Supplementary Material Text

The breath sample's CO_2 content correlates with oxygen uptake by mice.

We investigated determinants of the net amount of carbon dioxide in the collected sample by a new analysis of raw data that was acquired as part of the previous study of infected mice [1]. In brief, male adult BALB/c with the severe combined immunodeficiency mutation (C.B-17/Icr-*Prkdc*^{scid}/IcrIcoCrl from Charles River Laboratories) were infected with *Borrelia hermsii* strain CC1 on day 0 and then on days 4 and 5 breath samples were collected as described in the Methods section from mice in groups of 3. Quantitative polymerase chain reaction assays demonstrated that bacteremia had reached its peak on those days. The results for carbon monoxide and carbon dioxide are shown for all collections in the lower panel of Figure 3 of the previous study [1]. The analysis of the gases in the sample also included determination of the oxygen content of the sample using the same thermal conductivity detector that was used for CO_2. Oxygen (O_2) concentration was expressed as percent of total volume. There were two groups of uninfected mice and four groups of infected mice for the collections on day 4; in one of the groups of infected mice a mouse became moribund and was euthanized. The group had only two remaining mice and this basis was excluded from the measurements on day 5. For the present analysis we took the mean of the two determinations for each group of mice each day and then included the results for two days. The coefficients of determination (R^2) for the values between days 4 and 5 the groups that were sampled both days were 0.74 for O_2 and 0.65 for CO_2, and the corresponding paired, 2-tailed *t*-test *p* values were 0.76 and 0.46, respectively.

Figure S1 shows the direct linear relationship between uptake of oxygen by the mice during the sampling period and the total concentration of CO2 in the collected

sample by group of mice and condition. Oxygen uptake was estimated by subtracting the mean total O_2 content in the sample from the mean of duplicate blank O_2 determinations for the same day and time period. The mean O_2 concentration of the blank was 21.01% on day 4 and 20.99% on day 5. There was high correlation between the two gas determinations (ANOVA $F_{1,9}$ = 411; $p < 10^{-5}$). We interpreted this as evidence that for both infected and uninfected mice the CO_2 content of the collected sample provided an estimate of respiration. This linear relationship between the two parameters was not affected by the infection status. When the dependent variable was CO_2 content, the regression coefficient (95% confidence interval) for infection status as the independent variable was 0.016 (-0.151 to +0.184; p = 0.81).

Reference.

1. Barbour AG, Hirsch CM, Ghalyanchi Langeroudi A, et al. Elevated carbon monoxide in the exhaled breath of mice during a systemic bacterial infection. PLoS One 2013; 8:e69802.

Supplementary Material Figure Legend

Figure S1. Least-squares linear regression of total carbon dioxide (CO_2) in % on oxygen uptake, expressed as difference between blank and collection % volume O_2 values, for samples of collected breath from mice infected with *B. hermsii* and uninfected mice in groups of 3. The experiment is detailed in Barbour et al. [1]. The present analysis includes the results of O2 content determinations. The coefficient of determination (R^2) is shown. The inset indicates the symbols for status of infection.
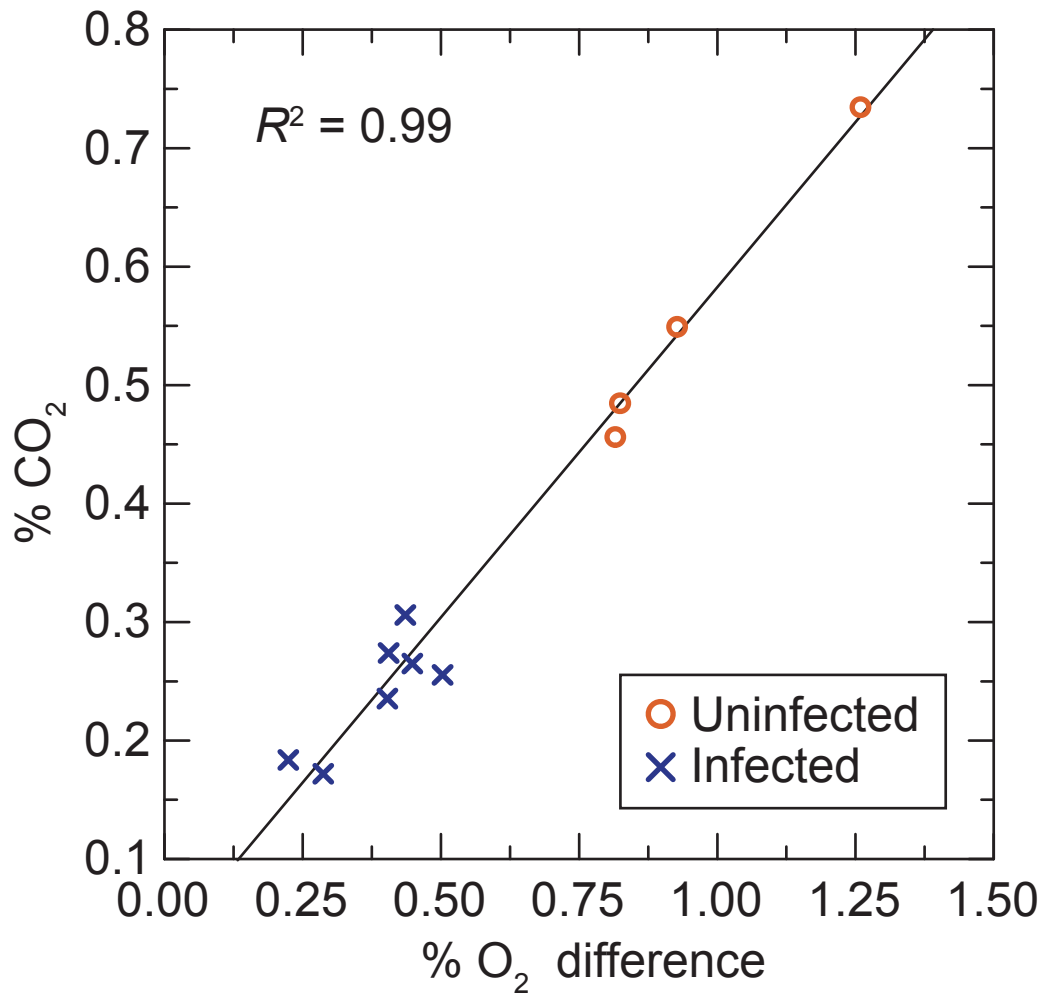
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