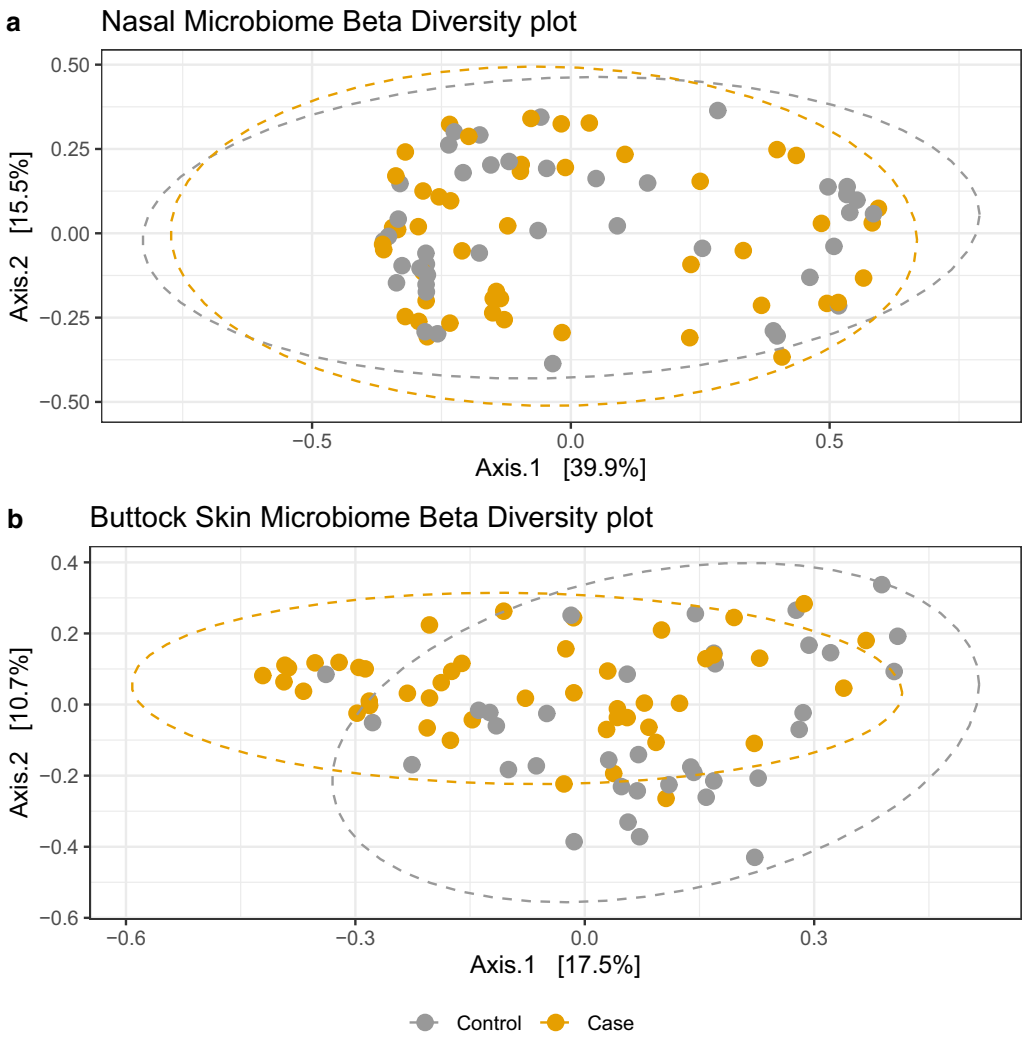

Figure 3. PCoA of beta-diversity. (**a**) PCoA plots of beta-diversity expressed by Bray-Curtis distance measures for the nasal microbiome. PERMANOVA test revealed an insignificant difference between control and cases with a $P = 0.983$. (**b**) PCoA plots of beta-diversity expressed by Bray-Curtis distance measures for the buttock skin microbiome. PERMANOVA test revealed significant difference between control and cases with a $P = 0.033$ after Benjamini-Hochberg adjustments. Orange represents persons with diabetes as cases and gray represents healthy controls. PCoA, principal coordinate analysis; PERMANOVA, permutational multivariable ANOVA.

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