Metabolic profiling of a *Schistosoma mansoni* infection in mouse tissues using magic angle spinning-nuclear magnetic resonance spectroscopy

Jia V. Li\textsuperscript{a,b}, Elaine Holmes\textsuperscript{a}, Jasmina Saric\textsuperscript{a,b}, Jennifer Keiser\textsuperscript{c}, Stephan Dirnhofer\textsuperscript{d}, Jürg Utzinger\textsuperscript{b}, Yulan Wang\textsuperscript{e,*}

\textsuperscript{a}Department of Biomolecular Medicine, Division of Surgery, Oncology, Reproductive Biology and Anaesthetics (SORA), Faculty of Medicine, Imperial College, London, UK

\textsuperscript{b}Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland

\textsuperscript{c}Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, Basel, Switzerland

\textsuperscript{d}Institute of Pathology, University Hospital Basel, Basel, Switzerland

\textsuperscript{e}State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Centre for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, The Chinese Academy of Sciences, Wuhan 430071, People's Republic of China

\textsuperscript{*}Corresponding author. Address: State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Centre for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Wuhan 430071, People’s Republic of China. Tel.: +86 27 8718 7143; fax: +86 27 8719 9291.

\textsuperscript{e-mail address:} yulan.wang@wipm.ac.cn (Y. Wang).

**A R T I C L E  I N F O**

Article history:
Received 9 July 2008
Received in revised form 13 October 2008
Accepted 14 October 2008

Keywords:
Chemosurveillance
Magic angle spinning
Metabolic profiling
Mouse
NMR spectroscopy
Schistosomiasis
*Schistosoma mansoni*

**A B S T R A C T**

In order to enhance our understanding of physiological and pathological consequences of a patent *Schistosoma mansoni* infection in the mouse, we examined the metabolic responses of different tissue samples recovered from the host animal using a metabolic profiling strategy. Ten female NMRI mice were infected with ~80 *S. mansoni* cercariae each, and 10 uninfected age- and sex-matched animals served as controls. At day 74 post infection (p.i.), mice were killed and jejunum, ileum, colon, liver, spleen and kidney samples were removed. We employed \textsuperscript{1}H magic angle spinning-nuclear magnetic resonance spectroscopy to generate tissue-specific metabolic profiles. The spectral data were analyzed using multivariate modelling methods including an orthogonal signal corrected-projection to latent structure analysis and hierarchical principal component analysis to assess the differences and/or similarities in metabolic responses between infected and non-infected control mice. Most tissues obtained from *S. mansoni*-infected mice were characterized by high levels of amino acids, such as leucine, isoleucine, lysine, glutamine and asparagine. High levels of membrane phospholipid metabolites, including glycerophosphoryl choline and phosphocholine were found in the ileum, colon, liver and spleen of infected mice. Additionally, low levels of energy-related metabolites, including lipids, glucose and glycogen were observed in ileum, spleen and liver samples of infected mice. Energy-related metabolites in the jejunum, liver and renal medulla were found to be positively correlated with *S. mansoni* worm burden upon dissection. These findings show that a patent *S. mansoni* infection causes clear disruption of metabolism in a range of tissues at a molecular level, which can be interpreted in relation to the previously reported signature in a biofluid (i.e. urine), giving further evidence of the global effect of the infection.

\textcopyright 2008 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Schistosomiasis is a complex of acute and chronic infections caused by a digenetic trematode flatworm of the genus *Schistosoma* that is widespread in tropical and sub-tropical environments (Gryseels et al., 2006; King and Dangerfield-Cha, 2008). An estimated 779 million individuals are at risk of schistosomiasis and more than 200 million are infected, yet schistosomiasis is often neglected (Steinmann et al., 2006). Clinical symptoms of acute and chronic schistosomiasis include abdominal pain, anorexia, nausea and diarrhoea, caused by the disturbance of gastrointestinal motility (Rogers et al., 2000; De Man et al., 2002). Enlargement of liver and spleen typically occurs in chronic cases (Ross et al., 2001). The pathogenesis of schistosomiasis is largely due to inflammatory reactions caused by trapped schistosome eggs. An adult female *Schistosoma mansoni* worm, for example, produces ~300 eggs per day (Moore and Sandground, 1956). Whilst about half of the eggs are excreted via faeces, the remaining eggs are trapped in the intestine and liver where they trigger a host immune response and subsequently cause granuloma formation around the eggs, which ultimately leads to fibrosis in these tissues (Boros, 1989; El-Garem, 1998; De Man et al., 2002; Ross et al., 2002; Gryseels et al., 2006).

The pathology of the intestine, liver and spleen due to a *Schistosoma* spp. infection, both in experimental animals and humans, has been investigated by histology, ultrasonography and magnetic resonance imaging (Freitas et al., 1999; Rogers et al., 2000; De Man et al., 2002; Ross et al., 2002; Gryseels et al., 2006).
infection with either $S. mansoni$ or $S. japonicum$ in mouse and hamster respectively. The urinary metabolic fingerprint in different tissue samples obtained from mice with a patent $S. mansoni$ infection and compared the metabolite profiles with those recovered from non-infected control mice.

2. Materials and methods

2.1. Host-parasite model

All experiments were carried out at the Swiss Tropical Institute (Basel, Switzerland), adhering to local and national guidelines and laws of experimental work with laboratory animals (Permission No. 2081). A total of 20 female mice (NMRI strain), aged ~3 weeks, were purchased from RCC (Itingen, Switzerland). Mice were randomly housed into four cages of five mice each and individually marked. Mice were acclimatized for 2 weeks, hence, at the onset of the experiment mice were ~5 weeks old, weighing between 17.3 and 27.3 g. Mice were kept under environmentally-controlled conditions (temperature, 22 °C; relative humidity, 60–70%; light/dark cycle, 12/12 h) and were fed on rodent pellets (Nafag; Gossau, Switzerland) and water ad libitum. Half of the mice were infected s.c. with ~80 $S. mansoni$ cercariae each. The remaining mice were left uninfected, and hence served as controls.

2.2. Tissue sample collection

Mice were killed by cervical dislocation on day 74 p.i. Control animals were killed at the same time point. Three types of intestinal tissues were collected, namely jejunum, ileum and colon. Small samples (~5 mm) were obtained from the middle part of each tissue in order to minimise variation due to possible topographical differences. Intestinal tissues were washed three times with PBS before freezing. Additionally, a small portion of the liver (~15 mg) was obtained from the edge of the left lobe, a small spleen sample (~5 mm$^3$) was cut from the distal part of the spleen, and the left kidney was removed from each mouse. Tissue samples were transferred into cryo-tubes, labelled with unique identifiers, immersed in liquid nitrogen and stored at ~80 °C pending spectroscopic analyses.

2.3. Histology and $S. mansoni$ worm burden

For histological investigations, the right kidney and a small portion of the spleen were obtained and transferred in Eppendorf tubes containing 4% formalin. Tissue samples were cut, stained with H & E and then examined under a light microscope. The results from tissue samples obtained from $S. mansoni$-infected mice were compared with those recovered from non-infected control animals.

In the infected mice, the $S. mansoni$ worm burden was quantified upon dissection by examination of the liver and the mesenteric veins surrounding the intestine, as described elsewhere (Utzinger et al., 2002).

2.4. $^1H$ MAS-NMR spectroscopic analysis of tissue

The kidney was separated into renal cortex and medulla, and samples of ~15 mg were taken from each region. Each sample was isolated and packed into a 4 mm outer diameter zirconia rotor (Bruker Analytische GmbH; Rheinstetten, Germany) with saline D$_2$O as the field lock. $^1H$ NMR spectra were acquired on a 600 MHz Bruker DRX spectrometer (Rheinstetten, Germany), operating at 600.13 MHz, equipped with a triple-resonance MAS probe. The spin rate of the rotor was regulated at 5000 Hz at 283 K. A $^1H$ NMR spectrum was acquired from each sample with Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence [relaxation delay (RD)-90°-($\tau$–180°–$\tau$)$_s$-acquire free induction decay (FID)], in order to attenuate signals from macromolecules with a short spin–spin relaxation time. A spin relaxation delay (2 $\pi t$) of 200 ms was used. The 90° pulse length was adjusted to 10 μs. The spectral width was 20 ppm and 128 transients were collected into 32 k data points. An exponential function was applied to the FID prior to Fourier transformation with a line broadening factor of 0.3 Hz.

Two-dimensional (2D) $^1H$–$^1H$ correlation spectroscopy (COSY) and $^1H$–$^1H$ total correlation spectroscopy (TOCSY) NMR spectra were acquired from selected samples for structural assignment of unknown metabolites. A total of 80 transients and 256 increments were collected into 2 k data points with a spectral width of 10 ppm. COSY and TOCSY data were multiplied by an unshifted sine-bell and a shifted sine-bell apodization function, respectively, prior to Fourier transformation.

2.5. Data reduction and multivariate data analysis

$^1H$ MAS-NMR spectra obtained from intestinal tissues were manually phased and corrected for baseline distortions. The spectra were referenced using the signal from anomeric $\alpha$-glucose proton at δ 5.223. The entire spectrum (δ 0.0–10.0) was digitized into ~40,000 data points with a resolution of 0.0005 ppm using an in-house developed MATLAB script (version 7.0). The region between δ 4.70 and δ 5.20 was removed in order to minimize the effect of the imperfect baseline caused by the water suppression. In addition, regions δ 0.0–0.3 and δ 9.0–10.0 containing only noise were removed.

et al., 2002; Shen et al., 2002; Bezerra et al., 2007; Lambertucci et al., 2008). Although histological techniques can offer visualization of differences between healthy and infected individuals, they fail to provide information at the molecular level, which might be important for an enhanced understanding of physiological and pathological consequences of a schistosome infection.

Metabonomic strategies find increasing application in systems biology, as they provide insight into metabolic responses of whole organisms to environmental, genetic and other factors at the molecular level, such as physiological and pathological stimuli, and drug and natural product interventions (Nicholson et al., 1999, 2002; Brindle et al., 2002; Lindon et al., 2004; Wang et al., 2005b; Schlotterbeck et al., 2006). Metabonomics typically employs high-resolution nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS), coupled with multivariate data analysis to explore and interpret complex spectroscopic data derived from biofluid or tissue samples. One of the advantages of NMR-based metabolic profiling is that small intact tissue samples can also be analysed with the aid of magic angle spinning (MAS). In MAS the major line broadening factors such as dipole–dipole interactions, NMR chemical shift anisotropy and magnetic field inhomogeneities are attenuated, thus producing $^1H$ NMR spectra comparable to those observed in the solution state NMR spectroscopy. MAS can therefore provide a metabolic link between histology and metabolic profiling of biological samples, and generate useful information on the metabolic consequences or mechanisms of external stimuli (Cheng et al., 1997; Waters et al., 2001).

A metabolic profiling strategy has been successfully applied to the investigation of altered urinary metabolites induced by an infection with either $S. mansoni$ or $Schistosoma japonicum$ in mouse and hamster respectively. The urinary metabolic fingerprint included a stimulated glycolysis process, reduced levels of the tricarboxylic acid cycle intermediates, altered amino acid metabolism and disturbance of the population and/or activities of gut microbiota (Wang et al., 2004, 2006). In order to relate the metabolic signature of a $S. mansoni$ infection to functional and structural changes in host tissues, and to obtain a deeper metabolic characterization of the infection, we examined the metabolic responses of different tissue samples obtained from mice with a patent $S. mansoni$ infection and compared the metabolite profiles with those from non-infected control mice.
Normalization to the total area of the remaining NMR spectrum was performed prior to multivariate data analysis. A supervised multivariate data analysis tool, i.e. orthogonal projection to latent structure-discriminant analysis (O-PLS-DA) (Trygg and Wold, 2002), was applied to the analysis of $^1$H NMR spectral data in a MATLAB environment. O-PLS-DA models were constructed using NMR data as the X matrix and the infection status (i.e. no infection versus infection) as the dummy Y matrix. One PLS component was calculated for each model with one orthogonal component using mean-centred data scaled to unit variance. The validation of the model was conducted using a sevenfold cross validation, i.e. iterative construction of models by repeatedly leaving out one-seventh of the samples and, subsequently, predicting them back into the model. The total explained variation for the X matrix is indicated by the goodness of fit ($R^2$) value and the corresponding cross-validation parameter is expressed as $Q^2$.

We used a multiblock hierarchical principal component analysis (H-PCA) and a hierarchical PLS-DA (H-PLS-DA) as a method for comparing several blocks (i.e. several PCA models from multiple tissues) derived from the same ‘objects’ (e.g. host animal). This method is ideally used for analysing variable-rich datasets (Westerhuis et al., 1998; Eriksson et al., 2004). Our data were divided into seven blocks according to the type of tissue samples (i.e. jejunum, ileum, colon, liver, spleen, renal medulla and renal cortex) obtained from the same mouse. A PCA model was constructed for the NMR data of each tissue type. All meaningful scores ($t_b$) from each PCA model (as determined using the optimal goodness of prediction ($Q^2$)) value) were combined into one super block. T. PCA or PLS-DA was then performed on T with $t_b$ denoted as variables and samples denoted as observations. The resulting super scores ($t_T$) plot shows the relationship between observations, and the super loading plot ($p_T$) indicates which scores ($t_b$) are most influential on the H-PCA/H-PLS-DA model, and hence facilitates visualization of differences and/or similarities in the metabolic responses of multiple tissues or organs.

3. Results

3.1. Gross observation, histology and S. mansoni worm burden

The S. mansoni worm burden in mice was assessed 74 days p.i. with ~80 cercariae each. On average, 36 worms (SD = 7) were found in each mouse. There was no significant difference in the mean body weight between non-infected control and S. mansoni-infected mice over the course of the entire experiment. Two mice in the infected group died at days 67 and 70 p.i.

Upon dissection of mice, thicker and rougher intestinal surface, enlargement of spleen and liver was observed in all S. mansoni-infected animals. Histological examination of spleen and kidney samples obtained from uninfected control mice showed normal cellular architecture (Fig. 1A and B). Spleen samples obtained from S. mansoni-infected mice showed pronounced lymphohistiocytic hyperplasia with markedly increased neutrophilic granulocytes (Fig. 1C), including splenic microabscesses. With the exception of one kidney characterized by pyelonephritis (Fig. 1D), most likely due to sepsis, the kidneys of the remaining S. mansoni-infected mice appeared normal.

3.2. $^1$H MAS-NMR spectra of intact tissue samples

Representative $^1$H MAS-NMR CPMG spectra from tissues (jejunum, ileum, colon, liver, spleen, renal cortex and renal medulla) from control mice are shown in Fig. 2 and provide a comprehensive description of tissue-specific low molecular weight components comprising these tissues. A number of metabolites, including a range of amino acids, acetate, creatine, glycerophosphoryl choline (GPC), choline, uracil, glucose, fumarate, inosine, phosphocholine (PC), nicotinurate, ascorbate, lipids and scyllo-inositol were identified based on 2D $^1$H NMR spectra and literature information (Fan, 1996; Wang et al., 2005a). Our findings concur with previous publications characterizing the metabolic profiles of the intact tissues investigated here. The chemical shift and peak multiplicities of all identified metabolites in each tissue type are listed in Table 1.

3.3. Multivariate data analysis of $^1$H MAS-NMR CPMG tissue spectra

Metabolic changes in each tissue type induced by a S. mansoni infection were established using an O-PLS-DA strategy, comparing $^1$H NMR CPMG spectra obtained from infected mice and corresponding controls. The total explained variation for the X matrix ($R^2$) and the corresponding cross-validation parameter ($Q^2$) for each model are listed in Table 2. Clear separation was achieved between samples obtained from non-infected controls and S. mansoni-infected mice for all tissues examined, as evidenced by the consistently high $Q^2$ values for all models.

The cross-validated scores plots depicting the distribution of samples based on their metabolic profiles are shown in Figs. 3 and 4 (left-hand side) together with the corresponding O-PLS-DA coefficient plots (right-hand side). These coefficient plots indicate the key metabolites giving rise to differentiation due to an infection with S. mansoni. Upward oriented peaks in the O-PLS-DA coefficient plots denote a relative high levels of metabolites in the infected group, whereas downward oriented peaks indicate a low levels of the metabolites. The colours on the plots represent the significance of metabolites in differentiation between S. mansoni-infected and non-infected control mice; red indicating a strong covariation with the infection, and blue indicating no or only little relation with the infection.

Table 2 summarizes those metabolites that were significantly correlated with a S. mansoni infection and their corresponding coefficients. An infection with S. mansoni induced similar metabolic effects on the jejunum and ileum, which included high levels of amino acids, such as glutamine, lysine, methionine, phenylalanine and tyrosine. Differential effects between these two tissues included low levels of choline, fumarate and scyllo-inositol in the jejunum and low levels of lipids together with relatively high levels of creatine, GPC, inosine, PC and xanthine in the ileum of S. mansoni-infected mice. Marked alterations in metabolic profiles of colon tissue samples were also observed, including high levels of GPC and PC, and a low level of scyllo-inositol in the infected mice.

The liver and spleen of S. mansoni-infected mice manifested a similar metabolic response, characterized by low levels of glucose and lipids, and high levels of amino acids, including alanine, asparagine, creatine, glutamine, glycine, lysine, methionine, phenylalanine and proline. Moreover, relative levels of ascorbate, fumarate and pyruvate were found to be higher in the livers of infected mice. The S. mansoni infection-induced changes in the metabolic profiles of both the renal medulla and renal cortex were characterized by high levels of ascorbate, asparagines, glutamine and lysine, and markedly low levels of choline and scyllo-inositol. Additionally, levels of ethanolamine and inosine were found to be lower in renal cortex obtained from infected mice.

3.4. Correlation of $^1$H NMR CPMG spectral data with S. mansoni worm burden

An O-PLS model was constructed using NMR spectra obtained from tissues as X matrix and S. mansoni worm burden as the Y matrix to enhance the recovery of metabolic features associated with severity of infection. One predictive component with one orthogonal component was calculated for each model. The results are summarized in Fig. 5, showing that there was a significant
correlation of the metabolite profiles for jejunum, liver and renal medulla with actual worm burden. The quality of the models, as expressed by $Q^2_Y$ and $R^2_X$, are summarized in Table 3. Generally, levels of various amino acids in these tissues were positively correlated with worm burden, whereas levels of glucose were positively associated with worm burden in renal medulla and jejunal tissues, but showed a negative association in liver tissue. In addition, glycogen levels in the liver and membrane component metabolites in the renal medulla, e.g. choline and GPC, were negatively related to *S. mansoni* worm burden.

3.5. PCA and H-PCA of tissue data

In order to assess the nature of the metabolic response to infection across several tissues, PCA models were constructed individually for each biological matrix and the significant scores from each model combined into a single new matrix to include all tissues. A total of three principal components were calculated for unit variance-scaled data obtained from jejunum and ileum and two principal components for the liver, renal cortex, renal medulla, spleen and colon. The number of components was decided by the point where $Q^2_Y$ reached a maximum value before declining. For ileum, the PCA model explained two-thirds of the total variance in the data matrix. More than 50% of the total variance was explained by PCA models for jejunum (62.8%) and liver (52.0%). Considerably lower percentages of the total variance were explained by PCA models for colon (45.7%), spleen (44.9%), renal cortex and renal medulla; i.e. 37.6% and 31.9%, respectively, indicating a more variable or less coherent response than in the ileum, jejunum and liver.

The scores ($t_b$) values for each model were combined into a ‘super block’ ($T$) and then PCA was performed on the super block with unit variance-scaled data, which is termed H-PCA (Westerhuis et al., 1998). A total of two principal components were calculated for the H-PCA model with 50.8% of total variance being explained. The H-PCA scores plot (Fig. 6A) showed a clear separation between the *S. mansoni*-infected and the non-infected control group of mice along the first principal components. To maximize the separation, a PLS-DA model was calculated for the same unit variance-scaled ‘super block’ dataset by using class information (i.e. infected and non-infected control) as the $Y$ variable, with two PLS components. The H-PLS-DA scores plot (Fig. 6B, $Q^2_Y = 0.94$) also showed clear discrimination between the infected and the non-infected control group along the first principal components. The corresponding loadings plot (Fig. 6C) represented associations between scores ($t_b$) (e.g. relating to the tissues which were most metabolically disrupted by the infection). The intestinal tissues, most clearly defined by the first ($t_{[1]}$) and third ($t_{[3]}$) components for the jejunum, the second ($t_{[2]}$) component for ileum and colon and the first ($t_{[1]}$) component of renal medulla were the highest positively weighting in the model and therefore, most discriminatory for the infected tissues. The first ($t_{[1]}$) component of the liver, spleen and ileum, and the second ($t_{[2]}$) component of the renal cortex exerted the most significant influence on the position of the control profiles in the scores plot. In order to investigate the key metabolic changes in each tissue, the corresponding loading plots of these important scores ($t_b$) were plotted (Figs. 6D–L). *S. mansoni*-infected mice showed higher levels of alanine, GPC, isoleucine, leucine, PC and valine in jejunum, ileum and colon, and
higher levels of ascorbate and glycine in renal medulla, whereas control mice manifested higher levels of glucose and lipids in the ileum, liver and spleen tissues, together with higher concentrations of glycogen in the liver.

4. Discussion

We have previously characterized the urinary metabolic profiles of mice infected with S. mansoni for 49 and 56 days. Preceding in vitro studies revealed that oviposition in S. mansoni occurs after 34–35 days (Clegg, 1965), and hence our previous findings pertain to metabolic changes due to a patent S. mansoni infection. In brief, characteristic metabolic changes included a stimulated glycolysis process, reduced levels of the tricarboxylic acid cycle intermediates, a metabolic disturbance of amino acids and systematic variation in the level of microbiota-related metabolites (Wang et al., 2004). Here, we extended our metabolic profiling strategy from urine to various tissue samples (i.e. liver, kidney, spleen, jejunum, ileum and colon) employing the same host-parasite model in order to probe the etiology of the observed changes in urine and to relate the changes in the urinary metabolic profile to potential mechanism of pathology. In addition, we have also employed an H-PCA analysis so as to efficiently connect information from multiple compart-
ments (e.g. different types of tissues). In the current study, the infection duration was extended to 74 days in order to ensure that overt pathology had been established based on literature reports.

Visual inspection of intestinal tissues revealed that the surface of the intestine in S. mansoni-infected mice appeared to be thicker and rougher than in non-infected control mice. These observations are consistent with previous studies in mice that noted a diffuse thickening of the smooth muscle layers of the intestine and disturbance of the architectural structure of the mesenteric plexus and the enteric nervous system due to a patent S. mansoni infection (Balembo et al., 2000, 2001; Bogers et al., 2000; De Man et al., 2002; De Jonge et al., 2003b). Higher levels of membrane phospholipid metabolites, such as GPC and PC found in the ileum and colon of infected mice in the current study may also be associated with structural changes in the intestinal architecture. Hence, high levels of membrane phospholipid metabolites may be responsible for cellular swelling, leading to the accumulation of intracytoplasmic fluids in cells and cellular overgrowth, as previously reported (Blennnerhassett et al., 1992; Bogers et al., 2000). For example, a recent study showed a transient increase of mast cells in mucosa in mice, reaching a peak value at 8 weeks p.i. (De Jonge et al., 2003a). Further effects on the intestinal structure can result from which cause granulomous inflammation and lead to tissue fibrosis (Lukacs et al., 1993; Weinstock, 1996). Intestinal fibrosis is particularly common in the distal ileum and the proximal colon (Weinstock, 1996) and is associated with structural changes including thickening of the ileal wall and a decrease in gastrointestinal transit, which could explain the elevated levels of GPC and PC observed in the ileum and colon from S. mansoni-infected mice in the current study. Biochemical changes associated with inflammation triggered by the S. mansoni infection appeared to be similar to those caused by chronic bowel diseases. For example, biopsies of colon

<table>
<thead>
<tr>
<th>Metabolites (key)</th>
<th>δH (multiplicity)*</th>
<th>Tissuesb</th>
</tr>
</thead>
<tbody>
<tr>
<td>leucine (1)</td>
<td>-CH; 1/2-CH2; 1/2-CH2; γ-CH2; δ-CH2; δ' -CH2</td>
<td>3.72(t); 1.96(m); 1.69(m); 0.91(d); 0.94(d)</td>
</tr>
<tr>
<td>iso-leucine (2)</td>
<td>-CH; 1/2-CH2; 1/2-CH2; γ-CH2; δ-CH2; δ' -CH2</td>
<td>3.68(d); 1.93(m); 1.25(m); 1.47(m); 0.99(d); 1.02(d)</td>
</tr>
<tr>
<td>valine (3)</td>
<td>-CH; 1/2-CH2; γ-CH2; γ'-CH2</td>
<td>3.62(d); 2.28(m); 0.99(d); 1.04(d)</td>
</tr>
<tr>
<td>lactate (4)</td>
<td>-CH; 1-CH2</td>
<td>4.11(q); 1.32(d)</td>
</tr>
<tr>
<td>threonine (5)</td>
<td>-CH; 1/2-CH2; γ-CH2</td>
<td>3.59(d); 4.27(m); 1.32(d)</td>
</tr>
<tr>
<td>alanine (6)</td>
<td>-CH; 1-CH2</td>
<td>3.77(q); 1.47(d)</td>
</tr>
<tr>
<td>lysine (7)</td>
<td>-CH; 1-CH2; γ-CH2; δ-CH2; ε-CH2</td>
<td>3.78(t); 1.92(m); 1.72(m); 1.47(m); 3.03(t)</td>
</tr>
<tr>
<td>arginine (8)</td>
<td>-CH; 1-CH2; γ-CH2; δ-CH2</td>
<td>3.76(t); 1.92(m); 1.65(m); 3.24(t)</td>
</tr>
<tr>
<td>acetate (9)</td>
<td>CH3</td>
<td>1.91(s)</td>
</tr>
<tr>
<td>proline (10)</td>
<td>-CH; 1/2-CH2; 1/2-CH2; γ-CH2; δ-CH2</td>
<td>4.13(t); 2.08(m); 2.37(m); 2.01(m); 3.38(t)</td>
</tr>
<tr>
<td>glutamate (11)</td>
<td>-CH; 1-CH2; γ-CH2</td>
<td>3.76(m); 2.07(m); 2.34(m)</td>
</tr>
<tr>
<td>methionine (12)</td>
<td>-CH; 1-CH2; γ-CH2; δ-CH2</td>
<td>3.87(m); 2.14(m); 2.63(t); 2.13(s)</td>
</tr>
<tr>
<td>glutamine (13)</td>
<td>-CH; 1-CH2; γ-CH2</td>
<td>3.78(m); 2.15(m); 2.45(m)</td>
</tr>
<tr>
<td>aspartic acid (14)</td>
<td>-CH; 1-CH2</td>
<td>3.89(m); 2.69(m); 2.80(m)</td>
</tr>
<tr>
<td>asparagine (15)</td>
<td>-CH; 1-CH2</td>
<td>4.01(m); 2.87(m); 2.95(m)</td>
</tr>
<tr>
<td>ethanolamine (16)</td>
<td>NH-CH2; HO-CH2</td>
<td>3.14(t); 3.82(t)</td>
</tr>
<tr>
<td>glycine (17)</td>
<td>-CH</td>
<td>3.55(s)</td>
</tr>
<tr>
<td>creatine (18)</td>
<td>N-CH3; CH2</td>
<td>3.03(s); 3.92(s)</td>
</tr>
<tr>
<td>GPC (19)</td>
<td>N=(CH3); γ-CH2; β-CH2; α-CH3; β'-CH; γ'-CH2</td>
<td>3.22(s); 4.32(t); 3.68(l); 3.61(dd); 3.90(m); 3.72(dd)</td>
</tr>
<tr>
<td>choline (20)</td>
<td>N=(CH3); γ-CH2; β-CH2</td>
<td>3.20(s); 4.07(t); 3.52(t)</td>
</tr>
<tr>
<td>taurine (21)</td>
<td>N-CH2; SO2-CH2</td>
<td>3.43(t); 3.27(t)</td>
</tr>
<tr>
<td>serine (22)</td>
<td>-CH; 1-CH2</td>
<td>3.84(m); 3.96(m)</td>
</tr>
<tr>
<td>uracil (23)</td>
<td>5-CH; 6-CH</td>
<td>5.80(d); 7.52(d)</td>
</tr>
<tr>
<td>tyrosine (24)</td>
<td>2.6-CH; 3.5-CH</td>
<td>7.18(d); 6.88(d)</td>
</tr>
<tr>
<td>phenylalanine (25)</td>
<td>2.6-CH; 1.5-CH; 4-CH; half Ar-CH2; half Ar-CH2; NCH</td>
<td>7.40(m); 7.13(m); 7.35(m); 3.17(dd); 3.30(dd); 3.99(dd)</td>
</tr>
<tr>
<td>α-glucose (26)</td>
<td>1-CH; 2-CH; 3-CH; 4-CH; half 6-CH2; half 6-CH2</td>
<td>5.24(d); 3.56 (dd); 3.70(t); 3.40(t); 3.83(m); 3.72(dd); 3.85(m)</td>
</tr>
<tr>
<td>β-glucose (27)</td>
<td>1-CH; 2-CH; 3-CH; 4-CH; half 6-CH2; half 6-CH2</td>
<td>4.65(d); 3.25 (dd); 3.47(t); 3.40(t); 3.47(dd); 3.78(dd); 3.85(dd)</td>
</tr>
<tr>
<td>deoxyuridine (28)</td>
<td>5-CH; 6-CH</td>
<td>5.88(d); 7.78(d)</td>
</tr>
<tr>
<td>fumarate (29)</td>
<td>CH</td>
<td>6.52(s)</td>
</tr>
<tr>
<td>inosine (30)</td>
<td>2-CH; 8-CH; 2'-CH; 4'-CH; 5'-CH; CH2i(2); CH2i(2)</td>
<td>8.34(s); 8.24(s); 6.10(d); 4.44(t); 4.28(q); 3.92(dd); 3.85(dd)</td>
</tr>
<tr>
<td>PC (31)</td>
<td>N=(CH3); 8-CH2; N-CH2; PO2-CH3</td>
<td>3.22(s); 3.61(m); 4.25(m)</td>
</tr>
<tr>
<td>lipids (32)</td>
<td>-CH3; (CH3)=; -CH2CH2CO; -CH2CH2CO; CH2C=CH2; CH2C=CH2; CH==CH=CH=CH</td>
<td>0.89(bs); 1.29; 1.59; 2.25; 2.03; 2.77; 5.32</td>
</tr>
<tr>
<td>icylo-inositol (33)</td>
<td>6x- CH</td>
<td>3.33(s)</td>
</tr>
<tr>
<td>xanthine (34)</td>
<td>CH</td>
<td>7.96(s)</td>
</tr>
<tr>
<td>cytidine (35)</td>
<td>5-CH; 6-CH</td>
<td>6.09(d); 7.85(d)</td>
</tr>
<tr>
<td>formate (36)</td>
<td>CH</td>
<td>8.45(s)</td>
</tr>
<tr>
<td>nicotinamide (37)</td>
<td>2-CH; 6-CH; 4-CH; 5-CH</td>
<td>8.92(s); 8.70(d); 8.24(d); 7.6(d); 3.99(s)</td>
</tr>
<tr>
<td>ascorbate (38)</td>
<td>half CH2; half CH2; CHO; CH; α-CH</td>
<td>3.76(d); 3.74(d); 4.03(dd); 4.52(d)</td>
</tr>
<tr>
<td>TMAO (39)</td>
<td>3x- CH</td>
<td>3.28(s)</td>
</tr>
<tr>
<td>pyruvate (41)</td>
<td>CH3</td>
<td>2.38(s)</td>
</tr>
<tr>
<td>glycerol (42)</td>
<td>γ-CH2; γ-CH2; β-CH</td>
<td>3.56(dd); 3.64(dd); 3.87(m)</td>
</tr>
<tr>
<td>glycogen (43)</td>
<td>CH</td>
<td>5.40(d)</td>
</tr>
</tbody>
</table>

PC, phosphorylcholine; TMA, trimethylamine; TMAO, trimethylamine-N-oxide.

* s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet; dd, double doublet; ddd, double doublet; bs, broad singlet.

b A, all; J, jejunum; I, ileum; C, colon; L, liver; S, spleen; K, kidney.
Enteric inflammation is commonly accompanied by disorders of the gastrointestinal tract. Khan and Collins (2006) demonstrated the association between inflammatory response and alteration in intestinal muscle contractility in a Trichinella spiralis-rat model. Hypercontractility can physically lead to a higher level of breakdown of proteins, facilitated by the assistance of enzymes from the liver and intestine (Kazura et al., 1981; Abdallahi et al., 2001). It is known that a S. mansoni infection induces free radicals in the liver and intestine (Kazura et al., 1981; Abdallahi et al., 1999), which leads to oxidation of lipids. Alternatively, oxidation of lipids can also provide energy for intestinal motility. Our observation was high levels of creatine were observed in the ileum of mice infected with S. mansoni after 74 days compared with non-infected mice. Phosphorylcreatine serves as an energy store and, together with ADP, is transferred into creatine and ATP via creatine kinase (CK) reaction. CK activity is higher in the ileum of mice infected with S. mansoni, especially in the ileum. This requirement of energy could be met via creatine metabolism and lipid oxidation. Indeed, relatively high levels of creatine were observed in the ileum of mice infected with S. mansoni compared with non-infected control mice. Phosphorylcreatine serves as an energy store and, together with ADP, is transferred into creatine and ATP in order to replenish the energy requirement (De Jonge et al., 2001). In the current study, lower levels of lipids were found in the intestine of S. mansoni-infected mice, especially in the ileum. It is known that a S. mansoni infection induces free radicals in the liver and intestine (Kazura et al., 1981; Abdallahi et al., 1999), which leads to oxidation of lipids. Alternatively, oxidation of lipids can also provide energy for intestinal motility. Our observation of a significant positive relationship between worm burden and energy-related metabolites such as glucose, lysine, glutamine and tyrosine underscores the high energy requirement of hypercontractility of the small intestine of S. mansoni-infected mice.
Furthermore, taurine was present in higher levels in the ileum, liver and spleen of infected mice, an observation that is in contrast to the decreased levels of taurine found in the ileum of rats in other helminth infections such as after infection with *Hymenolepis diminuta* (Novak et al., 1993). Taurine is a multifunctional metabolite and has been shown to have antioxidant (Das et al., 2008) and anti-inflammatory effects (Son et al., 1998) and is also known to be involved in several Ca^{2+}-dependent mechanisms, such as muscular contractility, control of cell volume and tissue osmolality (Huxtable, 1992; Martin et al., 2006). Therefore, elevated levels of taurine in the infected mice may play a protective role against free radical damage and cell expansion caused by inflammation. Interestingly, taurine conjugated bile acids such as taurocholic, tauroursodeoxycholic acid have been shown to increase egg production of *S. mansoni* in *in vitro* systems (Badr et al., 1999), which may relate to a host-parasite evolutionary strategy.

It is widely acknowledged that a *S. mansoni* infection causes inflammation of the liver and eventually leads to liver fibrosis, a crucial feature of chronic schistosomiasis in humans (Lambertucci et al., 2000). Liver fibrosis – regardless of its cause – is often associated with the deposition of extracellular matrix proteins, including collagen and a number of glycoproteins (Maher and McGuire, 1990). We found that levels of free proline, a precursor of hydroxyproline, as well as glycine and alanine, which are major amino acids in the synthesis of collagen, were elevated in the liver of infected mice, which might imply stimulated collagen synthesis activity. Previous *in vitro* investigations also proved that collagen synthesis by fibrotic liver slices grew as the concentration of free proline was increased in the medium (Dunn et al., 1977). The high level of free proline could be partially attributed to the production of *S. mansoni* eggs as shown in a previous study documenting a distinct increase in the proline production-involved enzyme activities of ornithine-δ-transaminase and Δ¹-pyrroline-5-carboxylic acid reductase in *S. mansoni* eggs (Isseroff et al., 1983). Furthermore, a high level of ascorbate and a reduced level of lipid were found in the liver of infected mice. Ascorbate is a reducing agent and plays an important role in free radical damage. The reduced levels of lipid could be due to the oxidation damage caused by free radicals generated in the infection and the oxidation of lipid could also be an important energy source when glucose and glycogen are largely depleted as observed in the liver and spleen in the current study. Alternatively, Cho and colleagues (2001) reported that the ratio of glutamine and glutamate complex to lipid increased as the severity of liver fibrosis progressed to the later stage, which is consistent with our results of higher levels of glutamine together with the lower concentration of lipids in the liver from infected mice. An egg granulomatous-induced liver fibrosis not only results in a structural alteration (Silva et al., 2000), but also causes functional alteration, such as decreased oxidative deamination of amino acids and a disturbance in ammonia metabolism (Daugherty et al., 1994). Previously, a decreased expression of enzymes related to the citric acid cycle, the fatty acid cycle, the urea cycle and amino acid metabolism and catabolism has been shown in mice 8 weeks p.i. with *S. mansoni* (Harvie et al., 2007). Here, a low level of glyco-
gen was found in the liver of *S. mansoni*-infected mice, which might be explained by either the inhibition of glycogen synthesis or an enhanced glycogen breakdown. It has been reported that glycogen phosphorylase for glycogenolysis was suppressed in mice due to an infection with *S. mansoni* (Ahmed and Gad, 1995). Moreover, stimulated glycolysis in *S. mansoni*-infected mice has been previously reported (Wang et al., 2004). Therefore, it is likely that the infection or infection-induced host responses lead to both the inhibition of the glycogen synthesis and stimulation of the glycogen breakdown, resulting in the depletion of the glycogen level in the liver recovered from *S. mansoni*-infected mice. Independent enzymatic assay on activities of glycogen phosphorylase and UDP-glucose pyrophosphorylase would verify whether the reduction in levels of glycogen is caused by both processes simultaneously or dominated by only one of these processes.

*Schistosoma mansoni*-induced inflammation is also reflected in an enlargement of the spleen, which was confirmed in our study by gross observation upon dissection of mice and further underscored by histological examination. Spleen enlargement is hypothesized to result from passive congestion of portal hypertension and/or cellular hyperplasia (Ross et al., 2001). Biochemical features associated with a *S. mansoni* infection included higher concentrations of glutamine, coupled with lower levels of lipids and glucose in the spleen of infected mice. Glutamine is an essential energy source in mitochondria and can also be utilised by lymphocytes in the spleen from infected mice. Glutamine is an essential energy source in mitochondria and can also be utilised by lymphocytes (Klimberg and McClellan, 1996; Yaqoob and Calder, 1997). Therefore, the high level of glutamine may indicate a hyperactive immune system triggered by *S. mansoni* eggs trapped in the tissue. Glycolysis is also carried out in lymphocytes and macrophages in order to meet the energy requirement of a stimulated immune system. As a consequence, the low level of glucose and the high level of lactate would be expected, consistent with our observations in the spleen of infected mice.

An epidemiological investigation carried out among schoolchildren in an area highly endemic for schistosomiasis found that renal disease was uncommon (Johansen et al., 1994). On the other hand, although neither the adult worms nor the eggs of *S. mansoni* directly caused a pathogenic effect in the kidney of hamsters, there was an immunological response at 6 weeks p.i., characterized by the deposition of immune complex and renal amyloidosis (Sobh et al., 1991). In our study, we found high levels of glutamine, GPC and choline in the renal medulla of *S. mansoni*-infected mice, which were positively

---

**Fig. 4.** Cross-validated score plots (left) and orthogonal projection to latent structure-discriminant analysis (O-PLS-DA) coefficient plots (right) derived from 1H NMR CPMG spectra of liver (A), spleen (B), renal cortex (C) and renal medulla (D) from non-infected (blue) and *Schistosoma mansoni*-infected mice (red). (For key of metabolites, see legend to Fig. 1 and Table 1).
correlated with worm burden upon dissection of mice. Glutamine and GPC have been reported to have osmotic function in the brain and inner kidney, respectively (Miller et al., 2000). These observations may indicate a counterbalance response and dysfunction of the kidney due to the parasitic infection. However, with the exception of one mouse, the cellular architecture in the kidney of the remaining S. mansoni-infected mice appeared normal.

Metabolic effects were observed in various intestinal tissues, liver, spleen and kidney of mice with a patent S. mansoni infection as revealed by a metabolic profiling strategy. Our findings indicate both localized and remote effects of the infection. We observed evidence of inflammation and hypercontractility of the intestine, damage to the liver and kidney and enlargement of the spleen in infected mice. These findings demonstrate that the integrated multivariate strategy employed provide a holistic approach to metabolic effects due to a S. mansoni infection in host organs at the molecular level and indicate a coordinated response in several tissues in response to the infection. Further work on enzymatic activities of glycogen phosphorylase and UDP-glucose pyrophosphorylase as well as total collagen content in the liver of S. man-

### Table 3

Metabolites that were correlated with Schistosoma mansoni worm burden upon dissection of mice, and $Q^2_Y$ and $R^2_X$ of each model from jejunum, liver and renal medulla.

<table>
<thead>
<tr>
<th>Metabolites (key)</th>
<th>O-PLS plots</th>
<th>Liver</th>
<th>Renal medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$Q^2_Y = 0.61$; $R^2_X = 0.46$</td>
<td>$Q^2_Y = 0.40$; $R^2_X = 0.41$</td>
<td>$Q^2_Y = 0.45$; $R^2_X = 0.35$</td>
</tr>
<tr>
<td>$b$-glucose (27)</td>
<td>+0.87</td>
<td>-0.54</td>
<td>+0.69</td>
</tr>
<tr>
<td>lysine (7)</td>
<td>+0.70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>glutamine (13)</td>
<td>+0.64</td>
<td>-</td>
<td>+0.85</td>
</tr>
<tr>
<td>$a$-glucose (26)</td>
<td>+0.86</td>
<td>-0.67</td>
<td>-</td>
</tr>
<tr>
<td>tyrosine (24)</td>
<td>+0.61</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>alanine (6)</td>
<td>-</td>
<td>+0.70</td>
<td>-</td>
</tr>
<tr>
<td>GPC (19)</td>
<td>-</td>
<td>-</td>
<td>-0.81</td>
</tr>
<tr>
<td>choline (20)</td>
<td>-</td>
<td>-</td>
<td>-0.67</td>
</tr>
<tr>
<td>glycogen (43)</td>
<td>-</td>
<td>-0.79</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 5. O-PLS plots derived from $^1$H magic-angle-spinning Carr–Purcell–Meiboom–Gill nuclear magnetic resonance (MAS CPMG NMR) spectra of jejunum (A), liver (B) and renal medulla (C) from Schistosoma mansoni-infected mice using worm burden as the class descriptor ($Y$ matrix).
soni-infected mice should provide in-depth understanding of the aetiology of the disease.

Acknowledgements

The authors thank Dr. Jacques Chollet for infection and dissection of mice, and Dr. O. Cloarec for access to MATLAB scripts for analysis of NMR data. This study received financial support from the Swiss National Science Foundation (J.V. Li, J. Saric and J. Utzinger, Project Nos. PPOOB–102883 and PPOOB–119129; J. Keiser, Project No. PPOOA–114941), Deputy Rector’s Award at Imperial College London (J.V. Li) and The Chinese Academy of Science (Y. Wang, KJXC2-YW-W11).

References


