

## Age-related Regional Variations of Human Skin Blood Flow Response to Histamine

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The process of ageing involves many changes in the skin. These changes are not necessarily uniform, so the pattern of regional variations may vary with ageing. The aim of the present study was to assess age-related regional variations in skin function, by measuring the cutaneous microvascular response to histamine. Histamine was topically applied to the back and forearm of a young and an aged group of volunteers ( $n=28$ , 14 in each group), and the response was quantified utilizing laser Doppler flowmetry. The following parameters were calculated from the data and compared between the two groups: (i) peak response; (ii) the time required to reach the peak; (iii) the time required to decay to half the peak flow; and (iv) the area under the response time curve.

For the young group, the magnitude of the maximum response, as well as the extent of the response as measured by the area under the response curve, were significantly greater on the back than on the forearm ( $p < 0.01$  and  $p < 0.05$ , respectively). In contrast, for the aged group, the two sites did not significantly differ from each other. The time required to reach the peak was longer in the aged group over both sites, and so was the time required to decay to half the peak flow.

These observations indicate regional anatomical or functional differences between old and young skin, which may provide insight into inherent differences influencing cutaneous manifestations of endogenous and exogenous diseases in various age groups.

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Inflammatory mediator-related processes may occasionally have a site predilection. Thus, in some cases of cold urticaria, the reaction is anatomically restricted (1, 2), suggesting local abnormalities of mast cells. Lahti (3) demonstrated regional variations in non-immunologic contact urticaria, and similar differences were noted by Maibach (4) in immunologic contact urticaria.

The back is regularly utilized for patch testing. The upper arm is also adequate, but the upper back is preferably used for routine patch tests, since false-negative tests are more common in the upper arm (5). That the back may show an enhanced allergic contact dermatitis reaction as compared to other sites is indicated in a report by Romaguera et al. (6). Patch testing a young boy (13-year-old), they got an "angry back" reaction, whereas the thigh did not react in such a manner. Clinically relevant regional variations were documented in young subjects. Thus, Smith et al. (7) who, studying a 20-year-old patient with contact urticaria from cobalt chloride, could only elicit an urticarial response above the waist but not below. They further studied 36 subjects, one of whom – a 9-year-old girl – developed

urticarial wheals following topical application of cobalt chloride to the back. The same application to the forearm and thigh elicited no response. Differences in the ability to elicit immunologic contact urticaria were noticed by Maibach in a young subject – a reaction appeared on the face, but could not be elicited on the forearm or dorsal hands (4).

A greater reactivity of the back was demonstrated in our previous study (8), when following topical and intradermal histamine administration, the back of young subjects reacted more than the forearm or the sole. In addition, we have observed that in young patients urticaria often involves the back, whereas in old patients the forearms are more frequently afflicted. To the best of our knowledge, such differences have not been reported in the literature, and in studies reviewing large numbers of patients with urticaria the sites of the lesions were not specified (9, 10).

The aim of the present study was to assess age-related regional variations in skin function, by measuring the cutaneous microvascular response to histamine over the back and forearm of young and aged volunteers. Laser Doppler flowmetry (LDF) was utilized to quantify the resulting reaction of the cutaneous microvasculature. This non-invasive optical technique has been used to evaluate the response of the cutaneous microvasculature to challenge (11–14).

### MATERIALS AND METHODS

#### Subjects

The subjects comprised two groups of 7 men and 7 women each: 1) fourteen young Caucasian volunteers aged 25–35 years (mean 28) and 2) fourteen aged Caucasian volunteers 64–74 years (mean 67). All were healthy and none were taking drugs with a known action on blood vessels or which interfere with inflammatory responses. The subjects signed an informed consent, the protocol of which was approved by the Israeli Ministry of Health Committee for the Conduct of Human Research.

#### Experimental design

Before and after topical application of histamine, the reaction was followed by measuring skin blood flow, utilizing a laser Doppler flowmeter (Periflux PF2, Perimed, Sweden).

The extravasated fluid ensuing on the presence of histamine restricts blood supply at the site of histamine administration (8, 11), resulting in decreased measurements at the center of the wheal. Therefore, measurements were taken 1 cm from the point of histamine administration. A distance of 1 cm appeared to generate the most sensitive and reproducible data according to our prior testing and that of others (3, 8, 11–12, 14). For the LDF measurements, a separate probe holder (PF 104, Perimed, Sweden) was positioned over each site and held in place with double adhesive discs (3M, Minnesota, U.S.A.). A 5-mm hole had previously been drilled in the probe holder at 1 cm distance from the center (Fig. 1). The probe holder was held in place during the whole period of the experiment.

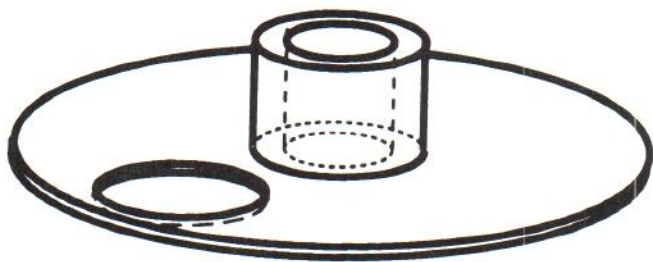


Fig. 1. The probe holder with a 5-mm hole drilled at 1 cm distance from the center.

#### Histamine provocation

After the baseline blood flow at each site had been established, 20 ml of 10% histamine chloride in saline was topically applied by a pipette into the hole (diameter = 5 mm) in the probe holder. The solution remained in contact with the skin surface for 2 min and was then gently dried. LDF measurements were started immediately and stopped upon reaching 50% of the maximum response in the decay phase. Measurements were taken by shifting the probe from one test site to another. For each subject, the test was randomly assigned either to the left or to the right side, and the sequence of measured anatomical site was random as well.

*Sites of histamine application:* i) the back over the spine of the scapula; ii) the ventral surface of the forearm, one third way from the cubital fossa to the wrist.

*Recording sites:* 1 cm proximal to the site of histamine application on the forearm, and at the same distance medially and diagonally down on the back.

*Environmental conditions:* all experiments were conducted in the same room under reasonably constant conditions, and during the same season.

*Characteristic parameters:* the response to histamine was characterized by four parameters: i) the magnitude of the maximum response ( $P$ ), expressed in arbitrary units; ii) the time required for the response to reach its maximum value ( $T_p$ ); iii) the time required for the maximum response to decay to half the maximum value ( $T_{1/2}$ ); and iv) the area under the time response curve from  $t = 0$  to  $T_{1/2}$  (A).

#### Statistics

Comparison between the parameters used to characterize the blood flow response (corrected for the baseline pre-administration value) involved analysis of variance.

## RESULTS

Mean LDF response curves for the two anatomical sites, as a function of time, are shown in Fig. 2. The characteristic response parameters and the results of the statistical analysis appear in Table I. LDF readings are expressed in arbitrary units. The values in the graphs are the means at each time point, and since the maximum response was not reached at exactly the same time for all volunteers, the graph reaches a maximum that is lower than the mean of the individual maxima reported in the table.

For the young group, the magnitude of the maximum response ( $P$ ), as well as the extent of the response as measured by the area (A), were significantly greater on the back than on the forearm ( $p < 0.01$  and  $p < 0.05$ , respectively) when measured by LDF. In contrast, for the aged group, the two sites did not significantly differ from each other. There were no significant

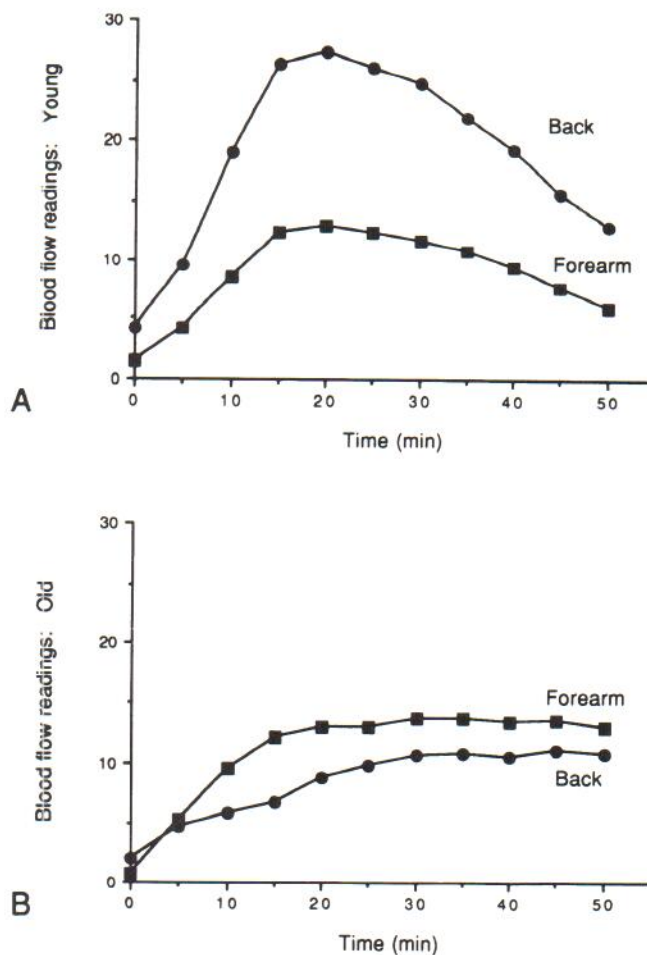


Fig. 2. LDF cutaneous blood flow measurements (arbitrary units) as a function of time, following topical application of histamine to young subjects (A) and old (B) on the back (circles) and forearm (squares).

differences between the two anatomical sites regarding the time parameters ( $T_p$  and  $T_{1/2}$ ) in each group. These parameters ( $T_p$  and  $T_{1/2}$ ) were longer in the aged group in both sites, as compared to the young group. There were no significant differences in any parameter between men and women.

## DISCUSSION

Differences between old and young skin relating to regional variations are indicated in this study. In young subjects, the back exhibited a greater response than the forearm, whereas in older subjects this was not so. The reaction took longer to decay in the aged group. A longer decay time was also noted by Kligman (15): following saline injection into the skin of young and elderly subjects the wheals took longer to flatten in the aged, the same pattern being exhibited both in the back and the volar forearms.

Topical application of histamine was employed by several researchers but contradictory results were obtained in regard to the regional dependence of the response. Shelley & Melton (16), applying 10% histamine topically, did not observe any difference in the degree of urticaria between the sites examined, but no information concerning the age of the subjects was given. Cronin & Stoughton (17), in contrast, found the back most

Table I. LDF response following topical application of histamine

Values are means SEM for the 20 volunteers. LDF readings are expressed in arbitrary units. P = the magnitude of the maximum response; Tp = the time required for the response to reach its maximum value; T1/2 = the time required for the maximum response to decay to half the maximum value; A = the area under the time response curve from t = 0 to T1/2.

	Young		Aged	
	Back	Forearm	Back	Forearm
P	29±6.3 <sup>1</sup>	14.2±4 <sup>1</sup>	16.9±5 <sup>3</sup>	17.3±4.6 <sup>3</sup>
A	832±291 <sup>2</sup>	324±148 <sup>2</sup>	869±384 <sup>3</sup>	998±351 <sup>3</sup>
Tp (min)	19±2 <sup>4</sup>	17.3±2.6 <sup>4</sup>	27±2 <sup>4</sup>	26.3±2.6 <sup>4</sup>
T1/2 (min)	31.1±3.9 <sup>4</sup>	25.4±4.2 <sup>4</sup>	35.1±4 <sup>4</sup>	48.4±4.2 <sup>4</sup>

<sup>1</sup> Back larger than forearm ( $p < 0.01$ )

<sup>2</sup> Back larger than forearm ( $p < 0.05$ )

<sup>3</sup> No significant differences between back and forearm.

<sup>4</sup> Aged longer than young ( $p < 0.01$ ).

responsive in terms of intensity of erythema and whealing, following topical application of 0.01 ml of histamine base in concentrations of 2%, 5%, 7% and 10%. On the back the response was three times greater than on the peroneal surface of the leg, whereas the arm reacted with an intermediate intensity. Their subjects were "mostly men from 20–60 years of age, and 23–26 volunteers were used for each group of experiments." Thus, no information is given about the ages of the volunteers for each separate experiment. Our results stress the importance of age and might shed some light on the seemingly contradictory results of various studies.

The process of ageing involves many changes in the skin. Preliminary findings revealed that, as a group, older subjects have a lower capacity to express certain inflammatory reactions (18). There is some evidence that chemicals pass through the senile epidermis more easily, but they are less readily removed by the circulation (19). Some previous reports showed that ageing decreases the sensitivity and/or responsiveness of vascular tissues to both contractile and relaxant agonists, including histamine (20). Our study indicates that these age-related changes are not uniform and vary between the two sites studied.

The observed differences may result from differences in permeability, although age-related regional differences in permeability between the forearm and upper back are unlikely (21). Differences in mast cell density and capacity to synthesize and store histamine may explain the variations observed. A reduction of mast cells in the dermis is known to occur during ageing (22), and this reduction might not be uniform. Differences in concentration of cyclic nucleotide that modulates histamine secretion from mast cells may also be a contributing factor (8). But the intensity of the wheal and flare response may be related to the local reactivity of the blood vessels once the histamine actually reaches them, and to their indirect dilatation via the axonal reflex. Montagna & Carlisle found that cutaneous nerves are little affected by age (23), and therefore it is probably not the nerve itself that causes the differences. The vasodilatory action of histamine on the human skin blood vessels involves H<sub>1</sub> and

H<sub>2</sub> receptors. Activation of either type of receptor can elicit maximum dilatation, but the responses differ in their sensitivity to histamine, in the duration of their effect and in the mechanism of their production. H<sub>1</sub> receptors, which reside on endothelial cells, have the higher affinity for histamine and mediate a dilator response that is relatively rapid in onset and short-lived. By contrast, activation of the H<sub>2</sub> receptors, located on vascular smooth muscle cells, causes dilatation that develops more slowly and is more sustained. The longer time parameters (Tp and T1/2) in the aged group might indicate a switch in the concentration of the two kinds of receptors, leading to the variations observed. This change in the proportion of the two kinds of receptors might explain the differences in regional variations between the two groups. However, there is still controversy as to whether cutaneous nerves are affected by age, and there are studies indicating a decrease in autonomic nervous system function in older persons (24). If these changes are site-dependent they may create the variations observed.

More studies are necessary in order to find the anatomical and physiological origins of the altered regional variations with ageing. The possibilities are: i) variations in the sensitivity or responsiveness of the blood vessels to mediators; ii) variations in the extent of the reduction of mast cells during ageing; iii) variable density of the cutaneous vasculature; iv) variations of the density and sensitivity of the receptors, v) site-dependence of the density of nerve endings; and vi) variable permeability. Hopefully, as more is learned about these micro-differences in skin function and their alteration with ageing, we will be better able to interpret some of the many localized patterns of skin disease, particularly when mediator-related processes are involved.

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