# Supporting Information

Wang *et al*. 10.1073/pnas.0801777105

## Main Experiment

### Materials and Methods

**Mouse Urine and Blood Collection.** Urine and blood collections were carried out at the same time throughout the study, i.e., between 0900 hours and 1100 hours, to avoid diurnal variations in urinary metabolites. At least 20 $\mu$l of urine were directly collected in a Petri dish by gently rubbing the abdomen of the mouse and transferred into small tubes kept on dry ice and stored at −40°C pending NMR analysis. Blood was collected from the tail of the mouse after cutting off its tip with scissors. At each time point, ≈40 $\mu$l of tail blood was collected into hematocrit tubes (Sodium [Na] heparin) and centrifuged at 4,000 × g for 4 min, using a model 1–15 centrifuge from Sigma (Osterode am Harz, Germany) The plasma was transferred into small Eppendorf tubes and stored at −40°C pending NMR analysis.

After the final urine and plasma collection time point (day 33 postinfection), mice were killed, and the liver and kidney were removed and transferred in small Eppendorf tubes containing 10% formalin. Sections were cut and stained with hematoxylin/eosin before examination by light microscopy.

Five microliters of mid-stream blood was also collected into an Eppendorf tube containing 20 $\mu$l of sodium-citrate from infected mice for determination of parasitemia. After thoroughly mixing, 5 $\mu$l of the homogenate was placed on a microscope slide and covered with a square-shaped cover slide (15 × 15 mm). The parasites were counted under a microscope at ×200 magnification using a counting grid. Based on the size of the grid, the thickness of the blood film and the dilution factor (5-fold), the parasitemia could be calculated.

**$^1$H NMR Spectroscopy.** Urine samples were prepared by mixing 20 $\mu$l of urine with 30 $\mu$l of phosphate buffer (pH 7.4) containing 50% $D_2O$ as a field frequency lock and 0.05% sodium 3-(trimethylsilyl) propionate-2,2,3,3-d4 (TSP) as a chemical shift reference ($\delta$ 0.0). Plasma samples were prepared by adding 30 $\mu$l of saline containing 50% $D_2O$ in an Eppendorf tube containing ≈20 $\mu$l of plasma. Samples were then transferred into 1.7-mm microNMR tubes using a microsyringe. All $^1$H NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer operating at 600.13 MHz. $^1$H NMR spectra of both urine and plasma were acquired with a Bruker 5-mm triple resonance probe with inverse detection employing the pulse sequence [RD-90°-$t_1$-90°-$t_m$-90°-ACQ]. $t_1$ was set to 3 $\mu$s and mixing time, $t_m$, was 150 ms. Water suppression was achieved via irradiation of the water peak during the recycle delay (RD = 2 s) and mixing time. The 90° pulse length was adjusted to ≈10 $\mu$s. A total of 256 transients were collected into 32,000 data points for each spectrum with a spectral width of 20 ppm. For plasma, an additional water-suppressed Carr-Purcell-Meiboom-Gill (CPMG) (1) spectrum was acquired for each sample using the pulse sequence [RD-90°-($\tau$-180°-$\tau$)$_n$-ACQ]. This acts as a $t_2$ relaxation filter to suppress signals from macromolecules and other molecules with constrained molecular motions. A total spin-spin relaxation delay (2n$\tau$) of 160 ms was used for all samples and water signal irradiation was applied during the relaxation delay. All free induction decays (FIDs) were multiplied by an exponential function equivalent to a 0.3 Hz line-broadening factor before Fourier transformation.

Metabolites were assigned from the literature (2) and from 2-dimensional (2D) NMR spectra including $^1$H-$^1$H COSY, TOCSY, and $^1$H J-resolved NMR spectra acquired for a selection of plasma sample using parameters described in previous studies (3).

### Results

**Histology and Parasitaemia.** From the main experimental cohort of mice, the numbers of trypanosomes were estimated under a microscope at five different time points postinfection (days 7, 14, 21, 28, and 33). On average, at days 7 and 33 postinfection, 115,300 and 164,900 trypanosomes were estimated per $\mu$l of blood from infected mice, respectively. There was a significant decrease in parasitemia from day 7 to day 14 postinfection (average: 41,300 parasites/$\mu$l blood), followed by a steady increase thereafter. However, we observed significant variation of parasitemia from one animal to another, and between different time points.

No significant differences in bodyweight were observed between the control and infected mice. One mouse died before day 28 postinfection, and 3 died before the end of the experiment (day 33 postinfection).

On termination, control mice showed no abnormalities in cellular architecture in either the liver or kidney. All infected animals manifested marked hepatic extramedullary hemopoiesis and renal interstitial nephritis (Fig. S1).

**Metabolic Changes Associated with *T. brucei brucei* Infection.** O-PLS-DA was carried out to characterize the metabolic features of *T. brucei brucei* infection. The key metabolic changes are summarized in Tables 1 (urine) and 2 (plasma) in the main text. An example coefficient plot obtained from the comparison of NMR spectra of preinfection and day 14 postinfection is shown in Fig. S2*A*. The O-PLS-DA coefficients plot can be considered as a difference spectrum between the samples at different time points. The direction of the signals corresponds to the relative concentrations of metabolites in one class (preinfection) compared with the other class (postinfection). The colors of the signal in the O-PLS-DA coefficient plot are associated with the significance of metabolites in separating the classes based on the correlation matrix, and the values can be obtained from the color scale on the right-hand side of the plot. Red denotes a more significant contribution to the separation beween classes than blue. A selected O-PLS-DA coefficient plot comparing plasma from the control and infected group at day 14 is shown in Fig. S2*B*.

**NMR Spectra of Plasma.** Plasma metabolic profiles showed alterations already 1 day postinfection. Elevated levels of lactate, alanine, branched chain amino acids, and acetylglycoprotein fragments were noted.

A systematic change in concentrations of valine and alanine were observed since the ratio of valine to alanine may report on the status of increased ketogenesis in the infected mice. Therefore, the valine to alanine ratio was measured by integration of the valine and alanine resonances in the plasma spectra and plotted in Fig. S3. The line plot showed a significant and enduring decrease in this ratio occurred from day 7 postinfection onwards.

**NMR Spectra of Urine.** Urinary concentrations of several microbial co-metabolites (e.g., hippurate) indicated a change of microbiotal activity.

**Metabolic Changes over Time.** Significant metabolic changes over time, associated with *T. brucei brucei* infection were detected in

plasma, by plotting averaged spectra of the infected animals in a series of time points on a PCA time trajectory. An additional satellite experiment was performed, based on the assumption, that the time-dependent shift was due to the parasite crossing the blood–brain barrier and aimed to specify the exact time point of this happening.

## Satellite Experiment

### Rationale

The rationale was to establish the time at which *T. brucei brucei* were established in the brain after experimental infection, to identify any metabolic signature specifically relating to this event.

### Materials and Methods

To correlate the changes in urinary and plasma metabolite profiles with disease stage and progression, an additional experiment was carried out to investigate the time at which the trypanosomes were established in the brain. A satellite group of 48 female mice (NMRI strain), aged ≈3 weeks, were acclimatized for 3 days. Half of the mice were infected i.p. with ≈20,000 *T. brucei brucei* (GVR 35 strain) each.

Infected mice were then split into three equal-sized groups ($n = 8$). At day 7 (group 1), day 14 (group 2), and day 21 (group 3) postinfection, mice were tested for parasitemia. All eight mice in group 1 were parasitemic, and therefore they were treated i.p. with 20 mg/kg diminazene aceturate (Sigma, Buchs, Switzerland). This drug at the dosage administered is known to kill *T. brucei brucei* parasites in the bloodstream, but the drug does not cross the blood–brain barrier. At day 10 postinfection, i.e., 3 days after the administration of diminazene aceturate, mice in group 1 were killed. The skull of each mouse was opened and the brain was removed and placed in a 15-ml Falcon tube filled with 10 ml of a PBS with glucose. The brain was homogenized and centrifuged for 10 min at 1,000 rpm to reduce the brain tissue into a pellet. The supernatant was taken and, sequentially, centrifuged for another 10 min at 4,000 rpm. Any trypanosomes present in the brain would now be concentrated in the pellet. Next, 0.5 ml of the pellet was injected i.p. into a new mouse. This procedure was carried out for each mouse in group 1, and a new group of mice (designated group A) was formed. The same procedures were followed for mice in groups 2 and 3 on days 14 and 21 after the initial infection, respectively, and designated groups B and C were formed. At days 4, 7, and 14 after mice in groups A–C were injected brain homogenate, they were examined for parasitemia.

### Results

From the satellite group of mice injected with brain homogenate from *T. brucei brucei*-infected animals, four mice died over the course of the experiment before administration of diminazene aceturate. Our temporal examination revealed that *T. brucei brucei* were established in the brain within 7 days, as all of the mice injected with brain homogenate of this first assessment time developed parasitemia as monitored over a 14-day course following injection of brain homogenate (Table S1).


1. Meiboom S, Gill D (1958) Modified spin-echo method for measuring nuclear relaxation times. *Rev Sci Instrum* 29:688–691.
2. Nicholson JK, Foxall PJD, Spraul M, Farrant RD, Lindon JC (1995) 750-MHz $^{1}$H and $^{1}$H-$^{13}$C NMR spectroscopy of human blood-plasma. *Anal Chem* 67:793–811.
3. Wang YL, *et al.* (2004) Metabonomic investigations in mice infected with *Schistosoma mansoni*: An approach for biomarker identification. *Proc Natl Acad Sci USA* 101:12676–12681.

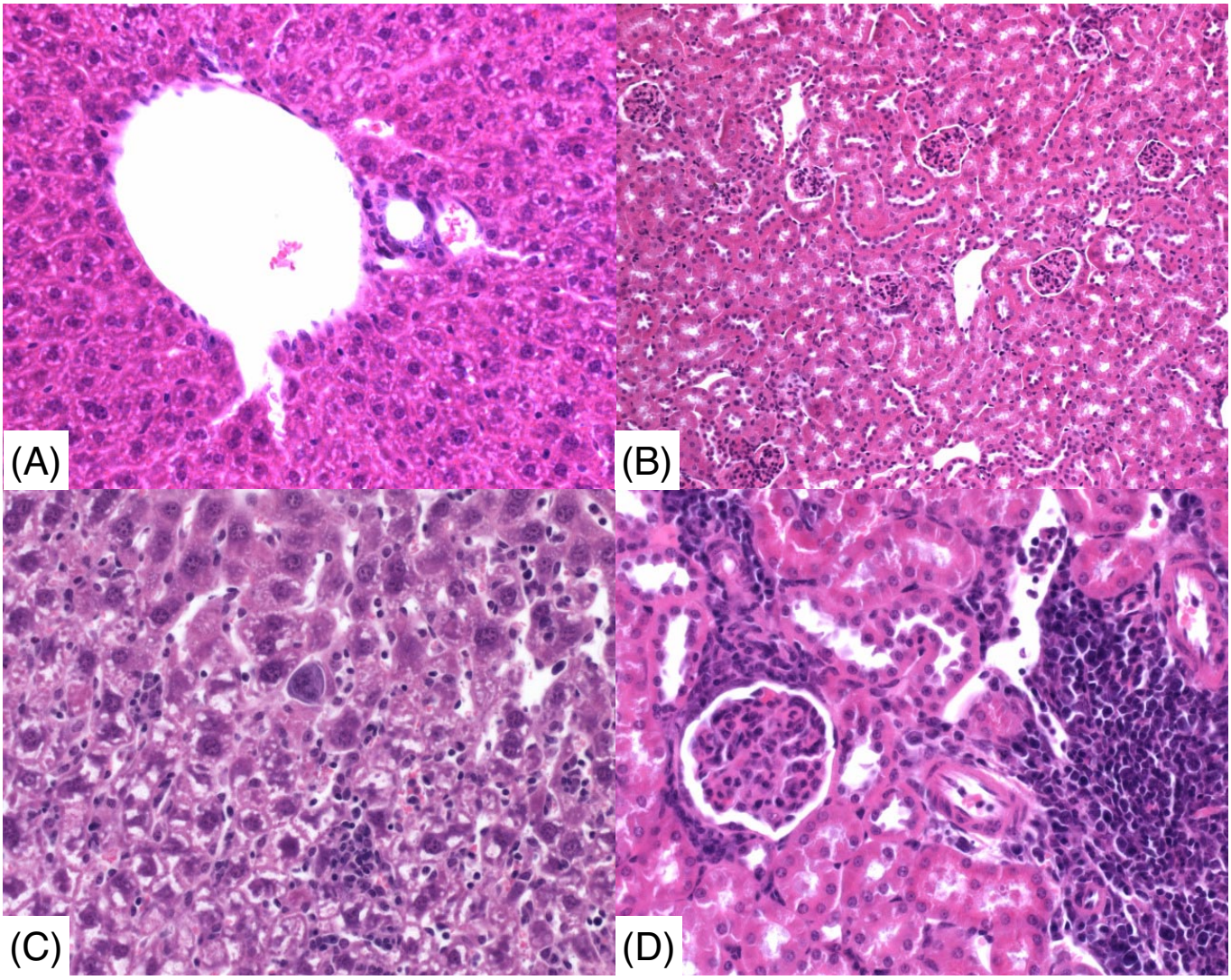


**Fig. S1.** Sections of liver (*A*) and kidney (*B*) from uninfected control mice. Histopathological alterations were seen in *T. brucei brucei*-infected mice both in the liver, i.e., hemopoiesis (*C*), and kidney, i.e., interstitial nephritis (*D*).

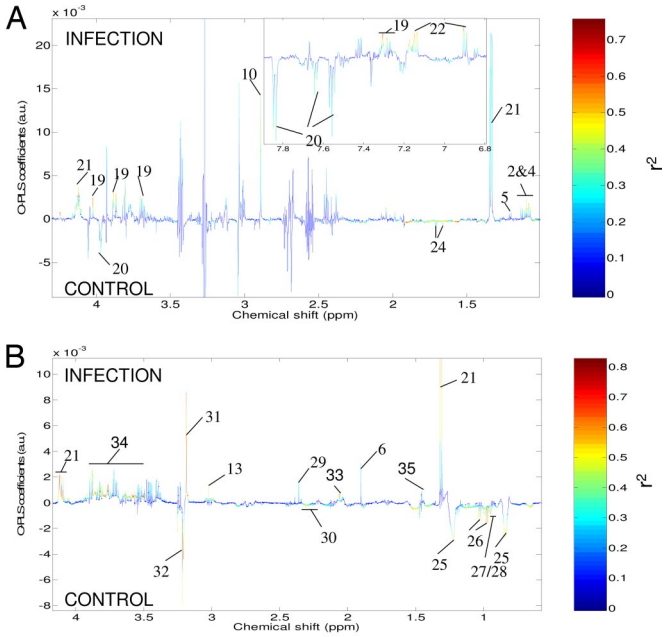


**Fig. S2.** Back-transformed coefficient plot calculated from O-PLS-DA modeling of the standard 1D NMR spectra of urine (*A*) and plasma (*B*) obtained from the preinfected mice, and at day 14 postinfection.

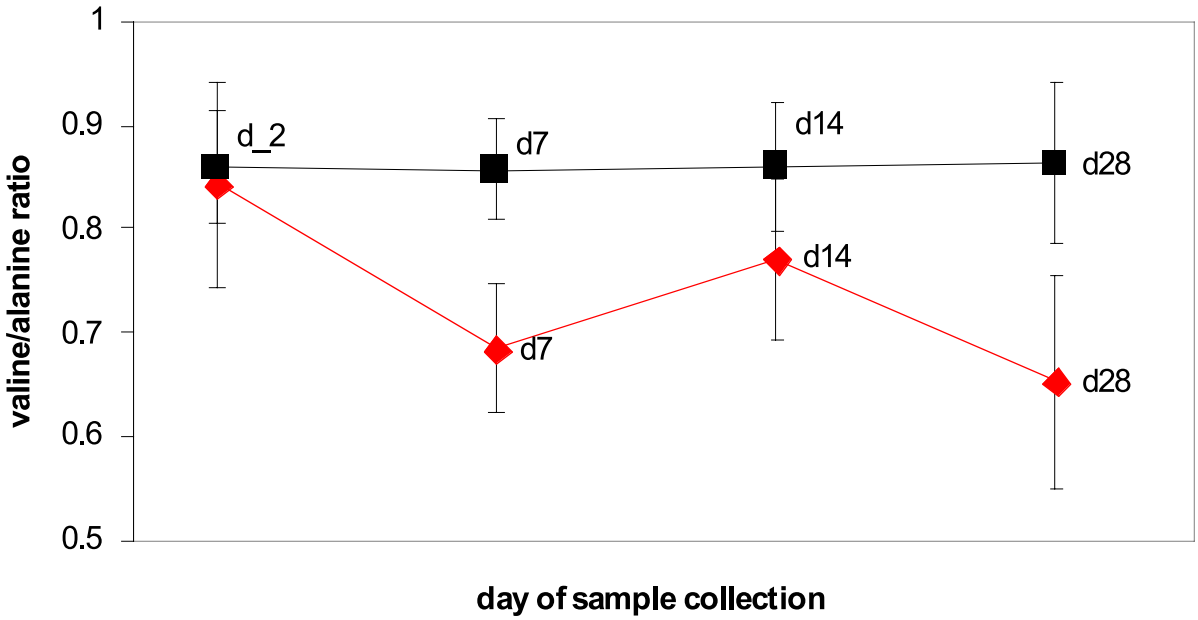


**Fig. S3.** Valine to alanine ratio for the control (black box) and *T. brucei brucei*-infected (red diamond) groups of mice calculated from the 1D CPMG NMR spectra of plasma.

**Table S1. Number of mice with parasitemia/number of mice examined at three different time points**

| | Days after injection of brain | | |
|---|---|---|---|
| | 4 | 7 | 14 |
| Group A (injected with brain from mice in group 1 that had been infected with *T. brucei brucei* for 7 days) | 4/8 | 8/8 | 7/8 |
| Group B (injected with brain from mice in group 2 that had been infected with *T. brucei brucei* for 14 days) | 6/7 | 7/7 | 5/7 |
| Group C (injected with brain from mice in group 3 that had been infected with *T. brucei brucei* for 21 days) | 5/5 | 4/5 | 5/5 |