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Differences in Hair Follicle Dermal Papilla Volume are Due to Extracellular Matrix Volume and Cell Number: Implications for the Control of Hair Follicle Size and Androgen Responses

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The size of a hair follicle is thought to be determined by the volume of its dermal papilla. The volume of the dermal papilla depends on the number of cells it contains and on the volume of the extracellular matrix. To establish which of these two variables is related to differences in hair follicle size we performed a stereologic study on 235 hair follicles from different sites, including male facial skin (beard), female facial skin, and scalp. In facial follicles there was a strong correlation between the area of the hair cortex and the volume of the dermal papilla. The area of the hair cortex also correlated with the number of cells in the dermal papilla and with the volume of dermal papilla per cell. In scalp hair follicles, where there was a smaller range of sizes, the correlations between these variables were weaker. In large male facial follicles the mean total dermal papilla volume was almost 40-fold higher than in vellus follicles from female facial skin. This difference was associated with a mean 17-fold greater number of cells in the dermal papilla and a 2.4-fold greater volume associated with each cell. Intermediate results were obtained in scalp follicles. In many regions of the skin hair follicles enlarge in response to androgens during adult life hair. Our results imply that the increase in the volume of the dermal papilla in these follicles is due to an increase in the number of cells, either through proliferation or through the migration of cells from the follicular dermal sheath, and to an increase in the amount of extracellular matrix per cell. As androgens are thought to act primarily on the dermal papilla, these changes may have a direct bearing on the mechanism of androgen-mediated alterations in hair follicle size.* Key words: androgens/hair. J Invest Dermatol 113:873–877, 1999*

The dermal papilla is a mesenchyme-derived structure situated at the base of the hair follicle. It is responsible for the induction and maintenance of growth and differentiation of epithelial cells in the hair bulb (Oliver, 1966, 1967, 1970; Jahoda *et al*, 1984) and is probably involved in regulating the hair cycle and in specifying the type of hair that is produced (Jahoda, 1992). There is also evidence that the size of the hair follicle and the volume of the hair fiber are determined by the volume of the dermal papilla. Van Scott and Ekel (1958) showed that the volume and mitotic activity of the hair bulb epithelium in human hair follicles correlate with the volume of the dermal papilla. Subsequently, Ibrahim and Wright (1982) explored the dynamics of the volumetric relationships between the papilla and the hair fiber in a study on rodent vibrissa follicles. In vibrissa follicles which regenerated following amputation of the hair bulb, the volume of the hair fiber correlated with the volume of the dermal papilla which had been reconstituted from the follicle dermal sheath. As only those follicles which reconstituted a dermal papilla resumed hair growth, their experiments suggest that the dermal papilla plays a primary part in this relationship.

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The major influence on hair follicle size during adult life is androgen action. Small vellus follicles in many body sites, such as the beard and pubic skin, enlarge in response to androgens, and the duration of anagen increases, resulting in the growth of thicker and longer terminal hair. On the scalp the opposite may occur as terminal follicles become miniaturized during balding. The effect of androgens on hair growth is thought to be mediated *via* the dermal papilla. Dermal papilla cells express androgen receptors, both *in vivo* and *in vitro* (Randall *et al*, 1992). In two immunohistochemical studies nuclear staining for androgen receptors in the lower part of the hair follicle was present only in the dermal papilla and was not seen in hair bulb epithelial cells (Choudhry *et al*, 1992; Itami *et al*, 1995a), although a third study using a different antibody showed more extensive androgen receptor expression including follicular epithelium (Liang *et al*, 1993). Cultured dermal papilla cells derived from hair follicles from different parts of the body also metabolize androgens in a pattern consistent with that expected from their site of origin (Itami *et al*, 1991; Hamada *et al*, 1996)

If androgens act primarily on the dermal papilla it follows that they must, in some way, alter its volume. The dermal papilla contains specialized fibroblast like cells. During the anagen phase of the hair cycle, these cells lie in an extracellular matrix that is rich in basement membrane proteins and proteoglycans (Couchman, 1986; Couchman *et al*, 1991; Messenger *et al*, 1991). Therefore, a change in dermal papilla volume could be due to a change in the number

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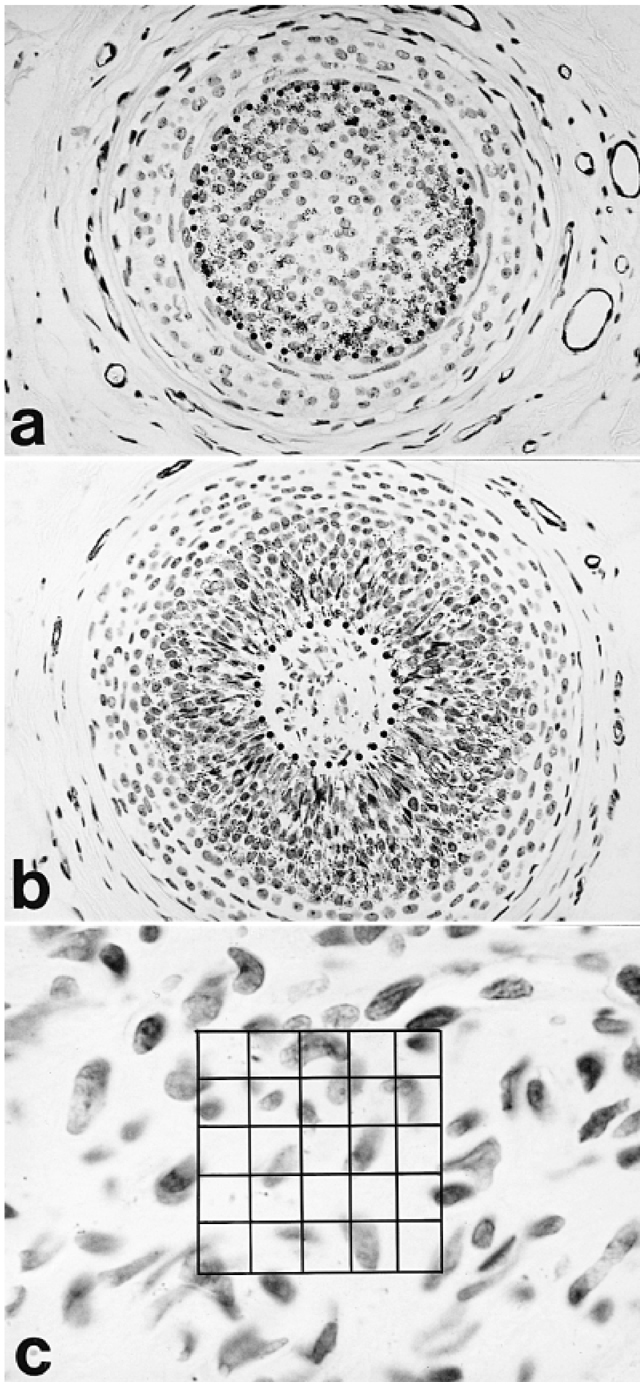

Figure 1. Sites of measurement on tissue sections. (*a*) Horizontal section through the upper hair bulb immediately above the upper pole of the dermal papilla. The hair cortex is delineated by the dotted line. (*b*) Horizontal section through the mid-region of the hair bulb. The dermal papilla is delineated by the dotted line. (*c*) A 6 × 6 grid placed over the image of the dermal papilla at high magnification. Vv is the ratio between the number of intersects overlying cell nuclei and the total number of intersects.

of cells within the papilla, a change in the relative amount of extracellular matrix, or both. In this study we have used a stereologic method to analyze the relationships between dermal papilla volume and the size of the hair cortex and to determine the relative contributions of cell number and extracellular matrix to the volume of the dermal papilla. We studied hair follicles from both human scalp and facial skin. The latter included small vellus follicles

Table I. Subject details

Biopsy	Age	Sex	Site[a]	Number of follicles
1	43	M	Face	10
2	28	M	Face	6
3	60	M	Face	6
4	43	F	Face	26
5	27	F	Face	13
6	14	F	Face	16
7	54	F	Face	12
8	30	M	Frontal	8
9	57	M	Occ	4
10	37	M	Occ	12
11	53	M	Vertex	12
12	61	F	Occ	15
13	10	F	Occ	10
14	25	F	Occ	11
15	48	F	Occ	14
16	53	F	Occ	16
17	18	F	Vertex	20
18	31	F	Vertex	25

[a]Frontal, frontal scalp; Occ, occipital scalp; Vertex, scalp vertex.

in female facial skin and large terminal follicles in male beard skin in order to assess the changes that occur during androgen-mediated changes in follicle size.

MATERIALS AND METHODS

Biopsies Biopsy samples were obtained from male and female scalp and from male beard and female facial skin as excess tissue removed during the routine excision of benign skin lesions (e.g., naevi). The samples were fixed in 4% formalin, processed to paraffin blocks and then sectioned horizontally with respect to the skin surface. Serial 5 µm step sections were taken through anagen hair bulbs at 10–25 µm intervals. To visualize endothelial cells (which may also be present in the dermal papilla), sections were stained for Factor VIII using a polyclonal antibody (Dako, Glostrup, Denmark) and an avidin–biotin immunoperoxidase method and then counterstained with hematoxylin. Light microscopic images were captured using a video camera and a personal computer equipped with a video-digitizing board and then color printed.

Stereologic analysis The following parameters were measured on the color prints.

In the section immediately above the upper pole of the dermal papilla the diameter of the hair cortex was measured (**Fig 1*a***). In each section through the dermal papilla were measured: (i) the diameter of the dermal papilla (**Fig 1*b***), and (ii) the volumetric density of cells in the dermal papilla (Vv). Vv was determined using the method described by Weibel and Gomez (1962). A 6 × 6 grid was placed at random over the image and the number of grid intersections overlying cell nuclei were counted (**Fig 1*c***). Vv is obtained from the formula:

$$Vv = \frac{\text{number of intersects overlying nuclei}}{\text{total number of intersects}}$$

A stable value for Vv was obtained by taking the mean value from 16 grids (which gave a total of 576 intersecting points).

The number of cells per unit area in the dermal papilla (Na) was obtained by counting the number of cell nuclei underlying each grid, the area of the grid being known. Nuclei underlying the top and right-hand margins of the grid were counted; those underlying the bottom and left-hand margins were excluded. Cells stained for Factor VIII were excluded.

The following were calculated from these measurements: (i) area of the hair cortex; (ii) volume of the dermal papilla (DPv); (iii) volume of dermal papilla per cell (1/Nv); (iv) number of nuclei per unit volume (Nv) in the dermal papilla; and (v) total number of cells in the dermal papilla (DPn).

To obtain DPv the segment of the dermal papilla between two successive step sections was regarded as the frustum of a cone whose volume (V) is calculated from the formula:

$$V = 3\overline{)\pi A(R^2 + Rr + r^2)}$$

where A is the distance between sections and R and r are the radii of the dermal papilla in the two sections. DPv is then the sum of the volumes of its constituent segments. Nv in the dermal papilla was obtained from:

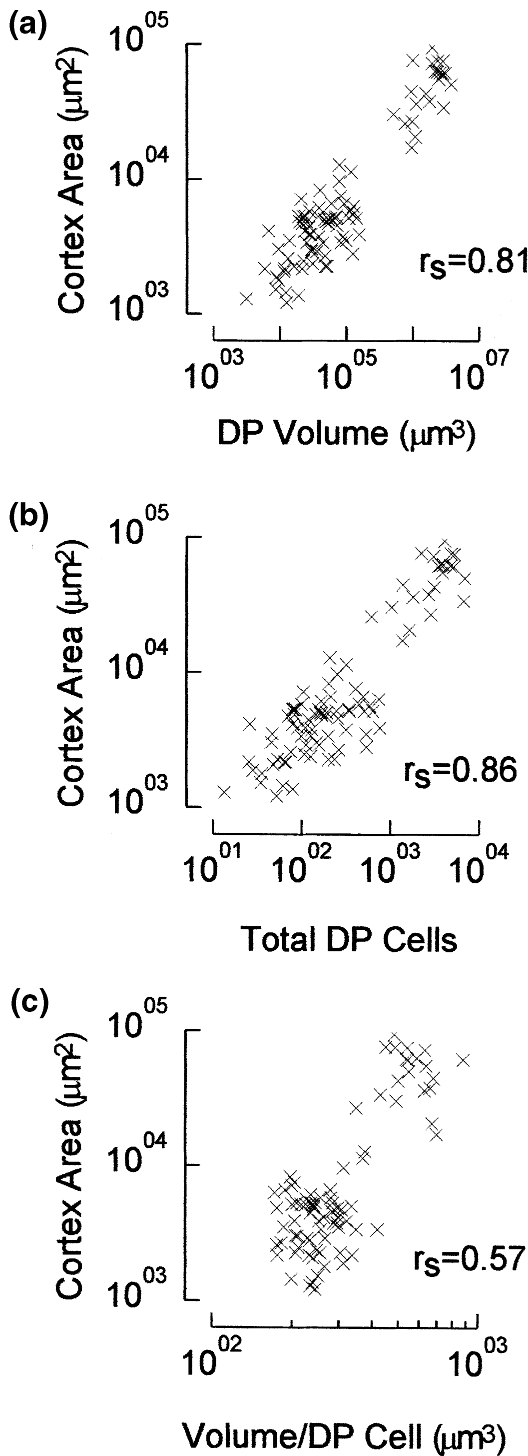

Figure 2. Male and female facial follicles. (*a*) Scatterplot of cortex area against dermal papilla (DP) volume; (*b*) scatterplot of cortex area against the total number of cells in the dermal papilla; (*c*) scatterplot of cortex area against the volume per DP cell (1/Nv).

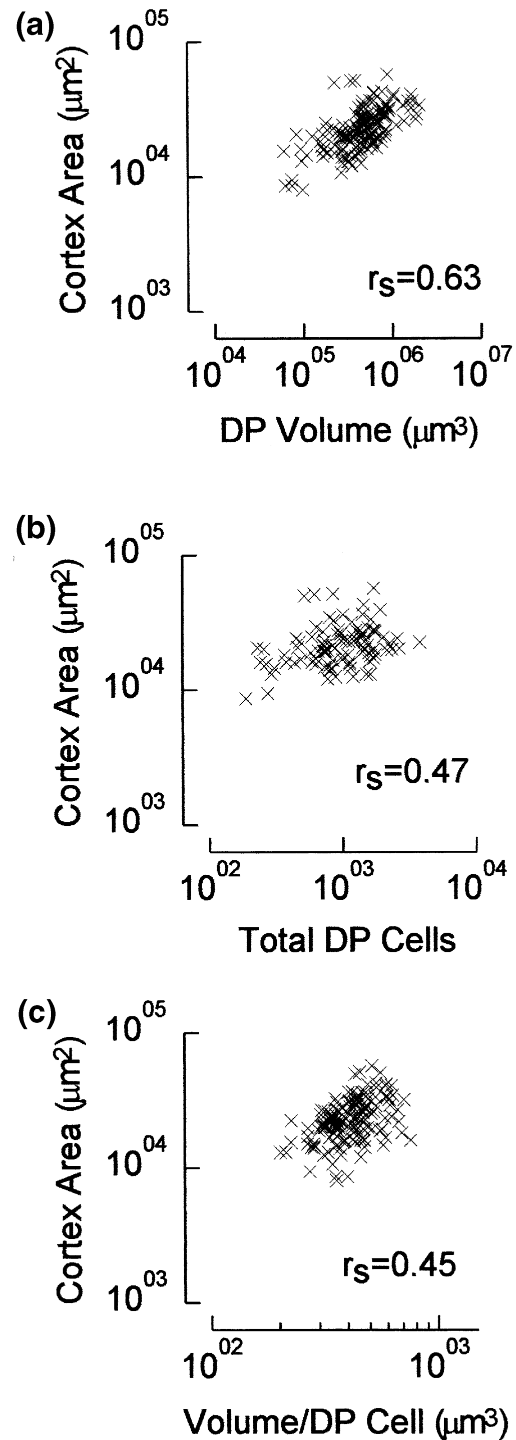

Figure 3. Scalp follicles. (*a*) Scatterplot of cortex area against dermal papilla volume; (*b*) scatterplot of cortex area against the total number of cells in the dermal papilla; (*c*) scatterplot of cortex area against the volume per DP cell.

$$Nv = \frac{K\ (Na)^{1.5}}{\beta\ (Vv)^{0.5}}$$

where K is the size distribution coefficient (1.2) and β is the shape coefficient (1.58). Values for K and β were obtained from Alcarez *et al* (1993). Total number of cells in the dermal papilla (DPn) is given by: DPn = Nv × DPv.

Statistical analysis Data were analyzed using nonparametric tests as sample values for facial hair follicles were not normally distributed. Correlations between variables were studied using the Spearman rank correlation. The sample means were first compared using a Kruskal–Wallis test. A Mann–Whitney U test was then used to compare sample means in male and female facial follicles.

RESULTS

We studied 235 hair follicles in 18 biopsy samples (**Table I**). The area of the hair cortex was taken as an indicator of hair follicle size. This varied widely between small vellus follicles in female facial

Table II. Dermal papilla volume[a]

	Female face		Scalp		Male face	
	Mean	CI	Mean	CI	Mean	CI
DP volume ($\mu m^3 \times 10^3$)	47.5	38.1–56.9	536.6	476.6–596.6	1878.1	1487.1–2269.1
Total DP cell number	198.2	154.3–242.1	1286.0	1153.1–1418.9	3344.3	2578.2–4110.5
Volume/DP cell (μm^3)	252.9	240.0–265.9	420.9	403.6–438.4	602.3	521.2–683.4

[a]Mean values and 95% confidence intervals (CI) for dermal papilla (DP) variables in hair follicles from male and female facial skin, and from scalp skin. There was a significant difference in sample means between the three groups for all three variables ($p < 0.001$, Kruskal–Wallis test). The differences in means between male and female facial follicles for all three variables were also statistically significant ($p < 0.0001$, Mann–Whitney U test).

skin to large beard follicles in male facial skin with intermediate values in scalp follicles. There was no significant difference in the distribution of cortex area in male and female scalp follicles and these were therefore analyzed together.

The results for facial follicles and scalp follicles were analyzed separately. In facial follicles (**Fig 2**) there was a strong correlation between the area of the hair cortex and the volume of the dermal papilla ($r_s = 0.81$). There were also strong correlations between the area of the hair cortex and the number of cells in the dermal papilla ($r_s = 0.81$) and the volume of the dermal papilla per cell (1/Nv) ($r_s = 0.57$). Similar relationships were observed in scalp follicles but the range of cortex areas was smaller and the correlations were weaker (**Fig 3**).

The mean total dermal papilla volume in male facial follicles was almost 40-fold higher than in female facial follicles. This was associated with a mean 2.4-fold greater volume of dermal papilla per cell. The mean dermal papilla cell number (which was derived from these two independent variables) was therefore about 17 times greater in male facial follicles than in female facial follicles. The values for these variables in scalp follicles were intermediate between those of male and female facial follicles (**Table II**).

Factor VIII positive cells were observed in the dermal papillae of all the male facial follicles and most scalp follicles, but none of the female facial follicles. Owing to their low numbers we were unable to assess accurately the contribution of Factor VIII cells to overall dermal papilla volume but, even in large male facial follicles, these cells formed only a small proportion of the total number of cells (< 1%) and their influence will have been negligible.

DISCUSSION

The point counting method for estimating the number of structures in a biologic sample from tissue sections has been validated experimentally (Weibel and Gomez, 1962). It is less accurate for counting structures whose diameter is less than 20 times the thickness of the tissue section, as will have been the case in this study, and this may have led to an overestimate of the number of cells in the dermal papilla. In view of the comparative nature of the study, however, the overall conclusions should not be invalidated.

Our results are consistent with the findings of previous studies in showing that the size of the hair follicle correlates with the volume of its dermal papilla (Van Scott and Ekel, 1958). They do not tell us whether the volume of the dermal papilla determines the size of the hair follicle or *vice versa*, although the experimental studies on the rat vibrissa follicle suggest that the dermal papilla is the primary factor in the relationship (Ibrahim and Wright, 1982). Our results also show that differences in the volume of the dermal papilla are due to differences in both the number of cells within the papilla and in the volume associated with each cell and that these variables also correlate with the size of the hair cortex. We assume that differences in volume per cell reflect differences in the amount of extracellular matrix. We cannot exclude the possibility that there are also differences in cell volume, although histologic observation indicates that the dermal papilla extracellular matrix is more abundant in terminal follicles than in vellus follicles.

These results imply that, as a follicle enlarges in response to androgens, there is an increase in both the number of cells in the dermal papilla and in the volume per cell of extracellular matrix. In volumetric terms the change in cell number has a larger influence on dermal papilla volume than the extracellular matrix. As the extracellular matrix may form a source of biologically active molecules, however, relatively small changes in its volume may have a disproportionately large functional effect. The apparent change in cell number during vellus-terminal transition is somewhat at odds with our previous understanding of dermal papilla biology. Wessels and Roessner (1965) found that thymidine labeling in the mesenchymal precursor of murine dermal papillae ceases at an early stage of follicular development, although Adelson *et al* (1992) demonstrated that cell proliferation in the dermal papilla does occur at a later stage in the development of the primary wool follicle in sheep. The observations of Wessels and Roessner have been interpreted to indicate that the number of cells in the dermal papilla is fixed during embryogenesis. Our results, however, suggest that this is not the case, at least in human hair follicles. The increase in cell number during vellus-terminal transition could be due to proliferation of cells within the dermal papilla or to recruitment of cells from the dermal sheath. There are no published studies demonstrating cell division within dermal papillae of adult hair follicles. Some cells showing thymidine labeling have been observed (Pierard and de la Brassinne, 1975), although, compared with the intense labeling seen in the epithelial matrix they are few in number and it is not clear whether these cells are endothelial or fibroblastic in type. If the increase in cell numbers during vellus-terminal transition is due to cell proliferation, it probably occurs only during early anagen development and over the course of several hair cycles. This will be difficult to detect in biopsy material.

The mechanisms underlying the putative role of dermal papilla volume in determining hair follicle size have yet to be established. Beard and axillary dermal papilla cells release keratinocyte mitogens, including insulin-like growth factor-1 (Itami *et al*, 1995b), when cultured in the presence of testosterone (Thornton *et al*, 1991; Itami *et al* 1995a). Thus, an increase in dermal papilla cell number in response to androgens may increase the local source of mitogens acting on hair matrix epithelium. Alternatively, dermal papilla volume may control the size of the hair fiber in a more simple mechanical fashion through modulating the surface area over which matrix epithelium interacts with the dermal papilla. Some support for this idea comes from recent experiments showing that testosterone enhances the release of mitogenic factors from human beard dermal papilla cells (but not cells from androgen-independent occipital scalp follicles), which stimulate the growth of other dermal papilla cells (Thornton *et al*, 1998), suggesting that autocrine mechanisms may operate to control dermal papilla volume.

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