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Metabolite Profiling of 5'-AMP-Induced Hypometabolism

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Abstract

We have demonstrated that 5'-adenosine-monophosphate (5'-AMP) can be used to induce deep hypometabolism in mice and other non-hibernating mammals. This reversible 5'-AMP induced hypometabolism (AIHM) allows mice to maintain a body temperature about 1°C above the ambient temperature for several hours before spontaneous reversal to euthermia. Our biochemical and gene expression studies suggested that the molecular processes involved in AIHM behavior most likely occur at the metabolic interconversion level, rather than the gene or protein expression level. To understand the metabolic processes involved in AIHM behavior, we conducted a non-targeted comparative metabolomics investigation at multiple stages of AIHM in the plasma, liver and brain of animals that underwent AIHM. Dozens of metabolites representing many important metabolic pathways were detected and measured using a metabolite profiling platform combining both LC-MS and GC-MS. Our findings indicate that there is a widespread suppression of energy generating metabolic pathways but lipid metabolism appears to be minimally altered. Regulation of carbohydrate metabolites appears to be the major way the animal utilizes energy in AIHM and during the following recovery process. The 5'-AMP administered has largely been catabolized by the time the animals have entered AIHM. During AIHM, the urea cycle appears to be functional, helping to avoid ammonia toxicity. Of all tissues studied, brain's metabolite flux is the least affected by AIHM.

Keywords

metabolomics; hypometabolism; 5'-AMP; non-targeted; plasma; liver

Introduction

Conserving energy by reducing the metabolic rate is a common strategy adopted by many animal species for survival when energy resources in the environment are limited. A variety of mammals, including several species of ground squirrels, prairie dogs, hamsters, and marsupials, innately enter into hypometabolic states such as hibernation and daily torpor to reduce energy expenditure during unfavorable environmental conditions such as cold winter. The mechanism of metabolic suppression in these instances is still largely unknown. Many other mammals, including humans, have yet to show a capability of entering a hypometabolic state naturally.

Several years ago, we demonstrated that a deep hypometabolic state is induced in non-hibernating mammals, such as mouse, rat and dog, upon receiving a significant dose of 5'-

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adenosine monophosphate (5'-AMP) (Zhang, et al., 2006; Daniels et al., 2010). This deep hypometabolic state, termed AMP induced hypometabolism (AIHM), can be maintained for 4–9 h in mice with a body temperature (T_b) near 16°C when kept at 15°C ambient temperature (T_a). Importantly, the process is reversible. The metabolic rates of mice in AIHM, as measured by oxygen consumption and carbon dioxide production, typically drop to less than 10% of the euthermic levels. In a separate study, we observed that during AIHM, greater than 99.9% of gene transcripts analyzed did not change significantly (Zhao et al., 2011), suggesting that regulation of the physiological changes observed during AIHM likely occur at the post-transcriptional level.

Here, we utilize metabolomics to investigate how the dramatic metabolic suppression induced by AIHM is accompanied by changes in the balance of metabolites *in vivo*. The dynamics of these metabolic pathways is reflected in the dynamics of the levels of their input and output metabolites in blood and tissues. Metabolomics technology provides a simultaneous measurement of the levels of hundreds of metabolites in tissues at a specific physiological stages, revealing the metabolic processes involved at the molecular level (Patti et al., 2012). We conducted non-targeted metabolomics profiling at different stages of AIHM to identify significant changes in metabolites in circulation, liver, and brain, in order to further the understanding of the mechanism of AIHM.

Methods and Materials

Animals

Mice (C57Bl/6) were purchased from Harlan Laboratories, Inc. (Indianapolis, IN). Mice were housed in a standard animal husbandry facility under a 12-h/12-h light/dark cycle with T_a between 23–25 °C. Female mice aged between 9 to 11 weeks were used in this study. All mouse studies were carried out under institutionally approved animal research protocols HSC-AWC-07-102.

AIHM procedure

The AIHM procedure is as previously described (Zhao et al., 2011). Briefly, each mouse was injected intraperitoneally with 0.5 mg/g body weight of freshly prepared 5'-AMP (Sigma catalog # A1752-25G) dissolved in phosphate buffered saline (PBS). Mice given 5'-AMP and maintained at a T_a of about 15°C entered into deep hypometabolism after about 90 min (Daniels et al., 2010). The T_b of these mice is usually 1–2°C above T_a . Mice usually stay in this deep hypometabolic state for 4–9 h. Arousal is apparent when the animal regains the ability to “reverse flip” or right itself on its feet when it is placed on its side or back. This behavior is used as an indicator for the arousal process in this study. Following arousal, the T_b of AIHM mice, measured by a rectal temperature probe, increased rapidly and normal grooming behavior become apparent within 1–2 h. Standard water and food supplies were provided *ad libitum* at all times.

Collection of tissue samples

Samples of about 250 µl of blood and 100 mg of liver and brain were collected from each animal from four groups of mice (n=7): (1) CTRL, euthermic control; (2) D-AIHM, when mice are in the deep AIHM (3 h post 5'-AMP administration); (3) A-AIHM, the initial arousal when an animal first displays the ability to right itself when placed on its side and prior to shivering; (4) R-AIHM, an early recovery stage when the animal starts to show normal behavior, about 15 min post-shivering behavior (Supplemental Figure S1). For collecting the blood, EDTA (4.5 mM) was used as anticoagulant. Plasma samples were separated from cells after centrifugation of blood samples at 1000 × g for 15 minutes and

frozen immediately. Tissue samples were frozen immediately in liquid Nitrogen. The frozen samples were shipped to Metabolon, Inc. (Durham, NC, USA) for metabolomics analysis.

Metabolomics data acquisition and analysis

Metabolite profiling of the collected samples was carried out at Metabolon on their standard platform as described previously (Evans et al., 2009; Daniels et al., 2010) and also included in supplemental information S2. Briefly, the low molecular weight fractions of a methanol extract were separated in parallel by gas-chromatography (GC) and liquid-chromatography (LC) in combination with detection by mass spectrometry (MS). Non-targeted metabolomics analyses were performed for both LC-MS and GC-MS outputs, where each detected compound was identified via searching its spectral files through metabolomics libraries of about 3000 compounds at Metabolon. Data for each compound were median-centered and inter-quartile-range-scaled. The company's technology platform strictly allows relative quantitation of peak areas for each metabolite. Changes in metabolite flux in liver, brain and plasma via snapshot were carried out and compared to the euthermic control group (CTRL) and groups for the following three stages, D-AIHM, A-AIHM, and R-AIHM. The analysis is based on the ratio changes when experimental animal groups are compared to the euthermic control animal group. All t-tests are two-sided applying a threshold of $P < 0.05$ for significance.

For a better appreciation of data distribution and deviation, we present all data as box plots. Each box plot shows the data distribution for each compound in all experimental groups, including the control. The data are relative quantifications scaled to the median value of all measurements (the 1.0 value on the y-axis) for that compound. Thus, the control in a box plot does not have a value of "1". For most of the figures, the box plots of the same compound from all three tissues are aligned in a horizontal line if it is detected in all tissues. However, data are not always available from all three tissues, hence the misalignment in parts of the figures. All related original metabolomics data are submitted as supplemental information.

Results and Discussion

Categories of metabolites identified

To investigate the possible AIHM-associated *in vivo* changes in metabolites and the metabolic processes they participate in, metabolites were extracted from plasma, liver, and brain tissue samples harvested during four stages: Euthermic control (CTRL), Deep AIHM (D-AIHM), Arousal-AIHM (A-AIHM) and Recovered AIHM (R-AIHM). The non-target metabolite profiling applied a method combining LC-MS and GC-MS. A total of 254, 282, and 199 named metabolites were identified in plasma, liver, and brain, respectively, and 76 unnamed metabolites were also identified in plasma. As shown in supplemental Figure S2, of all three tissues studied, the largest groups of named metabolites are lipids, followed by amino acids and carbohydrates. Other metabolites fall into metabolic pathway groups for nucleotides, energy metabolism, peptide factors, vitamins, and xenobiotics. Those compounds yet to have a counterpart in the compound library could not be identified currently and fall into the unnamed metabolites category.

Fate of 5'-AMP administered

The majority of changes in plasma purine catabolites resulting from 5'-AMP administration have been previously described (Daniels, et al., 2010). Adenosine is one of the catabolites of 5'-AMP. The rise in adenosine levels in plasma and in brain is not significant in euthermia and AIHM states. However, in the liver both adenosine and 5'-AMP are moderately and significantly increased (Figure 1). Given that the adenine nucleotide cannot be taken up

directly by nucleated cells, it is likely that adenosine in circulation is taken up by hepatocytes through CNT and ENT channels, and the rise in liver 5'-AMP could be a result of adenosine phosphorylation to 5'-AMP by adenosine kinase. The subsequent catabolism of 5'-AMP in the liver results in elevated levels of other downstream intermediates in the 5'-AMP catabolic pathway, including hypoxanthine, urate and allantoin. Not surprisingly, the end product of purine catabolism in mice, allantoin, is the most significantly elevated catabolite in all three tissues between euthermia and AIHM. Given the greater than 15 fold increase observed in the plasma, we cannot rule out that the rise in allantoin in liver and brain could be vascular. Our studies revealed that the levels of 5'-AMP were only moderately or insignificantly elevated in all the three tissues sampled. These observations suggest that the majority of the 5'-AMP administered has largely been catabolized by the time the animals have entered D-AIHM.

Energy Metabolism

Corresponding to the depressed oxygen consumption, the metabolite profile is consistent with an overall depressed energy consumption, as reflected in the changes in metabolites participating in glycolysis, tricarboxylic acid (TCA) cycle, fatty acid oxidation, and branched chain amino acid (BCAA) catabolism.

Glycolytic intermediates during AIHM—The levels of most of the plasma metabolites upstream of phosphoenolpyruvate (PEP), including glucose, glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), 3-phosphoglycerate, 1,3-dihydroxyacetone, 2,3-diphosphoglycerate and PEP, were significantly elevated during D-AIHM, and A-AIHM, while pyruvate and lactate appeared to be depleted (Figure 2A), as previously reported (Daniels, et al., 2010). In the liver, G6P, F6P and 3-phosphoglycerate were significantly elevated, while lactate was significantly reduced in the D-AIHM stage. These changes suggest a reduced glucose utilization when metabolism is significantly reduced during the D-AIHM stage. The metabolite profile could also indicate an increased production of glucose compared with its utilization during D-AIHM. Since the liver is the major organ for gluconeogenesis, the significantly elevated G6P is highly suggestive of an increased gluconeogenic process. Given that glucose is released by the liver during gluconeogenesis, it is not surprising that liver glucose during D-AIHM was not significantly different from control. In line with this observation, the level of lactate was significantly reduced in both liver and plasma consistent with a systemic reduced glycolysis and increased gluconeogenesis in the D-AIHM stage. By the A-AIHM stage, when arousal is underway, these metabolites in liver were largely restored to their control level. These adjustments suggest that the suppression of glycolysis is relieved to meet the gradually increased need for energy once the animals enter the arousal stage. In contrast to plasma and liver, the brain did display moderate, but not significant, elevated levels of G6P and its glucose level remained relatively constant in both control and D-AIHM animals. However, during A-AIHM, the level of glucose in the brain and plasma was significantly increased reflecting increased availability of this metabolite. Brain depends mainly on glucose for its energy expenditure. The rise in brain lactate level during A-AIHM is a further indicator of increased glycolysis. These observations suggest that during all stages of AIHM, brain glycolytic intermediates are maintained at relatively constant levels. By R-AIHM, the levels of almost all intermediates of the glycolytic pathway have been restored to their euthermic control levels in all tissues tested. These observations indicate that the biochemical and physiological changes are reversible between AIHM and euthermia.

TCA cycle—The levels of several plasma intermediates of the TCA cycle, including α -ketoglutarate, succinate, malate and isocitrate, were significantly decreased in D-AIHM and A-AIHM, suggesting a systemic depression of the TCA cycle (Figure 2B). In liver, while

most of the intermediates of the TCA cycle were unchanged, the significant accumulation of citrate and mesaconate in the D-AIHM stage appears to be the result of a stalled TCA cycle. Further, the significantly reduced levels of fumarate and dramatic accumulation of succinylcarnitine indicated a block in the TCA cycle at the succinate dehydrogenase step in the liver. Meanwhile, the high level of citrate in the liver during D-AIHM and A-AIHM suggests gluconeogenesis is favored over glycolysis since citrate inhibits the enzyme phosphofructokinase (PFK), a rate-limiting enzyme of glycolysis. Noticeably, the citrate level in A-AIHM was lower than that of D-AIHM, suggesting a partial recovery of the TCA cycle in the liver. This is also supported by the recovery of liver fumarate and malate levels in the A-AIHM stage. Interestingly, by R-AIHM, these altered levels of metabolites have largely returned to normal levels. In brain, the majority of the intermediates of the TCA cycle were not significantly changed.

Branched chain amino acids (BCAA)—There is evidence of a disconnect between BCAA catabolism and the TCA cycle that contributes to suppression of overall energy production. In liver, several of the carnitine and glycine conjugated metabolites of BCAA intermediates, such as isovalerylcarnitine, isobutyrylcarnitine, 2-methylbutyrylcarnitine, succinylcarnitine, hydroxyl-isovaleroyl carnitine, and isovalerylglycine, were significantly elevated in the D-AIHM and A-AIHM stages (Figure 2C). These changes along with decreased plasma levels of the BCAA alpha-keto acids, such as 3-methyl-2-oxobutyrates, 3-methyl-2-oxovalerate, and 4-methyl-2-oxopentanoate, are consistent with increased BCAA catabolism in liver. Under normal conditions, increased BCAA catabolism would result in an increased production of acetyl-coenzyme A (CoA) and succinyl-CoA that would then enter the TCA cycle. Accumulation of succinylcarnitine in liver and plasma, however, suggests that succinyl-CoA produced from BCAA catabolism largely failed to enter the TCA cycle to contribute to energy production. The fact that succinylcarnitine levels were elevated in all 3 tissues examined could indicate that the TCA cycle is stalled at succinyl-CoA when metabolic demand drops, while CoA is in short supply. Indeed, the levels of coenzyme A and 3'-dephosphocoenzyme A in liver were significantly lower in D-AIHM, A-AIHM and R-AIHM stages than control levels (Figure 2C). Utilizing ATP, 3'-dephosphocoenzyme A can be converted to CoA and ADP by dephospho-CoA kinase. The plasma levels of pantothenate (vitamin B5), a precursor of CoA, were lower than control in all three stages. This could imply that pantothenate is in limited supply (from food) thus resulting in insufficient CoA, exacerbated by stalling of endogenous CoA synthesis due to hypometabolism. The majority of metabolites involved in BCAA catabolism exhibited complete recovery, regaining euthermic control levels in the R-AIHM stage.

Urea cycle

The levels of urea were significantly elevated in plasma, liver and brain at the D-AIHM stage, followed by a progressive accumulation of urea in A-AIHM and R-AIHM stages in all tissues tested (Figure 3). The changes in urea levels are consistent with catabolism of BCAA during AIHM and production of NH_4^+ from amino acid transamination that is removed by the urea cycle. These observations suggest that the urea cycle must be functional during AIHM. In liver, the main tissue for the urea cycle, there was significant accumulation of n-acetylglutamate and citrulline in the AIHM stage. This suggests that argininosuccinate synthetase could have been slowed by AIHM or that the rate of citrulline production exceeded the enzymatic capacity for its removal, as the urea cycle intermediates after this enzymatic step were not significantly changed. By the A-AIHM stage, the citrulline level started to decline, accompanied by moderate increases in the levels of arginine and ornithine. The presence of a functional urea cycle therefore plays an important role in preventing ammonia toxicity during AIHM.

Kidney function

Plasma creatinine levels were elevated in D-AIHM and A-AIHM, implicating a reduced kidney glomerular filtration rate in these stages. The levels of creatinine in liver were also elevated in both D-AIHM and A-AIHM stages. Meanwhile accumulation of creatine, the metabolic precursor of creatinine, was observed in both plasma and liver (Figure 4A). Increased circulating levels of xenobiotic metabolites such as, hippurate, catechol sulfate, 4-methylcatechol sulfate, ethylphenylsulfate, cinnamoylglycine, and quinate further suggested a reduced kidney function during the D-AIHM and A-AIHM states (Figure 4B). The slower kidney activity is consistent with a systemic reduced metabolic rate. Again, the levels of all these metabolites returned to control levels by R-AIHM, the early recovery stage.

Stalled digestive system

In comparison to control, levels of diet-derived carbohydrates such as lactose, sucrose, raffinose, and stachyose were significantly elevated in plasma in the AIHM and early arousal states (Figure 5A). These changes likely result from decreased digestion of complex carbohydrates in these stages, as the animals were not fasted prior to AIHM. There was also a general accumulation of intermediates of inositol metabolism in all tissues tested under D-AIHM that persisted into the A-AIHM stage. The levels of myo-inositol, chiro-inositol, and inositol-1-phosphate were significantly elevated in D-AIHM and A-AIHM in plasma and liver, and to a lesser extent in brain (Figure 5B). These changes suggest an altered inositol metabolism under these conditions. Also notable are the very high levels of pinitol in all tissues tested and at all stages (Figure 5C). Pinitol is derived from plants and is added to the rodent chow. It is the precursor of other inositol intermediates. These changes suggest a suppressed metabolism of absorbed pinitol during the AIHM and early arousal states. The digestive functions are essentially restored by the R-AIHM stage, reflected by the recoveries of these metabolites to their normal levels.

Lipids and ketone bodies

Lipid profiles suggest a general lack of significant changes in lipid metabolism during AIHM, possibly due to the relatively short time frame of AIHM. Among the few measurable changes, two ketone bodies were found accumulated in the liver, but not in plasma or brain (Figure 6A). The elevated levels of liver acetoacetate in D-AIHM and 3-hydroxybutyrate in A-AIHM stages were fully recovered by the R-AIHM stage. Ketone bodies are typically generated when acetyl-CoA, produced from beta oxidation of fatty acids and BCCA catabolism, is in excess or fails to enter the TCA cycle. Reduced plasma carnitine levels are consistent with active fatty acid β -oxidation in the D-AIHM stage. Thus elevated ketone bodies provide further evidence of a stalled TCA cycle in D-AIHM. There was also a 3–4 fold accumulation of hexadecanedioic acid in AIHM and arousal stages (Figure 6B). This accumulation could be the result of bacterial fermentation in the gut, which has largely disappeared by the R-AIHM stage.

Other metabolites

The levels of corticosterone in all tissues tested at the D-AIHM stage were indistinguishable from those of the control, but elevated in the A-AIHM and R-AIHM stages (Figure 6C). In rodents, corticosterone is secreted from the adrenal cortex into the blood circulation in response to stress, and acts on organs and tissues throughout the body to mobilize energy and minimize non-essential functions in order to mitigate the stress. It may also suggest that regulation of energy metabolism in this system may involve signaling mediated by corticosterone, as its circulating levels were significantly elevated in A-AIHM and R-AIHM compared to the D-AIHM state. Also, there was a sharp drop in liver ophthalmate levels at the D-AIHM stage followed by an increase at the A-AIHM and R-AIHM stages that

correlated with a gradual decline in the reduced form of glutathione (Figure 6D). It has been suggested that ophthalmate is a promising reciprocal biomarker to indicate hepatic glutathione depletion (Dello et al., 2012). Thus, our data suggest the reduced form of glutathione could be in short supply during the arousal and recovery stages. Interestingly, oleamide, the metabolite found in the cerebral fluid of sleep deprived cats (Cravatt et al., 1995), was drastically elevated in the plasma of mice at R-AIHM, the early recovery stage (Figure 6E), while a similar change was not found in the brain or liver. Systemic oleamide has been shown to have a number of regulatory effects such promoting sleep, vasorelaxation (Hiley et al., 2007), analgesia and hypothermia (Fedorova et al., 2001). At present, the significance of a rise in plasma oleamide is unclear. A xenobiotic metabolite glucarate (also called saccharate) was elevated in both plasma and brain (Figure 6F). Interestingly, plasma glucarate was reported to have antioxidant effects that protect platelet (Saluk-Juszczak, 2010) and plasma proteins and lipids (Kolodziejczyk et al., 2011). The plasma levels of 1,5-anhydroglucitol were found to inversely correlate with the levels of plasma glucose, and have been considered a dynamic glycemic marker (Dungan, 2008). Plasma 1,5-anhydroglucitol decreased to about 40% of its normal level during A-AIHM (Figure 6G), suggesting an up-regulated glucose supply in plasma that serves to aid the recovery of the animal to the euthermic state.

Related issues and discussion

The observation that administration of 5'-AMP leads to a dramatic, yet safe and reversible metabolic suppression reignites the possibility that some form of hypometabolic state can be safely induced in humans for clinical applications (Lee, 2008). In order to understand the mechanism behind AIHM, we have conducted studies using various tools to characterize this phenomenon at the level of biochemical processes, gene expression and *in vivo* physiological processes in animal models. Our investigations showed that glycolysis suppression following 5'-AMP administration resulted in an elevated level of blood 2,3-bisphosphoglycerate (2,3-BPG), which allosterically inhibits hemoglobin affinity for oxygen. Thus, we have proposed that 5'-AMP mediated hypometabolism is triggered by reduced oxygen transport by erythrocytes initiated by uptake of 5'-AMP. Although reports by Swoap and colleagues suggested that adenosine, rather than 5'-AMP, induced the hypometabolism (Swoap et al., 2007 and Iliff and Swoap, 2012), our studies with adenosine receptor knockout mouse models demonstrated that adenosine is not a significant player in AIHM (Daniels et al., 2010). In addition, our studies with the CD73 knockout mouse model, further support that 5'-AMP, not adenosine, induces hypometabolism. An investigation of gene expression profiling demonstrated that the process of AIHM changes the expression level of less than 0.2% of transcripts profiled, many of which are immediate early genes (Zhao et al., 2011). We showed that temporal control of circadian genes is largely stalled during D-AIHM. In the early arousal state, the expression levels of a small population of additional genes are changed, presumably to aid the recovery process. At 2 days after the treatment, the gene expression pattern is almost the same as that of the pre-treatment state. Our observations to date suggest that AIHM is a reversible, induced physiological and cellular process.

Comparing the changes in metabolites at different stages of AIHM and earlier reports of metabolite changes during natural hibernation/torpor-arousal cycles, we noticed several similarities and differences. Keep in mind, when comparing metabolomics studies of similar metabolic processes by different investigators, often different groups of metabolites are being characterized depending on unique research conditions. A previous study focusing on the metabolic changes in liver of animals undergoing hibernation showed that short chain acyl-carnitines, including isovalerylcarnitine, isobutyrylcarnitine and 2-methylbutyrylcarnitine, were at their lowest levels in late torpor stage, while their levels in

euthermic and arousal stages were many fold higher (Nelson et al., 2009). In contrast, the levels of these short chain acyl-carnitines and several other acyl-carnitines were typically higher in D-AIHM and A-AIHM stages in our study, though the changes were usually within 2-fold. The marked suppression of sphingosine during the torpor and arousal stages in contrast to euthermic controls in ground squirrels was not paralleled in AIHM, though sphingosine is among the metabolites that were measured in this study. Among the three metabolites of redox reactions discussed in the same study, only biliverdin was detectable in liver in our study. In ground squirrels, the liver biliverdin level in torpor is similar to that of euthermic controls, while it increased about 20-fold during arousal. In contrast, in the D-AIHM stage a near 2-fold elevation of liver biliverdin, followed by a drop in the A-AIHM stage, was observed. The above comparisons illustrate some of the differences in metabolic changes between AIHM and natural hypometabolism, especially in lipid metabolism. On the other hand, these comparisons also revealed some similarities in changes of other liver metabolites. For example, the 3–4 fold accumulation of hexadecanedioic acid in the arousal stage of AIHM resembles a similar accumulation of hexadecanedioic acid seen in the late torpor and arousal stages in ground squirrels. This accumulation could be the result of bacterial fermentation, which largely disappeared by the early recovery stage. In a non-target analysis of metabolic changes in 13-lined ground squirrels by NMR, betaine was elevated in liver during the late torpor stage compared with the interbout stage (Serkova et al., 2007). Betaine is a metabolic product of membrane phospholipid catabolism, and is known to be a methyl group donor for S-adenosylmethionine, a compound that defends against oxidative stress (Gonzalez-Correa et al., 1997). In our current study, liver betaine was elevated in all stages of AIHM, suggesting that animals in AIHM may gain similar anti-oxidative protection from betaine. Further, accumulation of urate, an antioxidant (Ames et al., 1981 and Becker, 1993), and its catabolic derivative allantoin in the torpor phase have been previously described in hibernating animals (Tøien et al., 2001 and Epperson et al., 2011). In this study, accumulations of both are observed in all tissues tested during AIHM. Although catabolism of the administered 5'-AMP is the main source of urate and allantoin, the anti-oxidative function of urate could potentially play an important protective role during AIHM.

We also compared our results for circulating metabolites in AIHM to that of a metabolomics study of hibernating ground squirrels (Nelson et al., 2010). Among the shared metabolites analyzed, the levels of propionylcarnitine, butyrylcarnitine and isobutyrylcarnitine in the hypometabolic state were lower than those in the euthermic states in both studies. The observed accumulation of short chain acylcarnitines in the liver corresponded to their depleted circulating levels under AIHM and early arousal stages, indicating a blockade of short chain acylcarnitine release from the liver. In the same study in ground squirrels, methionine and tyrosine levels were lower while pantothenate was higher in later torpor stage compared to the euthermic state. We observed an elevation of methionine and unchanged tyrosine levels in the AIHM stage, while circulating pantothenate was almost unaffected. In contrast to naturally occurring hypometabolism behavior where lipid metabolism plays an important role, the levels of lipid metabolites failed to show significant changes during AIHM. This difference may be due to the fact that AIHM is a relatively short hypometabolic process. Interestingly, it was shown that Djungarian hamsters primarily utilize glucose in the initial phase of the daily torpor before switching to lipids (Heldmaier et al., 1999). This could imply that additional metabolic regulatory signals may be needed to activate selected lipid metabolic pathways in order to sustain animals during a prolonged hypometabolism. Together, these comparisons of metabolites in liver and circulation show that AIHM has both shared and unique metabolic profiles compared to naturally occurring torpor.

Concluding Remarks

This non-targeted metabolomics study provides us with snapshots of many metabolic pathways simultaneously in multiple tissues at each of the signature stages of AIHM. Our comparative analysis revealed that there is a widespread suppression of energy generating metabolic pathways in all tissues examined. In contrast to naturally occurring hypometabolic states such as torpor and hibernation, lipid metabolism appears to be minimally altered during induced hypometabolism. On the other hand, the animals seem to draw energy mainly from carbohydrate metabolic pathways in AIHM and the following recovery process. The urea cycle appears to be functional during AIHM, which could play a critical role in preventing ammonia toxicity. The majority of the significant changes in metabolite flux were observed in plasma and liver, while brain metabolites appeared to be relatively stable during AIHM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The fate of injected 5'-AMP as reflected in the changes of metabolites of the 5'-AMP catabolic pathways, with a simplified schematic outline of the pathway. Each box plot shows the data distribution for each compound for all experimental groups, including the control. The data are a relative quantification scaled to the median value of all measurements (the 1.0 value on the y-axis) for the compound. Thus in the box plot the control does not have a value of "1". For most of the figures, the box plots of the same compound from all three tissues are aligned in a horizontal line if it is detected in all tissues. However, data are not always available for all three tissues, hence the misalignment in parts of the figure.

Figure 2.

Energy metabolism during AIHM. A. The changes in metabolites of the glycolysis pathway in different stages of AIHM, with a simplified schematic outline of the pathway. B. The changes in metabolites of the TCA cycle pathway in different stages of AIHM, with a simplified schematic outline of the pathway. C. The changes in relevant metabolites in BCCA catabolism and CoA metabolism pathways at different stages of AIHM.

Figure 3. The changes in metabolites of the urea cycle at different stages of AIHM, with a simplified schematic outline of the pathway.

Figure 4.

Changes in relevant metabolites reflect changes in kidney function during different stages of AIHM. A. Accumulation of creatinine and its precursor creatine in liver and plasma during AIHM. B. Increased circulating levels of xenobiotic metabolites.

Figure 5.
The changes in relevant metabolites reflecting the function of the digestive system in different stages of AIHM. A. Levels of the circulating compound sugars; B. Metabolites associated with pinitol metabolism.

Figure 6.

Other metabolites that changed during AIHM. A. Elevated levels of ketone bodies in liver in D-AIHM and A-AIHM stages. B. Elevated hexadecanedioate levels in liver in D-AIHM and A-AIHM stages; C. Elevated levels of corticosterone in all tissues tested; D. Changes in liver glutathione and ophthalmate at all AIHM stages; E, Sharp elevation of circulating oleamide at the recovery stage, R-AIHM; F. The dynamics of plasma and brain glucarate (saccharate); G. Changes in plasma 1,5-anhydroglucitol (1,5-AG) levels in different stages of AIHM.