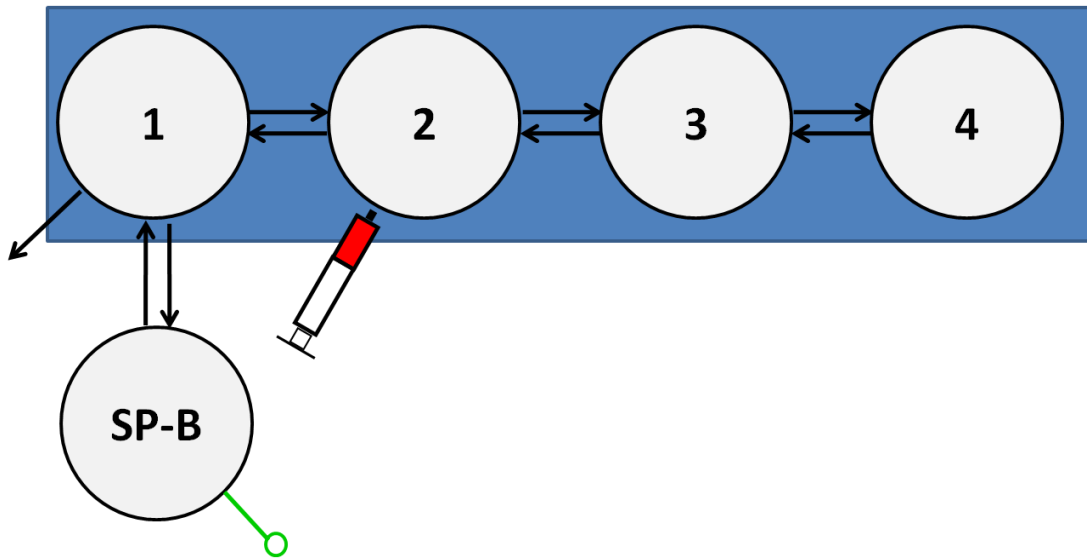
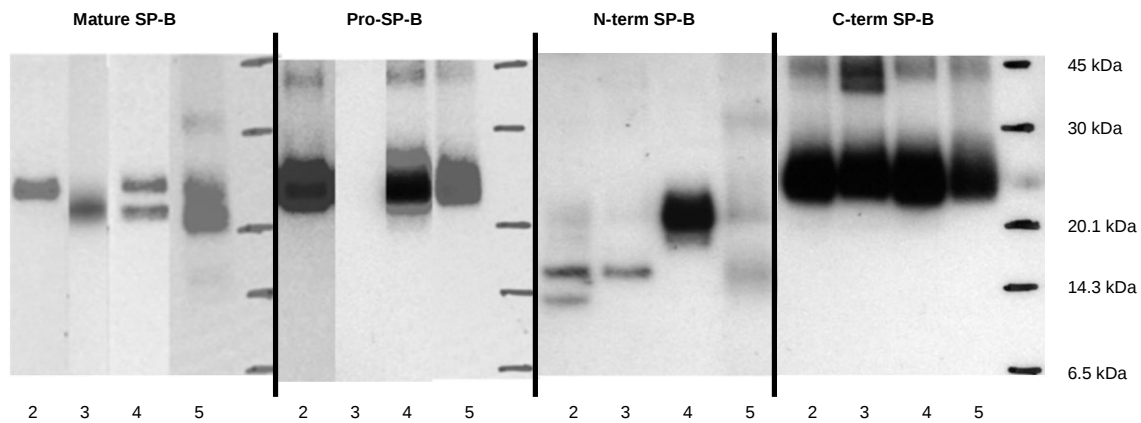


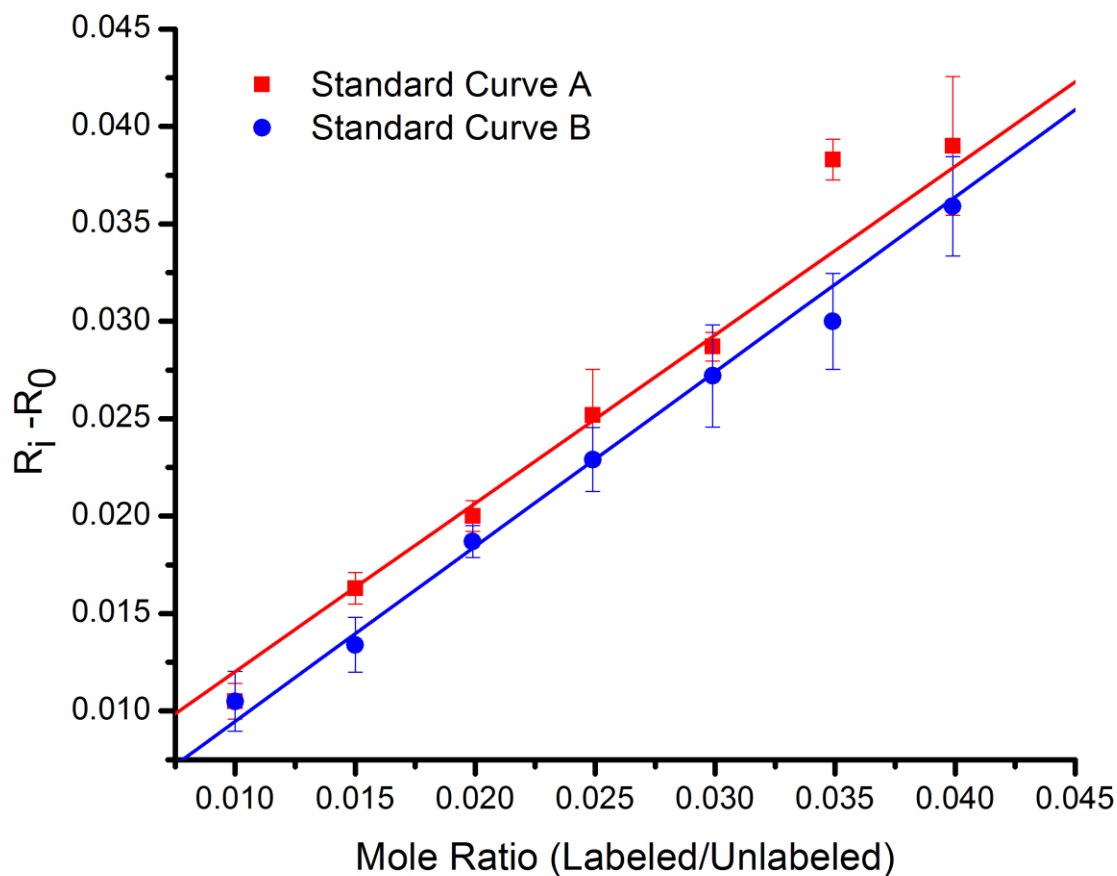
## Supplementary material



Supplementary Figure 1: Compartmental model used to calculate SP-B kinetics. We used a four pool compartmental model to estimate leucine kinetics (blue box) with the addition of a fifth pool for SP-B. The measured KIC enrichment in plasma was used as a proxy of the intracellular leucine enrichment in pool 1. A population approach was used to fit the SP-B enrichments using nonlinear mixed-effects modeling<sup>28</sup>. The population modeling enabled us to calculate individual rate constants even in patients with sparsely sampled data.

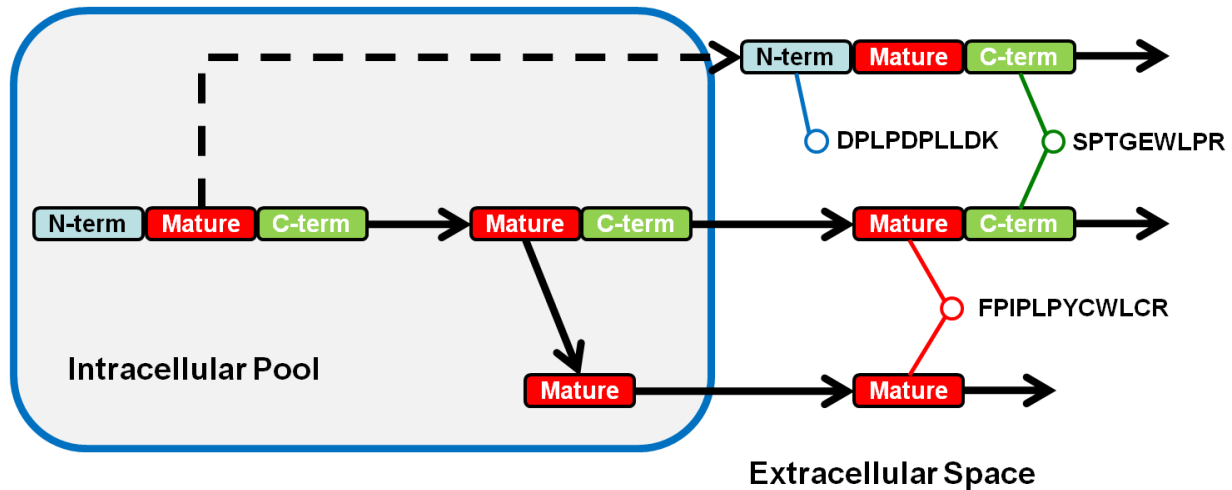


Supplementary Figure 2: Protein immunoblotting under non-reducing conditions for SP-B in unfractionated tracheal aspirates from Patients 2-5 using antisera directed against mature SP-B, proSP-B (Chemicon #AB3430), N-terminal proSP-B, and C-terminal proSP-B. In addition to mature SP-B, peptide fragments specific to the N- and C-terminal portions of proSP-B are present. Although it is difficult to determine exactly, the N- and C-terminal fragments likely correspond to the fragments represented by DPLPDPLLDK and SPTGEWLPR, respectively, identified with mass spectrometry.



Supplementary Figure 3. Measurement of the tracer:tracee ratio from synthetic peptide standards of known enrichment. The peptide SPTGEWLPR was synthesized with endogenous isotopes and enriched with  $^2\text{H}_3$ -leucine. The standards were mixed in known amounts on two different weeks to create two standard enrichment curves (A and B). The tracer:tracee ratio was calculated from the mass spectrometry data as described in the methods and plotted against the expected mole ratio of the standards. Points represent mean values ( $n=5$ ); error bars are  $\pm$  SD. Standard curve A had a regression that resulted in a linear equation of  $Y = (0.8645 \pm 0.0728) * X + (0.0034 \pm 0.0022)$   $r^2 = 0.9794$ . Standard curve B had a linear equation of  $Y =$

$(0.8966 \pm 0.0306) * X + (0.0005 \pm 0.0009) \quad r^2 = 0.9965$ . The sloped deviation from unity is probably a result of differences between the amount of peptide actually aliquoted by the peptide synthesis company and claimed. Note: these curves were only used to demonstrate a linear response and not to calibrate the instrument measurement because the source of the labeled amino acid in these peptides was different from that used as the tracer infusate.



Supplementary Figure 4: Model illustrating the forms accounted for by the measurement of individual tryptic peptides mapping to SP-B. The peptide DPLPDPLLDK sits on the N-terminus of the protein, the peptide FPIPLPYCWLCR is a partially tryptic peptide sitting on the N-terminal end of the mature SP-B peptide. The peptide SPTGEWLPR is present on the C-terminal portion of the proSP-B. The C-terminal peptide could have been derived from isoforms containing the N-terminus through to the C-terminus or following the cleavage and removal of the N-terminal portion of the protein. Given the Western blot data, the high abundance of the peptide SPTGEWLPR and the observation that the peptide DPLPDPLLDK is present at ~100x lower abundance, we assume that a majority of the SP-B in these tracheal aspirates are missing the N-terminus but contain the mature and C-terminal domains of the protein.

Supplementary Table 1: Measurements of SPTGEW(<sup>2</sup>H<sub>3</sub>-L)PR enrichment (MPE) from tracheal aspirates.

| Time points      | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Average | Stdev  |
|------------------|-------------|-------------|-------------|-------------|-------------|---------|--------|
| <i>Patient 1</i> |             |             |             |             |             |         |        |
| 0.00             | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000  | 0.0000 |
| 3.00             | 0.9808      | 0.9997      | 0.9549      | 1.1328      | 0.8221      | 0.9781  | 0.1110 |
| 6.00             | 2.3979      | 2.5389      | 1.9522      | 2.6381      | 2.1924      | 2.3439  | 0.2756 |
| 18.00            | 2.8447      | 2.4048      | 2.9990      | 3.0429      | 2.5727      | 2.7728  | 0.2760 |
| 34.00            | 1.0186      | 0.5236      | 0.9452      | 0.8877      | 1.1714      | 0.9093  | 0.2404 |
| 48.00            | 1.2920      | 1.1658      | 1.2514      | 1.2543      | 1.3541      | 1.2635  | 0.0685 |
| 62.00            | 0.9936      | 0.8598      | 0.9814      | 0.8081      | 0.8167      | 0.8919  | 0.0895 |
| 75.00            | 0.8119      | 0.9616      | 0.9241      | 1.0353      | 1.0548      | 0.9575  | 0.0973 |
| 96.00            | 0.9129      | 1.0655      | 0.8451      | 1.0860      | 0.9148      | 0.9649  | 0.1053 |
| <i>Patient 2</i> |             |             |             |             |             |         |        |
| 0.00             | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000  | 0.0000 |
| 22.67            | 1.6405      | 1.7925      | 2.0718      | 1.6721      | 1.6248      | 1.7604  | 0.1861 |
| 46.41            | 1.4410      | 1.3411      | 1.1796      | 1.2926      | 1.6118      | 1.3732  | 0.1632 |
| 59.50            | -0.0540     | 0.1958      | -0.1370     | 0.2116      | 0.3542      | 0.1141  | 0.2032 |
| 74.50            | -0.0742     | 0.0284      | -0.1181     | -0.1034     | -0.2546     | -0.1044 | 0.1016 |
| 80.50            | 0.1448      | 0.4845      | 0.3899      | 0.5022      |             | 0.3804  | 0.1646 |
| <i>Patient 3</i> |             |             |             |             |             |         |        |
| 0.00             | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000  | 0.0000 |
| 5.00             | 2.9273      | 2.5827      | 3.2303      | 3.1076      |             | 2.9620  | 0.2818 |
| 20.16            | 1.8593      | 1.9837      | 1.9189      | 1.4808      | 2.0403      | 1.8566  | 0.2208 |
| 26.00            | 1.1630      | 0.9069      | 1.4093      | 1.1087      | 0.7614      | 1.0699  | 0.2485 |
| 47.00            | 1.0472      | 1.4619      | 1.1338      | 0.8738      | 0.6072      | 1.0248  | 0.3165 |
| 56.00            | 0.3933      | 0.6851      | 0.6536      | 0.6321      | 0.2328      | 0.5194  | 0.1976 |
| 65.00            | 1.4180      | 1.3631      | 1.3179      | 1.6023      | 1.3142      | 1.4031  | 0.1190 |
| <i>Patient 4</i> |             |             |             |             |             |         |        |
| 0.00             | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000  | 0.0000 |
| 2.25             | -0.1193     | -0.0769     | -0.3065     | -0.2361     | -0.3426     | -0.2163 | 0.1154 |
| 10.25            | 2.7841      | 2.7953      | 2.3864      | 2.6503      | 2.4448      | 2.6122  | 0.1894 |
| 26.00            | 1.0613      | 1.0336      | 1.0848      | 0.8987      | 0.9539      | 1.0064  | 0.0779 |
| 34.16            | 0.7612      | 0.9048      | 0.8753      | 0.8219      | 0.8717      | 0.8470  | 0.0565 |
| <i>Patient 5</i> |             |             |             |             |             |         |        |
| 0.00             | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000  | 0.0000 |
| 0.25             | 1.3437      | 1.2696      | 1.0705      | 0.7560      | 0.8867      | 1.0653  | 0.2484 |
| 2.41             | 1.0560      | 0.8883      | 0.8768      | 0.8763      | 0.6616      | 0.8718  | 0.1400 |
| 4.25             | 1.4183      | 1.0619      | 1.0810      | 1.3321      | 1.0805      | 1.1948  | 0.1677 |
| 10.50            | 2.2442      | 2.4026      | 2.0539      | 2.4736      | 1.7895      | 2.1928  | 0.2773 |
| 21.71            | 1.7388      | 1.9192      | 1.8910      | 2.1019      | 2.3195      | 1.9941  | 0.2230 |
| 22.00            | 2.2496      | 2.3441      | 2.6114      | 1.8388      | 2.1507      | 2.2389  | 0.2819 |
| 25.25            | 1.9701      | 2.0194      | 1.7640      | 1.6456      | 1.7687      | 1.8335  | 0.1561 |
| <i>Patient 6</i> |             |             |             |             |             |         |        |
| 0.00             | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000      | 0.0000  | 0.0000 |
| 5.00             | 0.6934      |             | 0.6287      | 0.9227      |             | 0.7483  | 0.1545 |
| 13.00            | 2.1687      | 2.2543      | 2.3203      | 2.1814      | 2.1097      | 2.2069  | 0.0816 |
| 17.25            | 2.5211      | 2.3957      | 2.4627      | 2.1155      | 2.5770      | 2.4144  | 0.1802 |
| 21.00            | 1.9733      | 2.1946      | 2.0217      | 1.9704      | 2.0662      | 2.0453  | 0.0923 |
| 25.00            | 0.5565      | 0.4092      | 0.4734      |             |             | 0.4797  | 0.0738 |
| 33.02            | -0.0567     | 0.4202      | -0.2338     | 0.0706      | 0.0120      | 0.0425  | 0.2401 |
| 41.00            | 0.7698      | 0.1794      | 0.4413      | 0.0656      | 0.2001      | 0.3312  | 0.2808 |
| 49.00            | 0.3703      | 0.1702      | 0.5504      | 0.4044      | 0.3741      | 0.3739  | 0.1356 |
| <i>Patient 7</i> |             |             |             |             |             |         |        |

|                   |        |        |         |        |         |         |        |
|-------------------|--------|--------|---------|--------|---------|---------|--------|
| 0.00              | 0.0000 | 0.0000 | 0.0000  | 0.0000 | 0.0000  | 0.0000  | 0.0000 |
| 4.00              | 2.0529 | 1.6870 | 1.7796  | 2.9297 | 3.8890  | 2.4676  | 0.9339 |
| 9.00              | 2.9475 | 3.0426 | 2.8564  | 2.6300 | 2.5801  | 2.8113  | 0.2002 |
| 11.00             | 3.2057 | 2.2584 | 2.5355  | 3.1022 | 3.0674  | 2.8338  | 0.4138 |
| 18.00             | 2.6720 | 2.6406 | 2.5080  | 2.3227 | 2.5668  | 2.5420  | 0.1383 |
| <i>Patient 8</i>  |        |        |         |        |         |         |        |
| 0.00              | 0.0000 | 0.0000 | 0.0000  | 0.0000 | 0.0000  | 0.0000  | 0.0000 |
| 1.00              | 0.1841 | 0.1200 | 0.2617  | 0.2168 |         | 0.1957  | 0.0596 |
| 4.00              | 0.6378 | 0.5849 | 0.6542  | 0.5839 |         | 0.6152  | 0.0362 |
| 7.00              | 1.0182 | 1.0057 | 0.9729  | 0.8872 |         | 0.9710  | 0.0590 |
| 10.02             | 1.3461 | 1.4360 | 1.3666  | 1.4297 |         | 1.3946  | 0.0450 |
| 16.00             | 1.1910 | 1.1434 | 1.1393  | 1.1964 |         | 1.1675  | 0.0304 |
| 22.00             | 1.0116 | 1.0031 | 0.9582  | 0.9893 |         | 0.9906  | 0.0235 |
| 25.00             | 0.9063 | 0.8212 | 0.8125  | 0.8749 | 0.8371  | 0.8504  | 0.0393 |
| 28.00             | 0.6689 | 0.7062 | 0.6395  | 0.7861 | 0.7583  | 0.7118  | 0.0608 |
| 31.00             | 0.8077 | 0.6292 | 0.6888  | 0.8155 | 0.7517  | 0.7386  | 0.0795 |
| 34.50             | 0.6189 | 0.5106 | 0.6707  | 0.5849 |         | 0.5963  | 0.0671 |
| 43.00             | 0.4298 | 0.4658 | 0.4872  | 0.5453 | 0.4937  | 0.4843  | 0.0422 |
| <i>Patient 9</i>  |        |        |         |        |         |         |        |
| 0.00              | 0.0000 | 0.0000 | 0.0000  | 0.0000 | 0.0000  | 0.0000  | 0.0000 |
| 1.00              | 0.0959 | 0.0564 | -0.0262 | 0.0410 | -0.2082 | -0.0082 | 0.1201 |
| 4.00              | 0.4396 | 0.4035 | 0.2484  | 0.2067 | 0.3418  | 0.3280  | 0.0993 |
| 6.00              | 1.1307 | 1.1496 | 0.7979  | 0.5900 |         | 0.9170  | 0.2713 |
| 13.00             | 1.5349 | 1.8093 | 1.6798  | 1.6461 | 1.8273  | 1.6995  | 0.1212 |
| 16.00             | 1.4609 | 1.3110 | 1.3151  | 1.4247 |         | 1.3779  | 0.0764 |
| 18.00             | 1.4103 | 1.0983 | 0.8920  | 1.0708 | 0.9579  | 1.0859  | 0.1997 |
| 24.00             | 1.1046 | 1.1774 | 1.2278  | 1.2473 | 1.1531  | 1.1820  | 0.0575 |
| 27.00             | 0.7242 | 0.7041 | 0.9066  | 0.7955 | 0.8735  | 0.8008  | 0.0891 |
| <i>Patient 10</i> |        |        |         |        |         |         |        |
| 0.00              | 0.0000 | 0.0000 | 0.0000  | 0.0000 | 0.0000  | 0.0000  | 0.0000 |
| 2.08              | 1.1385 | 0.7458 | 1.0510  | 0.7952 | 0.5880  | 0.8637  | 0.2265 |
| 3.50              | 1.6143 | 1.2535 | 1.4958  | 1.7270 | 0.8394  | 1.3860  | 0.3524 |
| 10.50             | 3.5804 | 4.0821 | 2.7929  | 3.5704 |         | 3.5064  | 0.5323 |
| 15.00             | 2.6551 | 2.6935 | 2.5427  | 2.8746 | 2.4695  | 2.6471  | 0.1553 |
| 16.08             | 2.8007 | 2.6093 | 2.6102  | 2.3610 |         | 2.5953  | 0.1803 |
| 17.75             | 2.1434 | 2.0672 | 2.1659  | 2.2845 | 2.2505  | 2.1823  | 0.0868 |
| 18.75             | 1.9054 | 1.8123 | 1.6647  | 2.0308 | 1.6830  | 1.8192  | 0.1539 |
| 23.92             | 1.6655 | 1.5337 | 1.4117  | 1.4390 | 0.4938  | 1.3087  | 0.4663 |
| 28.50             | 1.1952 | 1.0741 | 0.9095  |        |         | 1.0596  | 0.1434 |
| 40.00             | 0.9843 | 0.9125 | 0.9732  | 0.9788 | 1.0152  | 0.9728  | 0.0374 |
| 46.00             | 1.0107 | 0.9103 | 0.9095  | 0.7807 |         | 0.9028  | 0.0942 |

Supplementary Table 2: Measurement of plasma  $^2\text{H}_3\text{-KIC}$  enrichment from a subset of the patients in this study.

|                  | Hours | $^2\text{H}_3\text{-KIC}$<br>TTR | $^2\text{H}_3\text{-KIC}$<br>MPE |
|------------------|-------|----------------------------------|----------------------------------|
| <i>Patient 2</i> |       |                                  |                                  |
|                  | 0     | 0.0000                           | 0.00                             |
|                  | 4.5   | 0.1114                           | 10.02                            |
| <i>Patient 3</i> |       |                                  |                                  |
|                  | 0     | 0.0000                           | 0.00                             |
|                  | 3     | 0.0634                           | 5.96                             |
| <i>Patient 4</i> |       |                                  |                                  |
|                  | 0     | 0.0000                           | 0.00                             |
|                  | 2.167 | 0.0772                           | 7.17                             |
| <i>Patient 5</i> |       |                                  |                                  |
|                  | 0     | 0.0000                           | 0.00                             |
|                  | 2     | 0.0559                           | 5.29                             |
| <i>Patient 8</i> |       |                                  |                                  |
|                  | 0     | 0.0000                           | 0.00                             |
|                  | 2.5   | 0.0510                           | 4.85                             |