Materials and Methods

Twenty-eight patients with the obvious clinical diagnosis of psoriasis vulgaris and capitis were recruited from the Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany. Half of the patients were included in the histological/stereological study and the other half in the immunohistochemical study. The demographic and clinical data of the patients are summarized in table 1. Each patient signed a consent form prior to inclusion.

Two 5-mm punch biopsies were obtained from involved and uninvolved skin of the scalp or the back area of two groups of 7 psoriatic patients. The biopsy localizations are summarized in table 2. The head and back areas were chosen as being among the most seborrheic areas of the human body, with a high frequency of sebaceous glands. The methodology of the study is summarized in figure 2.

A qualitative histological study of these biopsies was conducted to describe the presence or absence of the sebaceous glands and their number, distribution, and morphology (shape and size) in lesional and nonlesional skin.

The volume of the sebaceous glands was estimated by the Cavalieri principle $V_{\text{object}} = \sum_{t=1}^{n} A_t \cdot t$

A software-generated grid of points was superimposed over the HE-stained sections of skin, and all points falling within the sebaceous gland tissue were counted. Each point was associated with a known and predetermined area, a(p), and the area of sebaceous gland on each section was estimated by multiplying a(p) by the number of points counted. By analyzing parallel sections throughout the entire biopsy with known and fixed systematic intervals, the total volume of sebaceous gland could be expressed as the sum of the transect areas multiplied by the distance between sections, where At is the area of each transect equal to a(p) x ΣP, t is the average distance between the sections, and k is the inverse fraction of sections analyzed. Epidermal thickness was also estimated by a superimposed, software-generated grid of sine-weighted lines over the sections such that the lines crossed the epidermis 5 times in each sample. Then the 5 points where the lines crossed the basal layer of the epidermis were chosen, and the shortest distance between the basal and corneal layers of the epidermis was estimated. The thickness of the epidermis was evaluated as the average of the 5 assessments. In that way, it was ensured that the points for measuring the thickness were chosen randomly by the software and not by the investigator.

For the immunohistological study, biomarkers which recognize different differentiation stages of human sebocytes were applied: the proliferation marker Ki67 [1, 2], the early sebaceous differentiation marker keratin 7 [3, 4], the advanced sebaceous differentiation marker epithelial membrane antigen [3, 5], and the late differentiation marker epithelial

sialomucin [5, 6]. Involucrin was selected as the marker of keratinocyte differentiation, while it can sometimes be expressed by sebocytes [7, 8]. Table 3 summarizes the main antibodies used for immunohistochemical analysis.

Descriptive statistics are presented as means ± SD, medians with IQR, or percentages, as appropriate. Continuous variables were compared by using nonparametric tests, the two-sample Wilcoxon rank-sum (Mann-Whitney) test, and the matched-pairs Wilcoxon signed-rank test. Correlations were assessed using the nonparametric Spearman's rank correlation coefficient and the respective p value. It is generally considered that Spearman's rho of <0.20 indicates very weak correlation, 0.21--0.40 weak correlation, 0.41--0.60 moderate correlation, 0.61--0.80 strong correlation, and >0.81 very strong correlation. For hypothesis testing, a probability level of <0.05 was selected to indicate statistical significance. All statistical tests were two-sided. Stata software was used for the statistical analyses (Stata Corp., College Station, Tex., USA).

RESULTS

Supplementary table 1. Stereological estimation of the sebaceous gland volume between pathological psoriatic (psoriasis/lesional) and healthy psoriatic skin (healthy/nonlesional). The maximum number of sebaceous glands found in a biopsy was three (SG 1, 2, and 3). The nonlesional skin appears to have fewer sebaceous glands. Furthermore, the volume estimates appear to be much higher in the nonlesional compared to the lesional skin. Patients 3, 4, and 9 appear to lack sebaceous glands in the lesional skin. A stereological estimation of the thickness of the epidermis by biopsy follows (epidermis psoriasis vs. epidermis healthy). The reader can also observe the difference in sebaceous gland volume between lesional and nonlesional skin per patient (sebaceous gland healthy-psoriasis) as well as the difference in the epidermis thickness (epi pso-epi healthy). It is interesting to note that the larger the epidermis difference between lesional and nonlesional skin (the more severe psoriasis) was, the smaller was the sebaceous gland volume in the lesional skin.

psoriasis

		back							head						
		p1	**p2**	**p3**	**p4**	**p5**	**p6**	**p7**	**p8**	**p9**	**p10**	**p11**	**p12**	**p13**	**p14**
age		35	49	64	70	36	51	61	55	45	52	33	75	59	59
sex		m	m	f	f	m	f	m	f	m	f	m	f	m	f
	SG1 (mm^3)	0.001388	0.026196		0	0	0.028838	0.004903	0.001052	0.000985		0	0.089569	0.08661	0.004523	0.001478	0.006046
	SG2	0.03213					0.019211	0.001299						0.08296	0.00403	0.002284	0.004359
	SG3	0.003224						0.004344									
	Average	0.012247	0.026196		0	0	0.024024	0.003515	0.001052	0.000985		0	0.089569	0.084785	0.004276	0.001881	0.005203
epidermis psoriasis		*328.5121*	*335.3894*	*449.5276*	*		*205.8532*	*	*456.9433*	*325.107*	*477.7345*	*201.0271*	*264.831*	*255.1846*	*351.8502*	*340.4221*

healthy

		back							head						
		p1	**p2**	**p3**	**p4**	**p5**	**p6**	**p7**	**p8**	**p9**	**p10**	**p11**	**p12**	**p13**	**p14**
age		35	49	64	70	36	51	61	55	45	52	33	75	59	59
sex		m	m	f	f	m	f	m	f	m	f	m	f	m	f
	SG1 (mm^3)	0.029465	0.061302	0.00309	0.033249	0.006941	0.017912	0.046706	0.003291	0.017128	0.167045	0.16872	0.03213	0.076773	0.030719
	SG2		0.018559			0.047104	0.020509	0.017196	0.002239	0.101846	0.086616	0.165346	0.021584	0.047104	0.037078
	SG3		0.358108			0.092381	0.018024			0.101239				0.022216	0.044289
	Average	0.029465	0.14599	0.00309	0.033249	0.048809	0.018815	0.031951	0.002765	0.073405	0.12683	0.167033	0.026857	0.048698	0.037362
SG healthy-psoriasis		0.017218	0.119793	0.00309	0.033249	0.024784	0.0153	0.030898	0.00178	0.073405	0.037262	0.082248	0.02258	0.046817	0.032159
epidermis healthy		*56.4638*	*93.95569*	*63.15852*	*54.88412*	*78.34486*	*50.10517*	*83.54107*	*103.2759*	*61.40725*	*113.5996*	*119.5835*	*89.73941*	*89.65701*	*69.91259*
epi pso-epi healthy		272.0483	241.4337	386.369		127.5084		373.4022	221.8311	416.3273	87.42756	145.2475	165.4452	262.1932	270.5095

* epidermis fold, not able to measure

Supplementary table 2. Stereological estimations; correlations between sebaceous gland volume and thickness of psoriatic epidermis.

Correlation of **"sebaceous gland volume in psoriatic skin"** with **"thickness of psoriatic epidermis"**

Spearman's rho = –0.73; p=0.007 (strong inverse statistically significant correlation)

Correlation of **"sebaceous gland volume in psoriatic skin"** with **"[psoriatic epidermis – healthy epidermis]"**

Spearman's rho = –0.75; p=0.005 (strong inverse statistically significant correlation)

Correlation of **"[sebaceous gland volume in psoriatic skin – sebaceous gland volume in healthy skin]"** with "[psoriatic epidermis – healthy epidermis]"

Spearman's rho = –0.10; p=0.75 (not correlated variables)

Supplementary table 3. Summary of the number of patients who showed strong or slight differences in the immunohistochemical staining between lesional and nonlesional skin.

a.	Ki67	CK7	EMA	ESM	INV
p1	0	0	0	0	0
p2	0	0	0	0	0
p3	**1**	**2**	**2**	**2**	0
p4	0	**1**	**1**	**1**	0
p5	0	0	0	0	0
p6	0	0	0	0	0
p7	0	**1**	**1**	**1**	0
p8	0	0	0	0	0
p9	0	0	0	0	0
p10	0	0	0	0	0
p11	0	0	0	0	0
p12	0	0	0	0	0
p13	0	0	0	0	0
p14	0	0	0	0	0

** 0= no difference between lesional and non-lesional skin*
1= slight difference between lesional and non-lesional skin
2= strong difference between lesional and non-lesional skin

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