

Research article

## Plasma proteins in a standardised skin mini-erosion (II): effects of extraction pressure

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Published: 11 February 2002

Received: 25 September 2001

*BMC Dermatology* 2002, 2:4

Accepted: 11 February 2002

This article is available from: <http://www.biomedcentral.com/1471-5945/2/4>

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### Abstract

**Background:** A standardised suction technique has been used to sample plasma proteins in dermal interstitial fluid (IF) serially for 5 to 6 days from a suction-induced skin mini-erosion. Increased protein concentrations ascribed to inflammation have been shown from day 1 onward. In this study, we assessed the effect of two different extraction pressures on IF sample composition.

**Methods:** Total protein concentration and the concentrations of insulin, prealbumin, albumin, transferrin, IgG and alpha-2-macroglobulin were assessed daily in healthy volunteers. Samples were extracted at 50 mmHg and 200 mmHg below the atmospheric.

**Results:** At 0 h after forming the erosion, mean total IF protein content (relative to plasma) was lower in the samples extracted at -200 mmHg than at -50 mmHg (26 +/-13% (SD) vs 48 +/-9.8%;  $p < 0.05$ ). There were no significant differences at 24, 48, 72 or 96 h. Of the individual proteins, expressed as area units (AU) for area under the curve (AUC) from 0–96 h, albumin was lower in IF sampled at -200 mmHg (2.49 +/- 0.68 vs 3.08 +/- 0.36 AU;  $p < 0.05$ ), as was transferrin (1.91 +/- 0.52 vs 2.40 +/- 0.42 AU;  $p < 0.05$ ). Extraction volumes were significantly higher at -200 mmHg (AUC diff: 60%;  $p < 0.05$ ).

**Conclusions:** Samples of IF extracted at 0 h at -200 mmHg contained lower protein concentrations, indicating an increased water fraction and an intact sieve function of the vascular wall. The difference in protein concentration extracted at higher and lower pressure from 24 h onward was less pronounced. Lower pressure should be used to sample substances of greater molecular size.

### Background

A standardised suction technique has been used to sample plasma proteins in dermal interstitial fluid (IF) serially for 5 to 6 days from a suction-induced skin mini-erosion [1–3]. Increased protein concentrations ascribed to inflam-

mation have been shown from day 1 onward. In this study, we examined the effect of two different extraction pressures (50 mmHg and 200 mmHg below atmospheric) on the IF concentrations of different-sized plasma proteins (range 6–720 kDa) as a function of time (96 h).

**Materials and methods**

The study was undertaken using the same subjects, materials and methods as described in: "Plasma proteins in a standardised skin mini-erosion (I): permeability changes as a function of time" [4]. Separate erosions were used for the two different suction pressures.

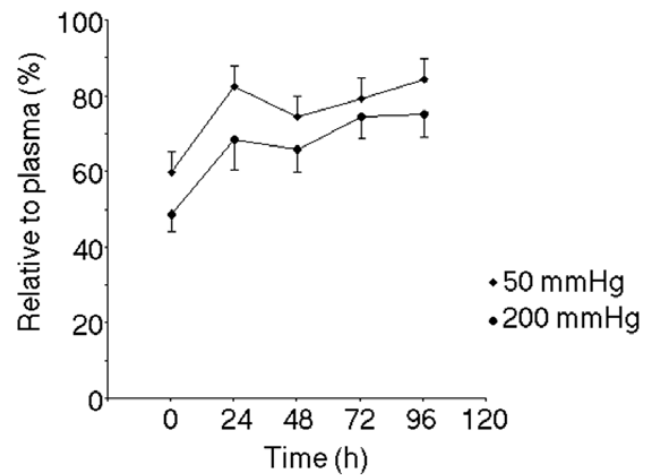
**Results**

There were no local complications. The IF samples were always clear and light yellow in colour with a more pronounced yellow tone from 24 h onward. Samples were never blood contaminated, but petechiae were noted in the erosions over time and more so in erosions exposed to sampling at -200 mmHg. After IF extraction, the erosions were transiently slightly elevated. Epidermal regeneration was complete in less than a week in all cases. An air leak prevented formation of one vesicle in the group of erosions sampled at -200 mmHg. In another subject, an air leak prevented IF sampling at -50 mmHg at 0 and 24 h.

**Total protein concentration**

At 0 h, the total protein content was significantly lower in the samples extracted at -200 mmHg than in those extracted at -50 mmHg ( $26 \pm 13\%$  (SD) vs  $48 \pm 9.8\%$ ;  $p < 0.05$ , diff: 46%). There were no other significant differences when comparing the two groups at 24, 48, 72 or 96 h.

**Concentrations of individual proteins**



**Figure 1**  
Albumin (66 kDa) concentration (mean ± SEM) in IF relative to plasma as a function of time.

Table 1 shows the concentrations of the individual proteins at different suction pressures over time. Figure 1 shows the relative concentrations (mean ± SEM) of albumin over 96 h. There were no significant differences when comparing any of the proteins in the two pressure groups, day by day.

**Table 1: Extraction volumes (µL/min) and IF concentrations, relative to those in plasma (%) of the six plasma proteins (means ± SD) using two different extraction pressures.**

	0 h	24 h	48 h	72 h	96 h
<b>Volume (µL/min)</b>					
50 mmHg	0,5 ± 0,5	1,0 ± 0,1	0,9 ± 0,1	1,8 ± 0,5	1,1 ± 0,4
200 mmHg	1,4 ± 0,2	1,9 ± 0,2	1,9 ± 0,4	2,6 ± 1,0	1,8 ± 0,5
<b>Insulin (%)</b>					
50 mmHg	45 ± 18	76 ± 27	55 ± 28	67 ± 36	69 ± 43
200 mmHg	65 ± 36	71 ± 24	66 ± 39	83 ± 38	68 ± 26
<b>Prealbumin (%)</b>					
50 mmHg	37 ± 15	64 ± 29	57 ± 22	61 ± 24	56 ± 18
200 mmHg	25 ± 13	50 ± 24	50 ± 15	78 ± 36	66 ± 19
<b>Albumin (%)</b>					
50 mmHg	59 ± 14	82 ± 14	74 ± 15	79 ± 15	84 ± 16
200 mmHg	48 ± 12	68 ± 21	66 ± 16	74 ± 15	75 ± 16
<b>Transferrin (%)</b>					
50 mmHg	43 ± 19	69 ± 22	57 ± 21	60 ± 18	63 ± 29
200 mmHg	31 ± 19	47 ± 25	42 ± 14	63 ± 22	49 ± 16
<b>IgG (%)</b>					
50 mmHg	47 ± 19	66 ± 17	62 ± 28	55 ± 16	64 ± 28
200 mmHg	31 ± 15	55 ± 30	45 ± 19	63 ± 20	55 ± 21
<b>Alpha-2-macroglobulin (%)</b>					
50 mmHg	45 ± 18	29 ± 29	28 ± 19	20 ± 8	24 ± 15
200 mmHg	20 ± 10	17 ± 9	15 ± 4	21 ± 9	21 ± 10

When comparing AUC, the concentration of albumin was significantly lower at -200 mmHg than at -50 mmHg ( $2.49 \pm 0.68$  vs  $3.08 \pm 0.36$  AU;  $p < 0.05$ , diff: 19%), as was the concentration of transferrin ( $1.91 \pm 0.52$  vs  $2.40 \pm 0.42$  AU;  $p < 0.05$ , diff: 20%). There were no other significant differences between the AUC of an individual protein extracted at different suction pressures.

#### Extraction volumes

Table 1 shows mean volume extraction rate over time for the two pressure groups. When comparing the AUC, the volumes were higher at -200 mmHg than at -50 mmHg ( $8.0 \pm 2.2$  vs  $5.0 \pm 1.9$  AU;  $p < 0.05$ , diff: 60%).

#### Discussion

The total protein concentration in IF at 0 h both at -200 mmHg and at -50 mmHg corresponds reasonably well with findings in earlier studies of interstitial fluid using other sampling techniques [5,6]. However, the significantly lower concentration at -200 mmHg at 0 h reflects an increased water fraction. It may indicate that the small pores are intact and that endothelial splits are rare. Thus, an ultrafiltrate of plasma without proteins may sieve out almost mainly through the small pores. The decrease in albumin and total protein concentration seen in the samples collected at -200 mmHg as compared to those collected at -50 mmHg correspond well with the decreases reported by Renkin et al at an approximately doubled lymphatic flow [7].

In the interval 24 to 96 h, there is an increased permeability in the erosion and the differences between the values obtained at -200 mmHg and -50 mmHg are less pronounced. This finding can be explained by an early increased permeability in venules and later by capillary leakage. In this situation, passage of plasma proteins through endothelial gaps may be predominant [8]. The findings are consistent with the two pore theory for exchange over the vascular wall and less consistent with theories of vesicular transport of macromolecules over the vascular wall. The degree of damage to the capillaries caused by repeated suctioning is not known, and although no significant differences were found in total protein content from 24 h onward, it may be noted that there was a tendency for total protein content to be greater in the -200 mmHg samples, which could indicate greater cellular and vascular damage.

The time course for the concentrations of the individual proteins was roughly similar in both extraction-pressure groups, indicating that the initial inflammatory stimulus was the determining factor. As figure 1 shows, increasing the suction pressure leads primarily to a parallel shift in the curve.

The extraction volumes were highly dependent on the extraction pressure used. The increased extraction volume over time may reflect increased endothelial area and permeability.

#### Conclusions

Samples extracted with higher negative pressure contained a lower protein concentration at 0 h, indicating an increased water fraction and that the sieve function of the vascular wall was intact. From 24 h onward, the difference was less pronounced. These findings indicate that temporal permeability changes were a result of the initial epidermal injury and that different extraction pressures and repeated sampling has only minor influence. Higher suction pressure may be used for sampling substances that are freely distributed, but substances of greater molecular size should be sampled at a lower pressure, perhaps optimally a continuous negative pressure approaching that of the interstitium.

#### Abbreviations

IF Interstitial fluid

AUC Area under curve

AU Area units

#### Competing interests

None declared

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