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Metabolic responses to water deprivation in C57BL/6J mice using a proton nuclear magnetic resonance-based metabonomics approach

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Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCAAs, branched chain amino acids; BW, body weight; FFAs, free fatty acids; GC-MS, gas chromatography-mass spectrometry; HCA, hierarchical cluster analysis; ^{1}H NMR, proton nuclear magnetic resonance; MAPK, mitogen-activated protein kinase; NAA, *N*-acetylaspartic acid; NEFA, non-esterified fatty acid; NMR, nuclear magnetic resonance; PLS-DA, partial least squares discriminant analysis; PVN, paraventricular nucleus; RAS, rennin-angiotensin system; ROS, reactive oxygen species; SD, Sprague Dawley; TP, total protein; UPLC-MS/MS, ultra performance liquid chromatography-tandem mass spectrometric; VIP, variable importance in the projection.

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Abstract

Water deprivation can occur in isolated conditions by a natural disaster or under normal living conditions. However, the mechanism underlying response or adaption to water deprivation in mammals is not fully understood. This study was undertaken to unravel this mechanism. Male C57BL/6J mice were treated without or with water deprivation for four time points (i.e., 1, 2, 3, and 4 days) before sacrifice. In four days of trial, no mice given with or without drinking water experienced any mortality. Body weight, serum total protein, albumin, and trans-aminase activity were determined at 24-h intervals for 96 h. During dehydration, mice body weight decreased consistently. Moreover, non-biased proton nuclear magnetic resonance (^{1}H NMR)-based metabonomics strategy was performed to evaluate the effects of water deprivation on responses of systemic hepatic metabolites in male C57BL/6J mice during a 96-h period. Water deprivation stress caused metabolic disturbance and changes in hepatic metabolites that are involved in the metabolisms of carbohydrates, lipids and amino acids. Under water deprivation stress, lactate and 3-hydroxybutylate might be the main energy sources. Increased taurine and branched chain amino acids (BCAAs) might confer tolerance and adaptability to mice under dehydration stress. Moreover, the increased free fatty acids (FFAs) caused by water deprivation probably contributed to counteracting dehydration related osmolality fluctuations. These results also reveal that the metabonomics strategy is a powerful tool to gain insight into the molecular mechanism of cellular response to environmental stresses.

Keywords: Water deprivation, hepatic metabolites, metabonomics, proton nuclear magnetic resonance, multivariate analysis

Introduction

Water availability is probably the primary factor for determining the distribution of terrestrial organisms on the land.[1] Water accounts for about 60-70% of the total body weight and is a major constituent of every cell from microbes to higher organisms. Water also plays a pivotal role in a wide range of basic physiological functions.[2] Dehydration may occur by excessive sweating, polyuria, severe diarrhea and hyperthermia.[3] Especially the older individuals and children are at a high risk of continued dehydration. Moreover, cancer patients, especially those with gastrointestinal tract cancer, are usually in a dehydrated state. Water deprivation may cause significant hormonal, physiological and biochemical changes in the body, and can impose stress on biomolecules, cells, and tissues.[4] A low water intake or dehydration might also disrupt mood and cognition,[2] cause renal[5] or liver dysfunction,[3] and affect the development of the rennin-angiotensin system (RAS) during pregnancy.[6] Moreover, dehydration state also influences the pharmacokinetics of therapeutic agents.[3,7] In the other hand, abstaining from water might also have beneficial effects on body, for example, water deprivation stimulated the release of endogenous vasopressin which can be used for the treatment of acute variceal bleeding.[8]

To survive the water deprivation, living organisms may regulate their molecular expression or concentration to response or adapt to the adverse environment. All living cells possess metabolic mechanisms that actively counteract the influences of hydration changes that otherwise threaten the integrity of the cell.[9] Some molecules or pathways have been reported to be correlated with the response to dehydration stress, such as ERK mitogen-activated protein kinase (MAPK) pathway signal transduction pathway.[4] Moreover, increased Ang Ⅱ in the brain can contribute to sustain systemic blood pressure under water deprivation stress;[10] increased glutamate uptake which might

restrict the dehydration induced activation of paraventricular nucleus (PVN) efferent pathways involved in activation of sympathetic outflow and release of neurohypophysial hormones in Sprague Dawley (SD) rats;[11] up-regulation of genes correlated with cellular recycling pathways and down-regulation of genes involved in general metabolism and ATP generation in larvae of *Belgica antarctica*, an Antarctic midge, in response to dehydration were detected by RNA sequencing.[12] The mechanism underlying the dehydration induced response or adaption is complicate and might involve many genes, proteins, metabolites and metabolic pathways. Although some progresses have been made, the responding or adapting molecular mechanisms are still poorly understood. Revealing metabolic responses to water deprivation by non-targeted global metabonomics approach can contribute to understand such molecular mechanisms.

Metabolomics or metabonomics strategy attempts to determine the dynamic multi-parametric metabolic changes of living systems to pathological states[13] or external stimuli[14] in an unbiased way to delineate the altered physiological states. Some works about the effect of drought or water loss on plants[15-18] or insects[12] using metabolomics strategy have been reported. For example, a non-targeted metabolomics analysis of drought acclimation in model and forage legumes was performed to understand mechanisms underlying the plants coping with water deprivation;[17] using GC-MS- and UPLC-MS/MS-based metabolomics platforms, metabolic profiling difference between desiccation-sensitive and desiccation-tolerant species of *Selaginella*, an ancient lineage of vascular plant, was also studied, and some compounds with critical roles in desiccation tolerance were determined.[18] These works demonstrated that the metabolomics or metabonomics strategy is a powerful tool to gain insight into the molecular mechanism of living organisms responded to dehydration. However, to date, no research result has been reported about using metabonomics

approach to elucidate the mechanisms of response or adaption to water deprivation in mammals. Nuclear magnetic resonance (NMR)-based metabonomics strategy can rapidly and noninvasively detect metabolic changes both *in vivo*[19] and *in vitro*[20] for the elucidation of mammal cellular response to water deprivation.

In this study, a proton NMR (^{1}H NMR)-based metabonomics strategy combined with multivariate data analysis were applied to determine the feasibility of detecting water intake restriction-associated global hepatic metabolite changes in male C57BL/6J mice, and to determine if the exact mechanism of response or adaption to water deprivation could be elucidated.

Materials and methods

Animals, treatments, and sample collection

Eight-week-old male C57BL/6J mice used in this study were purchased from Beijing Vital River Laboratory Animal Technology Co., LTD [animal license number: SCXK (Beijing) 2002-0003]. Animal breeding, care and all experiments were carried out in adherence to the Beijing animal experiment center guidelines. Male C57BL/6J mice were randomly divided into five groups according to the expected water fasting model establishment time, i.e., 0, 24, 48, 72, and 96 water deprivation hours, with five mice in the first four groups and six mice in the last group. For control mice (without water deprivation), food and water were supplied *ad libitum* throughout the study; for the model of dehydration, water was deprived for corresponding hours with free access to food. Body weights were recorded daily for each mouse. Trans-aminase activity, concentration of albumin and total protein (TP) contained in the serum were measured on a Hitachi 7170S automatic analyzer (Tokyo, Japan) every day throughout the study. After fasting overnight, mice

were killed by cervical dislocation at days 1, 2, 3, and 4, respectively for water deprivation groups, and at days 4 for control mice. Liver samples were took out immediately after killing the mice and stored at -80°C for ^{1}H NMR spectroscopic analysis.

Pretreatment of liver samples

Liver samples (0.25 g) were thawed, and homogenized in 2 mL of 50% acetonitrile prior to centrifugation (5070 g, 5 min). The supernatant was lyophilized to obtain liver tissue aqueous extracts; the pellet was resuspended in chloroform/methanol (3:1, v/v) solution (2 mL). The suspension was centrifuged at 5070 g for 5 min. The second pellet was dried by lyophilization to obtain liver tissue organic extracts.

^{1}H NMR spectroscopic analysis

Aqueous soluble liver tissue extracts were mixed with 550 μL of D_2O and 50 μL of 3-trimethylsilyl-[2,2,3,3-$^{2}H_4$]-propionate (TSP). Lipid-soluble liver tissue extracts were mixed with 450 μL of deuterochloroform ($CDCl_3$) and 150 μL of methanol-D (CD_3OD). Both aqueous and organic samples were centrifuged at 10000 g for 10 min to remove visible particles and then were placed into 5 mm NMR sample tubes. ^{1}H NMR spectra were measured at 600.13 MHz on a Bruker DPX-600 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) at a probe temperature of 300 K. TSP served as a chemical shift reference (δ 0.0) and D_2O provided a field-frequency lock signal for the NMR spectrometer.

Data processing and pattern recognition analysis

^{1}H NMR spectra were subdivided into 0.04 ppm designated regions, and each region was integrated. The generated integral intensities (variables) were imported into SIMCA package (Ver

10.0) (Umetrics, Umea, Sweden) for multivariate statistical analysis. Partial least squares discriminant analysis (PLS-DA) was carried out to the ^{1}H NMR data after mean centering and UV scaling, employing a default 7-fold internal cross validation, to discern control from water-deprived mice. A supervised PLS-DA was initially performed to obtain an overview of the ^{1}H NMR data from different water treated mice, and differences between the samples were detected in the PLS-DA score plots.

To specify the time-related metabolic variations, supervised PLS-DA pairwise comparison between control and water-deprived mice at each time point was performed. On the basis of loadings plots and variable importance in the projection (VIP) value threshold (VIP>1) from 7-fold cross-validated PLS-DA models, differential variables responsible for the distinguishing control and water-deprived mice groups were selected. Independent-Samples *t* test was performed on specific metabolites and their ratios to assess the statistical significance of the metabolic changes.

Disturbed metabolites elicited by water deprivation were extracted by PLS-DA models, and their geometrical means of all the biological repeats from each group were subjected for hierarchical cluster analysis (HCA) to assess the discriminability of PLS-DA models. HCA was performed using Euclidian distances and complete linkage grouping with the help of Gene Cluster 2.11, and visualized using TreeView 1.60.

Statistical analysis

Data were analyzed using SPSS13.0 software. Differences with *P*-values less than 0.05 were considered statistically significant.

Results and discussion

Water deprivation can occur in isolated conditions by a natural disaster,[21] such as earthquake etc., and under normal living conditions, such as polyuria, excessive sweating, severe diarrhea and hyperthermia.[3] There still remains the possibility that some particular populations may be at high risk of dehydration.[2] Dehydration could impair bodily functions[22] and attenuate aspects of basic physiology, and may cause significant hormonal, physiological and biochemical changes in the body.

Body weight changes induced by water deprivation

Water deprivation can cause a reduction in food intake[23] to prevent elevations in extracellular fluid osmolarity and sodium concentration.[21,24] In other words, to some extent, the reduction in food intake would alleviate the increased osmolarity. Lower food consumption combined with restriction to water can contribute to animal body weight loss after acute water deprivation. The outbred CD1 mice appearance and attitude began to deteriorate after 24 h acute water deprivation, and body weight loss exceeded 12%.[23] The body weight loss after 24 h water deprivation in mice (approximately 12%) is comparable to that detected in larger rodents including rats weighing about 10-fold more after 72 h water deprivation.[23,25] In this study, during water deprivation, mice body weight also decreased by approximately 12.7%, 19.1%, 22.8%, 23.4%, respectively, of their baseline body weight after 24, 48, 72, and 96 hours of water deprivation (P<0.001) (Fig. 1). However, though the appearance and attitude of C57BL/6J mice, declined beginning at 24 h, further deteriorated after 48 h of acute water deprivation, no mice died till 96 h of water deprivation.

Fig. 1

Changes of trans-aminase activity and concentration of TP and ALB contained in the serum induced by water deprivation

Restriction to water intake is associated with fluid reduction in both extracellular and intracellular compartments. During water deprivation, volume of circulating fluid including blood decreased, and plasma osmolality increased in mice that were denied access to water.[23] However, plasma osmolality would not continue to increase after the initial deprivation time point, most likely due to the loss of intracellular water.[23] The fluid change in the extracellular compartment was also reflected by increased serum TP and albumin at all four time points (i.e., 24, 48, 72, and 96 hours) (P<0.01) (Fig. 2a). Serum osmolality might be a sensitive index for indicating the changes in hydration status.[26] Moreover, fluid change in the intracellular environment could affect the activity of enzymes or hormones and finally influence the overall physiology of animals.[23] Changes in intra- or extra-cellular environment illustrated potential physiological distress.[23]

Fig. 2

Blood or urine chemistry data or microscopic examination of liver tissue illustrated that liver functions might be impaired in rats under water deprivation,[3] and tissue dysfunction might be due to the reduction in the organ perfusion rate.[21] In this study, both TP and albumin concentrations in water deprivation group mice increased significantly (P<0.01) with duration of water deprivation in comparison to control group mice (Fig. 2a), which was consistent with what reported by Bekkevold and his colleagues.[23] Intracellular dehydration can regulate the protein expression and nitrogen metabolism.[9] A loss of body water also induced an increase of serum content of TP and albumin in *Bos indicus* steers.[27] Water deprivation can decrease urinary excretion and promote the

accumulation of some protein waste products in the blood.[5] Albumin is synthesized in the liver and the most abundant protein in mammal blood. Serum proteins, especially albumin, can play roles as weak acids in blood and influence acid-base balance.[27] Increased total protein concentration caused by hemoconcentration secondary to dehydration would be a contributing factor for metabolic acidosis.[27] Moreover, the hypovolemia might result in a limited oxygen supply to tissues.[27,28] Increased lactate concentration measured by ^{1}H NMR (Fig. 3) also likely reflected a significant increase in liver anaerobic metabolism.

Fig. 3

Level of alanine transaminase (ALT) or aspartate transaminase (AST) did not show the similar change tendency with TP and albumin (Fig. 2b). Compared with control mice, mice deprived of water for 96 hours had higher level of AST (P<0.05), while mice in other water deprivation groups showed no significant increase of AST level (Fig. 2b). Moreover, compared with control mice, mice deprived of water for 48 and 72 hours had lower level of ALT (P<0.01), while mice in other water deprivation groups showed no significant change of ALT level (Fig. 2b).

Serum transaminase concentrations did not change in pigeons following 48 h of water deprivation, indicating no impairment in circulatory function.[29] In this study, the ALT concentration in all of subjects was in normal reference range of ALT for healthy male mice (26-70 U/L) (Fig. 2b); the AST concentration in all of subjects except mice deprived of water for 96 hours was in normal reference range of AST for healthy male mice (54-242 U/L) (Fig. 2b). Both serum ALT and AST concentrations did not exceed normal range before 72 h of deprivation, demonstrating no impairment in hepatic or circulatory function. However, compared with control mice, AST value increased remarkably (P<0.05) and exceeded the normal range following 96 h of

water deprivation, probably indicating impairment in hepatic or circulatory function after exposure to long time water deprivation. Above changes about body weight, serum proteins and trans-aminase activities preliminarily demonstrated potential physiological distress in C57BL/6J mice exposed to water deprivation.

Characterization of water deprivation on liver aqueous or organic extract metabolite profiles

Water deprivation can lead to significant physiological and biochemical changes *in vivo*.[3] Moreover, while encountering the hydration changes that would threaten the cell integrity, living cells may regulate their metabolic pathways to counteract such changes.[9] Liver is a metabolically active organ. Water deprivation could have systemic consequences and would induce a complex series of changes in liver metabolite levels. Changes of these disturbed metabolites could be detected by ^{1}H NMR-based metabonomics strategy for evaluating pathogenesis mechanism underlying the response or adaption to water deprivation.

Typical ^{1}H NMR spectra from control and water fasting mice liver aqueous or organic extracts were respectively shown in ESI Fig. S-1a and b, with metabolites indicated based on their chemical shifts. Partial least squares discriminant analysis (PLS-DA) scores plots were performed to represent the sample distribution in new multivariate space, on which the distinct clustering was observed between control and water-deprived mice (ESI Fig. S-2).

Changes of liver metabolites at each water fasting time point

To specify water deprivation-related liver aqueous or organic extract metabolic changes, PLS-DA pairwise comparison was performed. Control and water-deprived mice at all time points (i.e., 24,

48, 72, and 96 hours) for aqueous extract metabolites (ESI Fig. S-3a, c, e and g) or organic extract metabolites (ESI Fig. S-4a, c, e and g), were readily separated across the first component. The PLS-DA models were well constructed with excellent fit and satisfactory predictive power (ESI Table S-1). The major metabolic perturbations causing these clusters were determined from the loading plots of the first component of PLS-DA models (ESI Fig. S-3b, d, f and h) (ESI Fig. S-4b, d, f and h).

A metabolite with a variable importance in the projection (VIP) value greater than 1 demonstrates a significant contribution to the separation of groups within PLS-DA models.[30] The VIP plots demonstrated that some of identified metabolites contributed to the class separation (ESI Fig. S-5 and S-6). On the basis of the PLS-DA results with good pairwise discriminations between the control and water-deprived mice, a total of 28 differential aqueous compounds and some differential lipids were selected according to the cutoff VIP value (VIP>1).

The HCA plot of the identified differential metabolites reflected a clustering pattern that was similar to the PLS-DA model results (Fig. 4). The groups treated with short-term water deprivation (24 h and 48 h) were clustered and separated from the other groups; moreover, the groups treated with long-term water deprivation (72 h and 96 h) were also clustered and separated from the other groups (Fig. 4). This result was consistent with the PLS-DA models and also verified the predictive accuracy of the PLS-DA models.

Fig. 4

Restriction on water intake causes extracellular hyperosmotic state and then induces the decrease of the cellular volume which may regulate the intracellular metabolic pathways.[31] Compared with control mice, liver metabolisms in water-deprived mice were disturbed, especially

metabolisms of carbohydrates, amino acids, and lipids.

Water deprivation caused increase of plasma glycogen by 25% but no change on glucose in healthy men.[9] However, another report indicated that dehydration caused an increase of glucose content in the frog liver.[32] Cell shrinkage secondary to water deprivation can promote glycogenolysis and subsequent decrease of glycogen.[31] Moreover, though cell shrinkage can also promote glycolysis, the decrease of TCA cycle rate might contribute to the accumulation of glucose. In this study, compared with control mice, water-deprived mice at all four time points (i.e., 24, 48, 72, and 96 hours) had higher hepatic glucose level but lower hepatic glycogen level (Fig. 3).

Conversion from glucose to pyruvate can supply energy through TCA cycle. However, whole-body oxygen consumption decreases as the consequence of water deprivation-related hypoxic stress.[27,28] Tissue hypoxia secondary to hypovolemia induced more production of reactive oxygen species (ROS) synthesized directly by electron and oxygen. Limited oxygen supply and increased ROS can inhibit the TCA cycle and energy production through TCA cycle. To some extent, increased cis-aconitic acid in water-deprived mice (Fig. 3) probably also indicated the block of TCA cycle. To overcome ATP loss due to damage of mitochondria and inhibition of TCA cycle, lactate might be synthesized by water-deprived mice liver in large amount for rapid energy generation. Furthermore, liver can also provides ketone bodies (3-hydroxybutylate and acetoacetate) as the major energy source to extrahepatic tissues through β-oxidation or ketogenesis while blood glucose level is low after fasting.[33] In this study, hepatic 3-hydroxybutylate and lactate contents increased in water-deprived mice (Fig. 3) and possibly provided energy to extrahepatic tissues during water deprivation. However, contents of creatine and creatinine, which

can also supply energy to brain and heart, decreased in the mice that denied access to water (Fig. 3). These results demonstrated that lactate and 3-hydroxybutylate might be the main energy source during water deprivation. Moreover, more lactate and 3-hydroxybutylate contents made pH value drop in hepatocytes and further exacerbated liver acidosis.

Compared with control mice, water-deprived mice at all four time points (i.e., 24, 48, 72, and 96 hours) had higher hepatic levels of cystine, glycine, taurine, and branched chain amino acids (BCAAs; leucine, isoleucine, and valine) but lower level of alanine, serine, and threonine (Fig. 3). Furthermore, increased serum AST concentration after 96 h of water deprivation ($P<0.05$) (Fig. 2b) suggested increased transamination at this time point. The urea synthesis was also found to be down-regulated by dehydration, and such regulation of urea synthesis by water deprivation could be considered as a defense mechanism against intrahepatocyte hyperosomolality.[9] Down-regulation of urea synthesis further inhibited the degradation of amino acids. Both increased transamination and inhibition of the complete degradation of amino acids resulted in a relative higher concentration of some amino acids and free ammonia.

Taurine, a major free intracellular amino acid present in many mammalian tissues, is a potent free radical scavenger and can ameliorate the water deprivation induced oxidative damage of gastrointestinal mucosa and liver in rats.[34] Our results showed that concentration of hepatic taurine increased in mice that denied access to water ($P<0.05$). Increased hepatic taurine level was likely related to adaptive cellular responses to dehydration in mice.

BCAAs, especially leucine, can promote global protein synthesis through activating translation and inhibiting autophagic protein degradation.[35] In the water-deprived mice, increased BCAAs might also contribute to the high content of serum TP and albumin. BCAAs

supplementation reduced ROS production and hepatic oxidative stress in rats[36] and patients with liver cirrhosis[37] by activating antioxidative mechanisms.[36] Similar to taurine, BCAAs might also confer tolerance and adaptability to mice under dehydration stress. However, excess BCAAs in the blood can also result in profound neurological dysfunction.[35] Decreased *N*-acetylaspartic acid (NAA), a biochemical indicator of neuron damage, probably suggested the metabolic disturbance and injury of neuron.

Osmoregulation is vital for survival in all vertebrates.[38] Osmolality fluctuations can be influenced by glycerol, proline, trehalose, and free fatty acids (FFAs). Glycerol, one of the most common compounds that can counteract water loss and alleviate the stress,[39] can be a main fuel for liver gluconeogenesis under starvation state. However, as an osmoprotectant, glycerol content decreased in mice that denied access to water in this study (Fig. 3). Moreover, compared with that in control mice, content of glutathione, an antioxidant, also did not increase but decrease in mice deprived of water for 72 and 96 hours (Fig. 3). Interestingly, the contents of FFAs increased in mice that denied access to water (Fig. 3). Dehydration increased plasma non-esterified fatty acid (NEFA) content in dromedary camels.[40] Increased FFAs in this study might be also forced by water deprivation. Arachidonic acid can inhibit the cell shrinkage of Ehrlich ascites tumor cells in the process of regulatory volume decrease.[41] The increased FFAs caused by water deprivation probably contributed to counteracting dehydration related osmolality fluctuations.

Conclusions

In summary, the present work illustrated the utility of a ^{1}H NMR-based metabonomics strategy in the study of the C57BL/6J mice response to water deprivation. Water deprivation stress mainly

caused changes in metabolites that are involved in the metabolisms of carbohydrates, lipids and amino acids. Lactate and 3-hydroxybutylate might be the main energy source during water deprivation. Increased taurine and BCAAs might confer tolerance and adaptability to mice under dehydration stress. Moreover, the increased FFAs caused by water deprivation probably contributed to counteracting dehydration related osmolality fluctuations. These results also reveal that the metabonomics strategy is a powerful tool to gain insight into the molecular mechanism of cellular response to environmental stresses.

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Electronic supplementary information (ESI) available: ESI Table S-1 and ESI Figs S-1-S-6. ESI associated with this article can be found in the online version.

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Figure Captions

Fig. 1. Effect of water deprivation on the body weight of C57BL/6J mice.

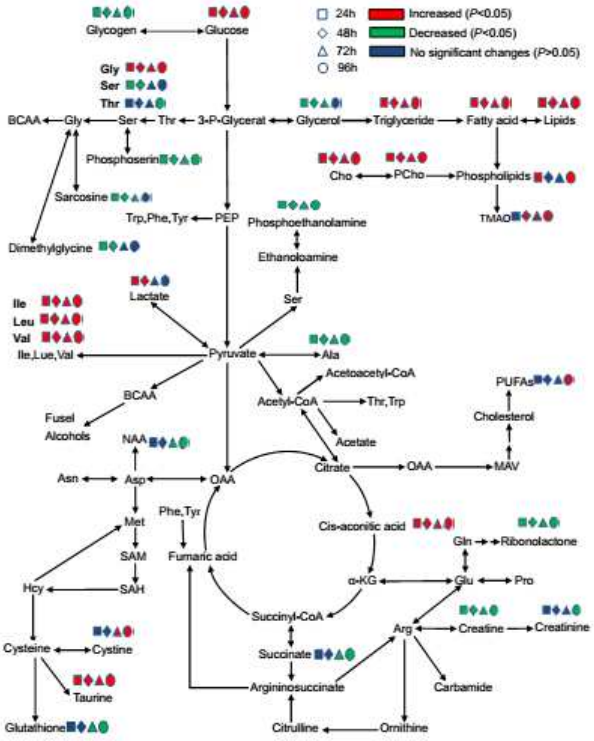
Fig. 2. Effect of water deprivation on the serum concentration of total protein and albumin and trans-aminase activity of C57BL/6J mice. (a) Total protein and albumin; (b) trans-aminase activity.

Fig. 3. Water deprivation-induced metabolic changes in C57BL/6J mice described by changes in hepatic metabolite levels. Red symbols denoted significant increases (P<0.05) and green ones denoted significant decreases (P<0.05), while black ones denoted no significant changes in metabolite levels (P>0.05). Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; BCAA, branched chain amino acids; Cho, choline; Gln, glutamine; Glu, glutamic acid; Gly, glycine; HCY, homocysteine; Ile, isoleucine; α-KG, α-ketoglutarate; MAV, methyldihydroxypentanoic acid; Met, methionine; NAA, N-acetylaspartic acid; OAA, oxaloacetate; PCho, phosphorylcholine; PEP, phosphoenolpyruvate; Phe, phenylalanine; Pro, proline; PUFAs, polyunsaturated fatty acids; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine; Ser, serine; Thr, threonine; TMAO, trimethylamine N-oxide; Trp, tryptophan; Tyr, tyrosine; Val, valine.

Fig. 4. Hierarchical cluster analysis of the identified differential metabolites.

Graphical Abstract

^{1}H NMR-based metabonomics approach is effective for elucidating underlying response or adaption to water deprivation in mammals. PLS-DA revealed a group classification and pairwise discrimination, and 28 differential liver aqueous compounds and some differential lipids with VIP value greater than 1 were extracted by PLS-DA. The metabolic relevance of these compounds in the response of C57BL/6J mice to water deprivation was discussed.



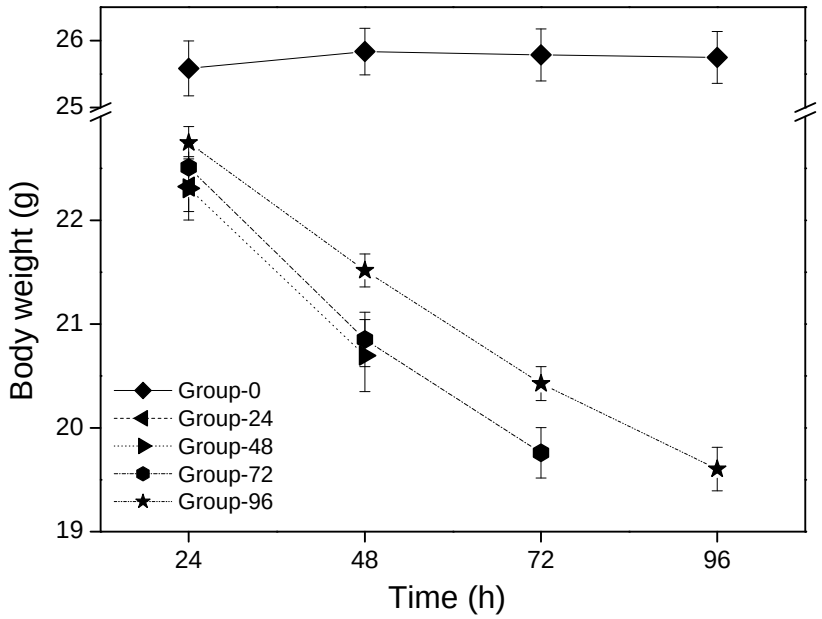


Fig. 1

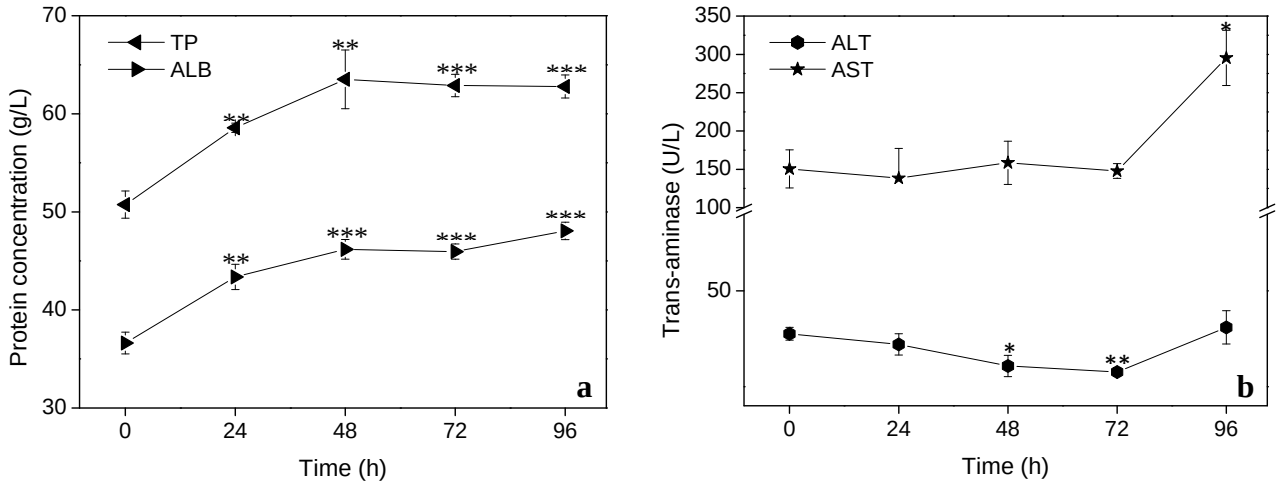


Fig. 2

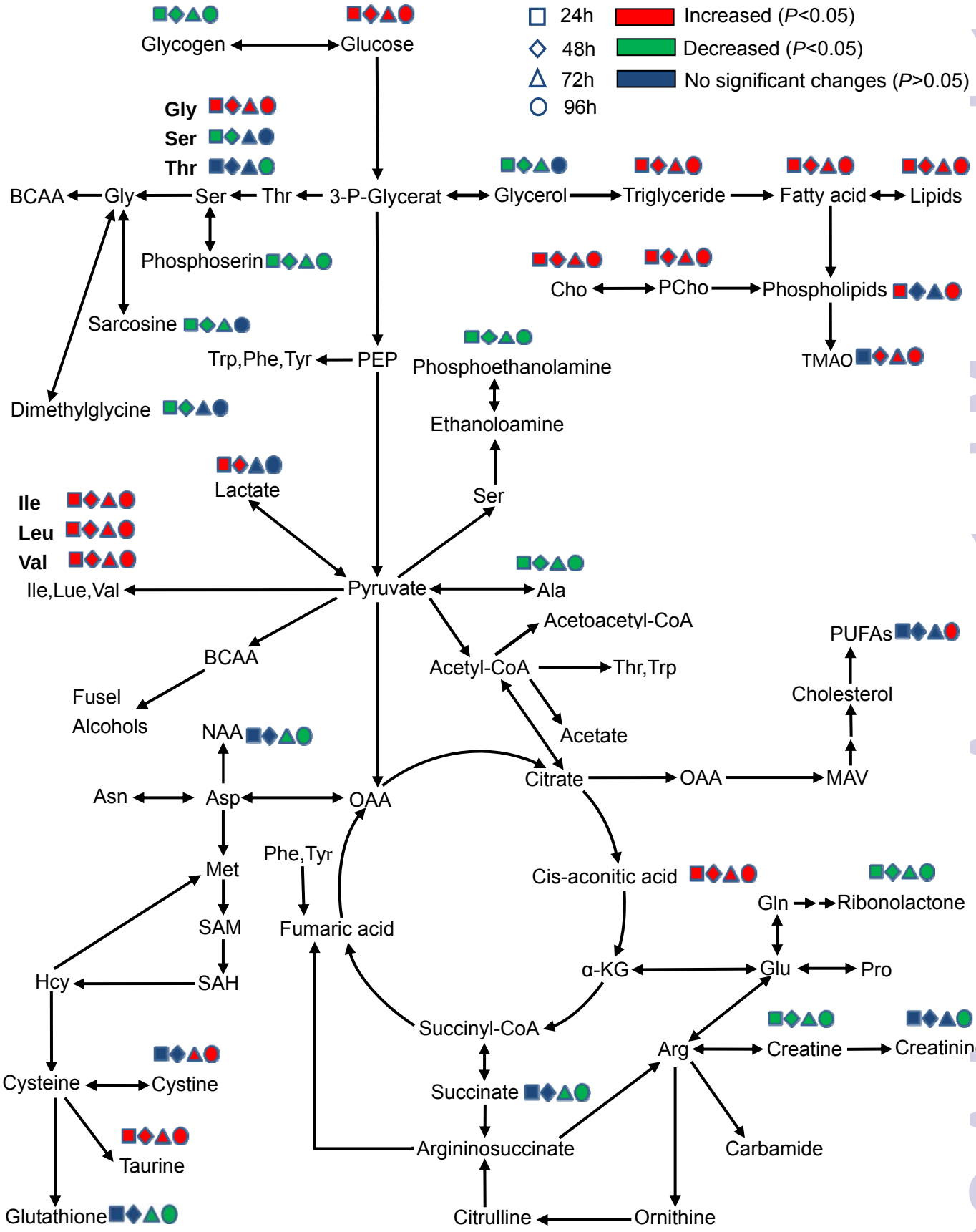


Fig. 3

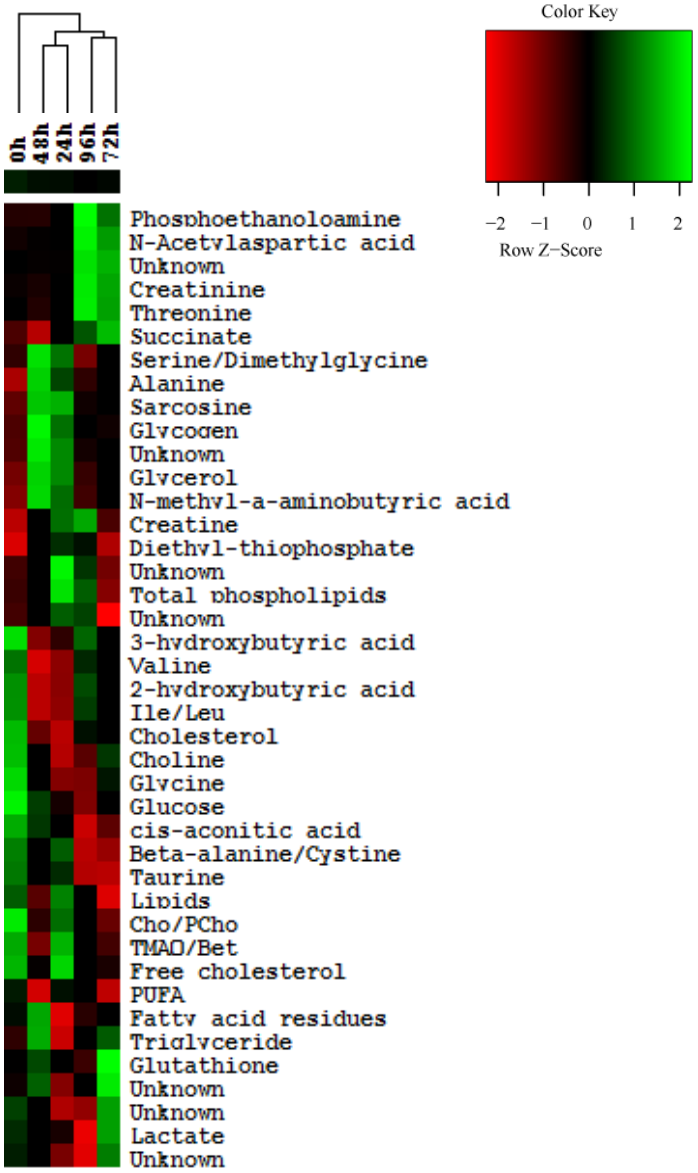


Fig. 4