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ORIGINAL ARTICLE

Lipidomics Reveals Mitochondrial Membrane Remodeling Associated with Acute Thermoregulation in a Rodent with a Wide Thermoneutral Zone

Qian Pan · Min Li · Yao-Long Shi · Huwei Liu · John R. Speakman · De-Hua Wang

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Abstract Mongolian gerbils (*Meriones unguiculatus*) have high physiological flexibility in response to acute temperature changes, and have the widest thermoneutral zone (TNZ, 26.5–38.9 °C) reported among small mammals. At the upper critical temperature (T_{uc}, 38.9 °C), body temperatures of gerbils were significantly increased (39–41 °C) while metabolic rates were maintained at the basal level. In contrast, below the lower critical temperature (T_{lc}, 26.5 °C), metabolism was elevated and body temperature stable. Rapid changes in mitochondrial membrane lipidome were hypothesized to play an important role during acute thermoregulation of gerbils. Taking advantage of a recent lipidomic technique, we examined changes in the membrane phospholipids environment and free fatty acids (FFA) production in mitochondria between 38 and 27 °C (in the TNZ), and between 27 and 16 °C (below the TNZ). At 38 °C, acute heat stress elicited distinct remodeling in mitochondrial membrane lipidome which related to a potential decrease in mitochondrial respiration and membrane fluidity compared to 27 °C. At 16 °C, a sharply decreased unsaturation index and increased chain lengths were detected in mitochondrial FFA production both in muscle and brown adipose tissue. Our results suggest that mitochondrial membrane lipid remodeling may stabilize membrane function and activity of respiration related membrane protein to maintain a stable metabolic rate at T_{uc}, and improve heat production by decomposing less fluid fatty acid conjugates of membrane lipids under acute cold exposure. These data therefore imply an important role of membrane remodeling during acute thermoregulation in a non-hibernating endotherm.

Keywords HPLC–ESI-MS · Lipidomics · Mongolian gerbils · Acute thermoregulation · Mitochondrial membrane lipid · Thermoneutral zone

Abbreviations

BAT	Brown adipose tissue
BMR	Basal metabolic rate
C	Thermal conductance
CerPCho	Sphingomyelin
COX	Cytochrome *c* oxidase
ESI-MS	Electrospray ionization-mass spectrometry
FFA	Free fatty acid(s) (non-esterified fatty acids)
HPLC	High pressure/performance liquid chromatography

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Q. Pan · Y.-L. Shi · D.-H. Wang (✉)
State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beichen Xilu, Chaoyang, Beijing 100101, China
e-mail: wangdh@ioz.ac.cn

Q. Pan · Y.-L. Shi
University of the Chinese Academy of Sciences, Beijing, China

M. Li · H. Liu
Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing, China

J. R. Speakman
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beichen Xilu, Chaoyang District, Beijing, China

J. R. Speakman
Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, UK

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MR	Metabolic rate at each ambient temperature
MS	Mass spectrometry
MUFFA	Monounsaturated FFA
PC1	Principal component 1
PC2	Principal component 2
PCA	Principal component analysis
PtdCho	Phosphatidylcholine
PtdEtn	Phosphatidylethanolamine
PtdGro	Phosphatidylglycerol
Ptd_2Gro	Cardiolipin
PtdIns	Phosphatidylinositol
PtdSer	Phosphatidylserine
PUFFA	Polyunsaturated FFA
QToF	Quadrupole time-of-flight
RER	Respiratory exchange ratio
RER_{BMR}	RER at basal metabolic rate
RER_{min}	Minimum RER during each metabolic rate measurement
T_{lc}	Lower critical temperature
TNZ	Thermoneutral zone
T_{uc}	Upper critical temperature
UI	Unsaturation index
VCO_2	CO_2 production
VO_2	Oxygen consumption rate

Introduction

Capacity for thermoregulation of endotherms when dealing with hourly acute temperature changes is reflected by the widths of their thermoneutral zones (TNZ) [1, 2]. TNZ is defined as the range of ambient temperatures over which the metabolic rate is maintained constant at the basal level (i.e. the lowest level for an endotherm to stay conscious, also referred to as the basal metabolic rate, BMR) [3, 4]. In mammals, animals with large body sizes are more likely to have wide TNZ (more than 10 °C) as a result of insulation effects [1], although there are exceptions (e.g. the human TNZ ranges only from 28 to 31 °C [5]). Small mammals usually have TNZ with a width no more than 6 °C (for instance, mice, *Mus musculus*, about 26–32 °C [3] and rats, *Rattus norvegicus*, about 28–34 °C [6]), because they are more sensitive to temperature fluctuations as a result of their larger surface to volume ratios. Yet, with similar body size, some desert rodents have TNZ wider than 10 °C and are, therefore, considered to have good capabilities for acute thermoregulation [7].

Mongolian gerbils (*Meriones unguiculatus*) distributed in the Inner Mongolian grasslands of China, Mongolia and the Baikal region of Russia [8, 9] where the fluctuation of ambient temperature is large. The daily temperature range in summer is from 3 °C during the night to 52 °C during the day in the sun [9]. For more than 50 years, it has been known that this rodent has the widest TNZ reported among small mammals (30–40 °C reported by Robinson [2]; 26–38 °C by Wang et al. [9]). In the TNZ, they can maintain the BMR even when their body temperature is elevated to 40 °C [2, 9]. This high physiological flexibility has been suggested as being an adaptive mechanism for surviving the high daily temperature fluctuations encountered in the desert environment [7], but the mechanism underpinning it still remains unknown.

At the cellular level, changes in metabolic rate, independent of whether they are mediated via the nervous system or by endocrine factors, derive mostly from changes in mitochondria, including mitochondrial density, activity of membrane protein underlying metabolic pathways, the inner mitochondrial membrane surface area and mitochondrial membrane lipid profiles [10]. Among these factors, mitochondrial membrane lipids are considered the most effective candidate in hourly acute thermoregulation since they provide a specific functional hydrophobic environment for an electron transport chain and all the other transporters and channels associated with oxidative phosphorylation [11–15]. Moreover, membrane lipids are suggested as being able to change very rapidly with minimal cost [16, 17] and altering the profile of associated phospholipids can effect large changes in protein functions [18–20]. For instance, the protozoan, *Paramecium multimicronucleatum*, can increase its membrane fluidity in seconds in response to a cooling stimulus [21]. Mussels, *Mytilus californianus*, can adjust the membrane order on hourly time scales during tidal cycles to offset the thermal variations [22]; Ground squirrels, *Ictidomys tridecemlineatus*, remodeled mitochondrial membrane rapidly during arousal from hibernation, which lasts for only 2–3 h [23].

For rodents whose body temperatures are maintained relatively stable, remodeling of membranes may not require such big changes as ectotherms or hibernators for acute thermoregulation. At T_{uc} where gerbils' body temperature was elevated by only 2–4 °C (still quite enough to cause a significant increase in metabolic rate in most mammals [1]), the mitochondrial membrane remodeling in response may not be detectable by traditional methods which simply estimate the proportions of fatty acid compositions or the phospholipid classes. The relatively large individual variations among wild rodents also impair the sensitivity of such methods to detect subtle changes after acute treatments. Therefore, the hybrid technique of high pressure/performance liquid chromatography (HPLC) and electrospray ionization-mass spectrometry (ESI-MS) was used to analyze the small changes in phospholipid molecule populations without breaking the molecules into polar heads and fatty acid tails [24]. The mitochondrial

membrane is not permeable to ionized long-chain free fatty acids (non-esterified fatty acids, FFA) in the cytoplasm. Fatty acid content enters into mitochondria and proceeds to β-oxidation in the form of acylcarnitines and acyl-CoAs [25], which cannot be detected by the lipidomic method used in our study. Therefore, the FFA in isolated mitochondria, which are produced by membrane lipid metabolization [26], were also analyzed to detect minor changes in membrane fatty acid conjugates.

The objective of our investigation was to observe membrane remodeling during acute thermoregulation of gerbils. It has been established that ectotherms remodel membrane lipids to counteract the temperature effect on membrane fluidity, a process known as homeoviscous adaptation [12]. Similarly, in endotherms, the membrane unsaturation index was found to be positively correlated with interspecific metabolic rates, and membrane unsaturation is suggested as increasing membrane protein activity by increasing membrane fluidity, known as the membrane pacemaker hypothesis [27]. We hypothesized that Mongolian gerbils respond to rapid ambient temperature changes by reversible mitochondrial membrane lipid remodeling. This remodeling may stabilize membrane function when body temperature is rapidly elevated at T_{uc}, and may also contribute to the elevated metabolic rate for heat production below the TNZ. Metabolic features of Mongolian gerbils were first measured to confirm previous observations [2, 9]. Then comparative analysis of mitochondrial membrane lipidome and FFA production were performed between 38 and 27 °C, and between 27 and 16 °C. Mitochondria in liver, skeletal muscle and brown adipose tissue (BAT) were measured because these organs contribute large proportions of the total metabolic rate [10, 28, 29]. Our study was the first exploration of the role of membrane remodeling during acute thermoregulation in non-hibernating endotherms.

Materials and Methods

Experimental Animals

All animal use procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences (Permit Number: IOZ11012). All researchers and students had been certified before performing animal studies.

Mongolian gerbils (*Meriones unguiculatus*, Milne-Edwards 1867) were from our laboratory colony, which were the offspring of Mongolian gerbils trapped in Inner Mongolian grasslands in 1999 and raised in the Institute of Zoology, Chinese Academy of Sciences. They were housed in plastic cages (30 × 15 × 20 cm) at a room temperature of 23 ± 1 °C under a photoperiod of 16L:8D (lights on at 0400 h) with sawdust as bedding. Commercial standard rat pellet chow (Beijing KeAo Feed Co) and water were provided ad libitum. Adult gerbils (11–12 months) were housed individually for at least 2 weeks before any treatment. About 5 weeks before the experiment, six gerbils were implanted intraperitoneally with a temperature transmitter (15.5 × 6.5 mm; 1.1 g) (Mini Mitter Model G2 E-Mitter), as described by Chi et al. [30].

Experimental Procedures

Measurement of Metabolic Features of Mongolian Gerbils

Twenty gerbils (Mb = 70.0 ± 2.6 g, mean ± SEM) were used to determine the TNZ of this species, among which six animals were implanted with transmitters and data of core body temperature and activity were recorded simultaneously during the metabolic trials. Metabolic rates, body temperatures, total activity and respiratory exchange ratios (RER) of 11 gerbils were first measured at 5.2, 10.5, 13.8, 19.6, 24.8, 29.2, 34.1 °C (the order of temperature exposure was randomized). Then nine other gerbils (6 implanted) were measured at 24.8, 29.2, 34.1 °C. Together, all 20 gerbils were used to identify the lower critical temperature at 26.5 °C. The 20 gerbils were then measured at 37.9, 38.9, and 39.8 °C, with at least a 2-day interval between each measurement. As gerbils began to show signs of stress at 38.9 and 39.8 °C, we only measured 12 gerbils at these two temperatures. At 39.8 °C two gerbils died during or immediately after the metabolic rate measurement, therefore, the RMR measurements were stopped at this temperature. The TNZ was defined by comparison of the metabolic rates at each temperature from 24.8 to 39.8 °C.

Estimation of Changes in Mitochondrial Membrane Lipidome and Energetic Parameters During Acute Thermoregulation

To minimize the influence of individual variation on the metabolic rate, BMR of 24 gerbils (16 of which were also used in experiment 1) were measured near the lower critical temperature (27 °C) and upper critical temperatures (38 °C). They were then divided equally into three groups (8 gerbils, half of them male in each group) matched for body weight and BMR. 3 days after the measurement, two groups were exposed to 27 and 38 °C, respectively, for 3 h without food and water after fasting for 3 h to minimize the specific dynamic action of food. They were then sacrificed immediately. Samples of liver, interscapular BAT and forelimb red skeletal muscle were dissected from gerbils in each group and weighed before processing for mitochondrial isolation. After measurements of mitochondrial

protein content, mitochondrial respiration, and cytochrome c oxidase (COX) activity, mitochondrial suspensions were kept at −80 °C until they were used for total lipid extraction and membrane lipidomic analysis (including FFA production).

After the comparison of metabolic parameters between 27 and 38 °C, gerbils in the third group were put under 16 °C for 3 h (with 3 h's fasting) and then sacrificed for mitochondria isolation from BAT and muscle tissue, total lipid extraction, and estimation of FFA production.

Metabolic Rate, Body Temperature and Activity Measurements

Metabolic rates (quantified as oxygen consumption rate, VO_2) and CO_2 production (VCO_2) were measured using an open-flow respirometry system (TSE Labmaster Calorimetry System, Germany) with a overall flow rate of 0.8 l/min and a sample flow rate of 0.39 l/min. RER were calculated as VCO_2/VO_2 [31]. An incubator (Sanyo, MIR-554) was used to maintain the respiratory chambers (TSE, type I for mice, volume 2.7 l) at a constant temperature (±0.5 °C). Real time ambient temperature in the incubator was recorded (±0.2 °C) during metabolic measurements. Body weights and rectal temperatures (body temperature) of animals were recorded before and after each measurement. Rectal temperature of gerbils without transmitters was measured using a TES-1310 digital thermometer (TYPE-K, TES Electrical Electronic Corp., Chinese Taipei) by inserting the probe into the rectum to a depth of 3 cm. All measurements were made daily between 08:00 and 20:00 h.

Each metabolic rate measurement lasted for 3 h with each animal fasted for 3 h before each measurement to minimize the specific dynamic action of food. Food and water were not provided during the measurements. BMR was calculated for each individual as the average of two lowest consecutive oxygen consumption recordings (values of 5 min) at every temperature by using the data of last 2 h of metabolic rate measurement [8]. RER calculated using recordings where the average of two consecutive oxygen consumptions is the lowest was defined as RER_{BMR} (RER at the basal metabolic rate), and as RER_{min} (minimum RER during the entire metabolic rate measurement) using recordings where the average of two consecutive RER values was the lowest.

Isolation of Mitochondria and Determination of Protein Concentration and Cytochrome C Oxidase Activity

Mitochondrial isolation was carried out immediately after death of the animal to prevent further remodeling of the membrane lipids. Liver mitochondria were prepared as described in Li et al. [8], BAT mitochondria as described in Zhao and Wang [32], and muscle mitochondria as in Scorrano et al. [33, 34]. Total mitochondrial protein content was determined by the Folin phenol method [35] with bovine serum albumin as standard. State 3 and 4 mitochondrial respiration rates were determined polarographically at 30 °C using a Clark-type electrode (Hansatech Instruments, UK) according to the description of Estabrook [8, 36]. State 4 respiration in three organs was measured substrate-dependently and succinate was used as the substrate. Then ADP was added to determine state 3 respiration [8]. COX activity was also measured at 30 °C with the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., England) according to Li and Wang [37].

Total Lipid Extraction

Synthetic lipid standards purchased from Avanti Polar Lipids (Alabaster, AL, USA) were: 1,2-dimyristelaidoyl-*sn*-glycero-3-phosphocholine (14:1/14:1 PtdCho), 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine (DMPS) and 1-heptadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (17:0 Lyso-PtdCho). *N*-hexane, isopropanol and chloroform of HPLC grade from CNW Technology (Germany). Ammonium formate was from Sigma-Aldrich (USA).

Total lipids in the mitochondria were extracted by a modified Folch extraction method [24, 38]. Briefly, mitochondria suspensions containing approximately 1.6 mg of mitochondrial protein (calculated by the mitochondrial protein concentration) were added to 8 ml of 2:1 vol/vol chloroform–methanol (1 mg mitochondrial protein: 5 ml Folch solvent) [11, 39]. Appropriate amounts of internal standards [14:1/14:1 PtdCho (0.4 μg), DMPG (0.4 μg), DMPE (0.4 μg), DMPS (0.4 μg), 17:0 Lyso-PtdCho (0.4 μg)] for phospholipids analyses were added to the crude solution. Samples were vortexed for 15 s and agitated ultrasonically for 15 min. The solvent was washed with a 20 % volume (1.6 ml) of 0.25 % KCl (w/v) solution to remove the water-soluble compounds. After vortexing for several seconds, the mixture was centrifuged at low speed (2,000 rpm, 10 min, 4 °C) to separate the two phases. The upper phase (water–methanol phase containing mitochondria protein) was removed and the lower phase was dried by evaporation under nitrogen. The samples were then stored dry at −80 °C.

Membrane Phospholipids Profile Analysis

Prior to phospholipid analysis, lipid extracts were redissolved in 0.4 ml of 2:1 vol/vol chloroform–methanol.

The phospholipids profile and free fatty acid composition in mitochondrial lipids was analyzed using the HPLC–ESI-MS/MS technique according to the first dimension analysis by Nie et al. [24]. The experiments were performed on an Agilent 1200 series HPLC system equipped with quaternary pump, online degasser, autosampler and thermostatted column compartment (Agilent 10 Technologies, Karlsruhe, Germany). The LC was coupled online to an Agilent 6520 Accurate-Mass Quadrupole Time-of-Flight mass spectrometry (QToF MS) equipped with an Agilent ESI source (Agilent Technologies, CA, USA). MS data of phospholipids profiles from all 12 samples (data of one sample of BAT mitochondrial lipidome was lost due to instrument malfunction) were extracted by MassHunter Qualitative Analysis software (B.03.01) and analyzed by Minitab software. The information of the location of the first double bond for each fatty acid was determined by gas chromatography described in Kang and Wang [40]. Unsaturation index (UI) and molar percentage (mol%) of each free fatty acid were calculated as in Ocloo *el al*. [39]. Relative molar amounts of phospholipid species were calculated by dividing their integrated peak areas by the peak area of the appropriate phospholipid standard [24]. Mol% of each phospholipid species was calculated as (100 % mole phospholipid)/(sum of mole phospholipid for all phospholipids). No undetected phospholipid contributed more than 0.0001 % of the total phospholipids present.

Statistical Analysis

Data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) except the membrane phospholipid profile data. Prior to all statistical analyses, data were examined for normality using the Kolmogorov–Smirnov test. In experiment one, parameters across different temperatures were compared using one-way repeated measures ANOVA (RM-ANOVA), with least significant difference tests estimated paired comparison, followed by Tukey's honestly significant difference post hoc comparisons. In experiment two, differences in parameters of different groups were examined using two-way (temperature × gender) ANOVA, followed by Tukey's honestly significant difference post hoc comparisons. Independent-samples T test were used when there was no significant effect of gender or gender × T interaction. Principal component analysis was performed to visualize the variation of lipidomic profiles between 27 and 38 °C groups and identify lipid biomarkers for acute thermoregulation in the TNZ. Pearson correlation analysis was performed to determine whether these biomarkers correlate with mitochondrial respiration and COX activity.

Results

Metabolic Rates and Respiratory Exchange Ratios

There was no significant difference between implanted and unimplanted gerbils for the parameters we measured at all ambient temperatures. Therefore data from the two groups were pooled for analysis. Metabolic rates were relatively low and stable from 26.5 to 38.9 °C (no significant difference was detected, Fig. 1a), which was regarded as the TNZ. Although there was no significant difference for metabolic rate between 25.5 and 26.5 °C ($P > 0.1$), the difference between 25.5 and 38.9 °C was significant ($P < 0.01$). The iterative fitting method [9] also showed that the inflection of the gradient between metabolic rate and T_a was around 26.5 °C (27.1 °C). BMR in the TNZ averaged 87.0 ± 8.0 ml O_2/h. Below T_{lc}, metabolic rates had a strong negative correlation with ambient temperature ($R = –0.904$, $P < 0.001$) and metabolic rates at 13.8 °C were about two times of those at T_{lc}.

Body temperatures of gerbils were not significantly different between 5.2 and 29.2 °C, and began to increase at some point between 29.2 and 34.1 °C (Fig. 1b). Lethal body temperature for Mongolian gerbils was 42.0 °C. Below the TNZ, mean body temperature was 37.5 ± 0.2 °C. At or above 35 °C, gerbils showed clear signs of increased evaporation and heat dissipation mechanisms as described by Wang et al. [9] and Robinson [2]. After 3 h of measurement of the metabolic rate at or above 34.1 °C, almost all of their fur on the abdominal surface was wet. Body weight losses after each metabolic measurement around T_{uc} (38.9 °C, 3.33 ± 0.25, $P < 0.001$; 38.9 °C, 3.70 ± 0.17 g, $P < 0.001$) averaged about 2 g more than at T_{lc} (26.5 °C, 1.14 ± 0.15 g, $F_{(4,8)} = 52.460$, $P < 0.001$, RM-ANOVA).

RER_{BMR} and RER_{min} varied significantly with T_a over the range of 5–40 °C (RER_{BMR}, $F_{(7,70)} = 7.552$, $P < 0.001$, Fig. 1c; RER_{min}, $F_{(7,70)} = 12.716$, $P < 0.001$, Fig. 1d). Compared to 34.1 °C, gerbils had higher levels of RER ($P = 0.046$ for RER_{BMR}, and $P = 0.020$ for RER_{min}) at T_{uc}, which continued to increase above T_{uc} (at 40 °C, $P = 0.006$, as for RER_{BMR}, and $P < 0.001$, as for RER_{min}). Least significant difference tests showed that compared to 34.1 °C (0.856 ± 0.017), RER_{min} increased by 0.033 at T_{uc} (0.889 ± 0.016), and by 0.096 at 39.8 °C (0.950 ± 0.024). The RER_{min} (0.806 ± 0.015 at 34 °C) had a similar pattern and increased by 0.032 at T_{uc} (0.838 ± 0.012), and by 0.117 at 39.8 °C (0.923 ± 0.021).

Body Temperature Fluctuations and Activity at T_{uc} and T_{lc}

In all cases, body temperature varied almost simultaneously with activity and metabolic rate at the T_{lc} but in

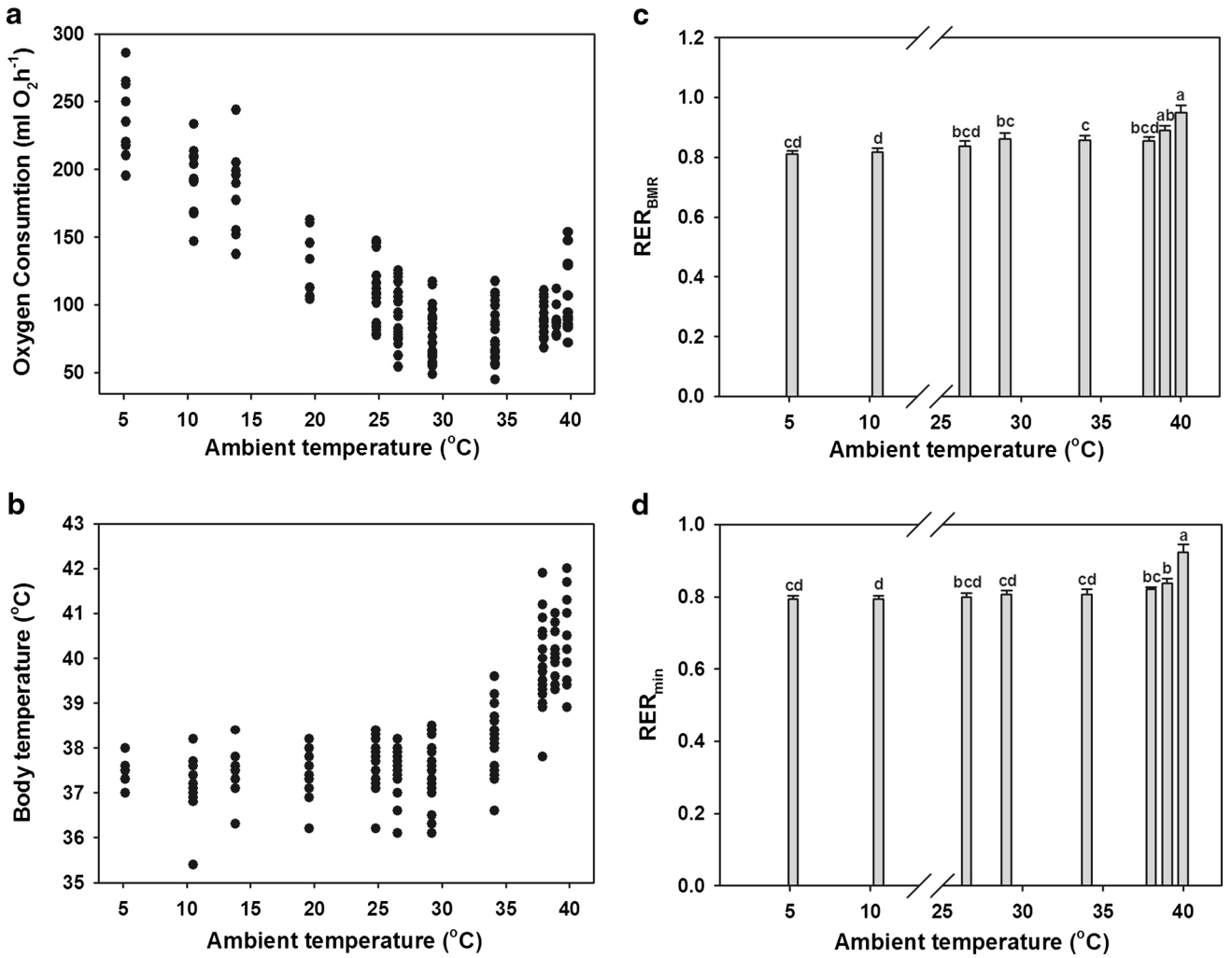

Fig. 1 Thermoneutral zone and metabolic features of Mongolian gerbils (*Meriones unguiculatus*). 5–20 °C, $n = 11$; 25–38 °C, $n = 20$ (6 implanted); 39–40 °C, $n = 12$ (4 implanted). **a** Metabolic rates of gerbils over 5–40 °C normalized for body mass. **b** body temperatures of gerbils over 5–40 °C. **c**, **d** Respiration exchange ratio (RER) variations of Mongolian gerbils at different ambient temperatures. Values are means ± SEM. Different letters above the *bars* are significantly different (by RM-ANOVA)

contrast showed little association with activity or metabolic rate at the T_{uc}. Typical examples at T_{uc} and T_{lc} are shown in Fig. 2a, b, respectively. At the T_{uc}, gerbils could maintain their T_b above 40 °C for 1–2 h, and could even cool themselves down to 38.5 °C from 41.5 °C (Fig. 2a). It is interesting to note that no matter how high the core body temperature became, the metabolic rate was maintained relatively low and stable. A low metabolic rate appeared to be crucial for survival in high temperatures, as beyond T_{uc} (at 40 °C), where the metabolic rate increased by a small amount, most gerbils could not sustain this state for more than 3 h.

Mitochondrial Respiratory Parameters

Body weight ($F_{(2,21)} = 0.546$, $P = 0.587$, calculated by one-way ANOVA) and metabolic rate ($F_{(2,21)} = 0.018$, $P = 0.982$, calculated by one-way ANCOVA with body mass as covariant) had no significant difference among the 38, 27 and 16 °C group. No significant difference was detected between 38 and 27 °C groups in the mitochondrial protein content, COX activity or mitochondrial respiration state 3 and 4 (Table 1).

Mitochondrial Membrane Lipid Composition

Phospholipid Profiles of Mitochondrial Membrane at 38 and 27 °C

To extract clear effects of temperature on mitochondrial lipids without dilution of insensitive phospholipids, the phospholipid profile was analyzed by principal components analysis using phospholipids that had the top and bottom 5 % of *T* values when comparing the two groups

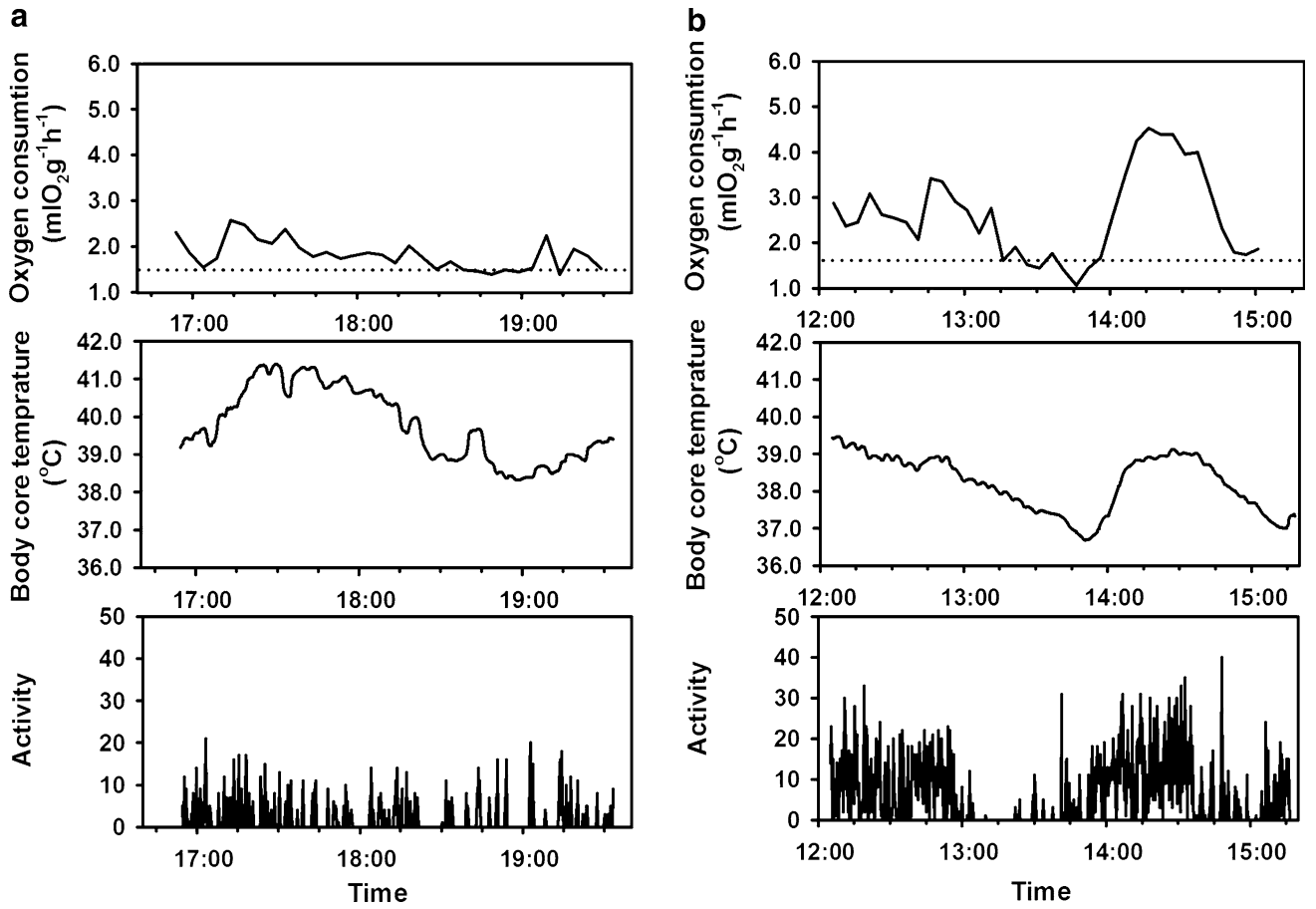

Fig. 2 Metabolic rate, body core temperature and activity variations of Mongolian gerbils during metabolic measurement processes. The BMR for each gerbil is indicated by the *dotted lines*. **a** Real-time data of one gerbil at the upper critical temperature. **b** Real-time data of one gerbil at the lower critical temperature

Table 1 Comparisons of mitochondrial protein content (Mito. Prot. Conc.), COX activity and mitochondrial respiration (resp.) in three organs of gerbils at 38 and 27 °C

	38 °C	27 °C	*T*	*df*	*P*
Liver					
Mito. Prot. Conc. (mg/g tissue)	12.81 ± 1.16	13.13 ± 1.68	−0.147	12	NS
COX activity (nmol O_2/min/mg Mt protein)	85.82 ± 25.02	84.80 ± 22.66	0.030	12	NS
State-3 respiration (nmol O_2/min/mg Mt protein)	17.12 ± 2.65	14.62 ± 1.10	0.912	11	NS
State-4 respiration (nmol O_2/min/mg Mt protein)	7.11 ± 2.99	7.16 ± 1.95	−0.012	11	NS
State-3 respiration/state-4 respiration	4.90 ± 1.56	4.97 ± 2.59	−0.030	13	NS
BAT					
Mito. Prot. Conc. (mg/g tissue)	13.34 ± 0.56	10.78 ± 1.44	1.156	13	NS
COX activity (nmol O_2/min/mg Mt protein)	211.38 ± 30.62	348.63 ± 122.93	−1.016	13	NS
State-4 respiration (nmol O_2/min/mg Mt protein)	68.10 ± 11.01	88.58 ± 15.01	−1.072	13	NS
Muscle					
Mito. Prot. Conc. (mg/g tissue)	5.78 ± 0.57	5.66 ± 0.53	0.147	13	NS
COX activity (nmol O_2/min/mg Mt protein)	341.51 ± 61.64	319.04 ± 71.35	0.235	13	NS
State-3 respiration (nmol O_2/min/mg Mt protein)	31.08 ± 5.86	46.30 ± 7.14	−1.617	13	NS
State-4 respiration (nmol O_2/min/mg Mt protein)	30.55 ± 4.96	21.74 ± 3.22	1.527	13	NS

Values are means ± SEM. Significant difference is defined if $P < 0.05$ by independent-samples *T* test

(calculated by independent T tests). Principal components of each sample within a treatment group were plotted to yield a group of points that can be circumscribed spatially to determine if acute temperature treatment at 38 °C is physiologically distinct from that at 27 °C. The resultant areas of different treatment groups were compared to see if there is any overlap. Our results showed that two areas generated by the treatment clusters did not overlap indicating a distinct effect of acute temperature changes on mitochondrial membrane lipid in muscle and BAT (Fig. 3). Variation along principal component 1 (PC1), which elevated significantly at 38 °C, accounted for the separation of the 38 °C treatments from the 27 °C controls in both muscle and BAT (PC1. $t = 4.202$, $df = 10$, $P < 0.01$ in muscle; $t = 2.655$, $df = 9$, $P < 0.05$ in BAT). The eigenvalue and proportion of variance explained by the first seven principal components are shown in the supplementary material Table S1. The phospholipids comprising PC1 were listed in Table 2 according to their values of loadings.

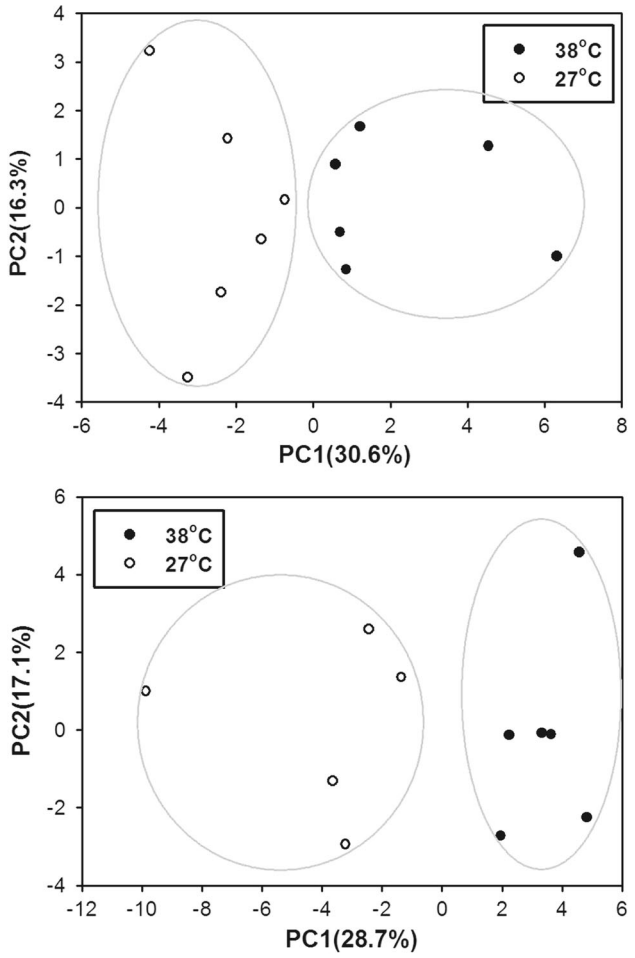

Fig. 3 2D principal component analysis (PCA) score plot separating T_{uc} and T_{lc} samples. Proportions of each component are shown in the *parentheses*

The pattern of the change in phospholipid profile showed a clear decrease of zwitterionic phospholipids [Phosphatidylethanolamine, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine, Phosphatidylcholine and 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (PtdEtn, PakEtn, PtdCho and PakCho)] in muscle as all phospholipids with significant negative loadings were zwitterionic phospholipids.

In muscle mitochondria, the mol proportion of Lyso-PtdCho(14:0), which had the highest loading value in PC1, correlated negatively with state 3 respiration (Fig. 4a); PakEtn(42:4), which had a negative loading value, correlated positively with state 3 respiration (Fig. 4b). In BAT mitochondria, the mol proportion of Lyso-PtdSer(20:4) and PtdCho(44:5) in membrane phospholipids correlated positively with state 4 respiration (Fig. 5a, b), and the latter had also a strongly positive correlation with mitochondrial COX activity (Fig. 5c). These two phospholipid species all had a negative loading value in PC1.

Lipidomic analysis between 38 and 27 °C groups also showed that among the 40 kinds of fatty acids we monitored in muscle, the digital negative of the T values were mostly (38 fatty acids) different between each free fatty acid and the same fatty acid conjugate in lysoglycerophospholipids (Table 3).

Free Fatty acid Profiles in Mitochondria

Potential changes ($P < 0.1$) in FFA production and related global FFA indices between 38 °C group and 27 °C group in mitochondria of muscle, BAT and liver are shown in Table 4.

In muscle mitochondria, FFA(21:3) and FFA(16:1) potentially decreased at the T_{uc} (Table 4) and positive relationship with mitochondria state 3 respiration was found in FFA(21:3) production (Fig. 4c). When the effect of gender was put into calculation, n-6 FFA, which constituted 25 % of total FFA production, showed a significant increase in muscle mitochondria at 38 °C. A potential increase ($P = 0.069$) in FFA(18:2), the major n-6 FFA species, was believed to have contributed to the increased n-6 FFA proportion.

In BAT mitochondria, acute heat stress at 38 °C resulted in significant reductions in the production of FFA(21:5) and n-3 FFA (22:5), both FFA correlated strongly and positively with mitochondrial state 4 respiration (Fig. 5e, f). FFA(24:6) in BAT mitochondria, which potentially decreased ($P = 0.058$) at 38 °C, also had a strong and positive correlation with mitochondrial state 4 respiration (Fig. 5d).

Only one FFA(20:2) in the liver showed potential changes ($P < 0.1$, Table 4) between the 38 and 27 °C groups. This FFA species did not show any correlation with any parameters regarding liver mitochondrial respiration. Given that liver mitochondria also had hardly any variation

Table 2 Coefficient of phospholipids in principal component 1 (Co. PC1) in muscle and BAT mitochondria from gerbils at 38 and 27 °C analyzed from HPLC-Q-TOF MS and their fold change analysis

Muscle mitochondrial membrane				BAT mitochondrial membrane			
Phospholipid	Co. PC1	*P*	Fold change	Phospholipid	Co. PC1	*P*	Fold change
PtdCho(44:4)	−0.191	0.002	0.46	Lyso-PtdEtn(19:0)	−0.197	0.043	0.44
PtdCho(42:5)	−0.186	0.049	0.84	Lyso-PtdGro(16:1)	−0.195	0.016	0.38
PtdCho(O-42:4)	−0.172	0.067	0.67	Lyso-PakEtn(20:2)	−0.189	0.093	0.51
PakCho (35:6)	−0.172	0.168	0.84	PtdSer(30:0)	−0.185	0.006	0.58
PakEtn(44:6)	−0.172	0.050	0.63	Lyso-PtdEtn(20:3)	−0.183	0.113	0.63
PtdCho(37:03)	−0.164	0.129	0.73	Lyso-PtdGro(16:0)	−0.183	0.002	0.60
PtdEtn(35:4)	−0.158	0.091	0.81	Lyso-PtdEtn(24:1)	−0.182	0.085	0.50
PakCho(38:2)	−0.157	0.059	0.57	Lyso-PtdEtn(22:4)	−0.180	0.084	0.61
PakEtn(42:4)	−0.153	0.109	0.41	PtdSer(41:4)	−0.177	0.062	0.52
PtdCho(44:6)	−0.149	0.165	0.74	PtdSer(41:5)	−0.176	0.015	0.63
PtdCho(42:3)	−0.143	0.066	0.66	PtdIns(40:8)	−0.175	0.016	0.51
PtdCho(40:7)	−0.134	0.057	0.86	Lyso-PtdGro(18:1)	−0.171	0.036	0.57
PtdEtn(39:3)	−0.131	0.066	0.70	Lyso-PtdGro(18:0)	−0.171	0.043	0.69
PtdCho(36:3)	−0.128	0.068	0.88	**Lyso-PtdSer(20:4)**	−0.166	0.086	0.62
PtdEtn(36:4)	−0.123	0.117	0.84	PtdSer(40:2)	−0.164	0.009	0.50
PtdEtn(36:3)	−0.122	0.053	0.79	PakIns(38:5)	−0.160	0.039	0.55
PtdEtn(34:1)	−0.114	0.163	0.90	PtdSer(37:1)	−0.156	0.012	0.70
PtdEtn(O-39:5)	−0.109	0.127	0.77	PtdSer(33:1)	−0.150	0.052	0.49
PtdEtn(34:3)	−0.108	0.132	0.80	PtdEtn(34:1)	−0.138	0.069	0.82
PtdEtn(34:2)	−0.102	0.138	0.88	PtdIns(38:7)	−0.129	0.043	0.60
PtdEtn(34:4)	−0.091	0.088	0.49	**PtdCho(44:5)**	−0.118	0.105	0.51
PakEtn(34:5)	−0.076	0.174	0.27	CerPCho(20:1)	−0.114	0.052	0.79
PakCho(36:0)	0.105	0.074	1.34	PtdEtn(38:4)	0.080	0.174	1.30
PtdSer(40:5)	0.114	0.170	1.76	PtdCho(40:3)	0.096	0.158	1.70
PtdEtn(32:0)	0.127	0.096	1.24	PtdCho(40:4)	0.108	0.154	1.45
PakSer(38:5)	0.130	0.132	1.80	Lyso-PtdCho(14:1)	0.111	0.137	2.16

Table 2 continued

Muscle mitochondrial membrane				BAT mitochondrial membrane			
Phospholipid	Co. PC1	*P*	Fold change	Phospholipid	Co. PC1	*P*	Fold change
PtdSer(40:4)	0.131	0.182	1.98	PtdGro(36:4)	0.114	0.124	1.44
PtdGro(42:1)	0.141	0.180	1.36	PakEtn(35:5)	0.114	0.054	1.46
Lyso-PtdCho(22:4)	0.145	0.162	1.40	PtdCho(40:2)	0.118	0.049	1.64
Lyso-PtdCho(20:2)	0.150	0.025	1.77	PtdCho(42:3)	0.120	0.063	1.41
Lyso-PtdCho(20:3)	0.150	0.140	1.29	PtdCho(36:1)	0.122	0.185	1.27
PtdIns(36:5)	0.152	0.052	1.82	PakCho(38:2)	0.123	0.028	1.22
Lyso-PtdEtn(24:1)	0.157	0.001	/0	PakEtn(40:3)	0.126	0.074	1.39
PtdCho(46:5)	0.158	0.066	3.18	PtdCho(38:4)	0.126	0.157	1.45
Lyso-PtdCho(22:3)	0.165	0.018	4.34	PtdGro(35:1)	0.127	0.117	1.20
Lyso-PtdCho(20:4)	0.166	0.119	1.41	PtdCho(40:8)	0.132	0.176	1.49
Lyso-PtdCho(18:3)	0.167	0.073	1.34	PtdCho(42:1)	0.134	0.103	1.49
Lyso-PtdCho(22:6)	0.177	0.069	1.46	PtdGro(34:0)	0.136	0.107	1.33
Lyso-PtdCho(20:5)	0.178	0.110	1.33	PtdCho(44:4)	0.137	0.066	2.08
CerPCho(18:2)	0.179	0.195	/0	PtdGro(42:10)	0.144	0.128	1.31
Lyso-PtdCho(22:5)	0.183	0.098	1.36	PtdGro(36:2)	0.156	0.060	1.30
PtdIns(32:1)	0.190	0.030	2.81	PtdCho(36:2)	0.170	0.065	1.31
PtdEtn(30:0)	0.191	0.073	1.57	CerPCho(18:2)	0.172	0.077	1.30
Lyso-PtdCho(14:0)	0.195	0.064	1.36	PtdGro(36:1)	0.176	0.043	1.38

Phospholipid species are listed according to their values of loadings in principal component one with a dashed line separating the ones with negative loadings and those with positive loadings. Lipid species that correlated significantly with mitochondria parameters are showed in bold type

Fold change was calculated by dividing the mean of proportions of each phospholipid from 27 °C by that from 38 °C group. *P* value represented the effect of temperature analyzed by group × gender-two-way ANOVA. "/0" means there was no molecular detected in 27 °C group

detected in the mean value of mitochondrial respiratory parameters between 38 and 27 °C, no further analysis of liver mitochondria was made beside the FFA estimation.

The sudden drop of ambient temperature from 27 to 16 °C led to acute changes in FFA which decreased the unsaturation index by 46 % in muscle mitochondria ($T = 8.894$, $df = 10$, $P < 0.001$) and by 36 % in BAT mitochondria ($T = 3.454$, $df = 10$, $P = 0.006$) (Fig. 6). Average chain length (ACL) of FFA increased two carbons on average in both muscle ($T = -9.906$, $df = 10$, $P < 0.001$) and BAT ($T = -8.828$, $df = 10$, $P < 0.001$). The pattern of cold response in FFA production was slightly different between these two organs: the n3/n6 index of muscle FFA increased ($T = -14.119$, $df = 10$, $P < 0.001$), but did not differ in BAT due to decreasing n-3 FFA ($T = 3.406$, $df = 10$, $P = 0.007$). Also, monounsaturated FFA (MUFFA) decreased to be much lower ($T = 17.572$, $df = 10$, $P < 0.001$) at 16 °C in muscle resulting in a decrease in the ratio of MUFFA/polyunsaturated FFA (MUFFA/PUFFA) ($T = 8.997$, $df = 10$, $P < 0.001$), both of which did not show any temperature effect in BAT mitochondria (Fig. 6).

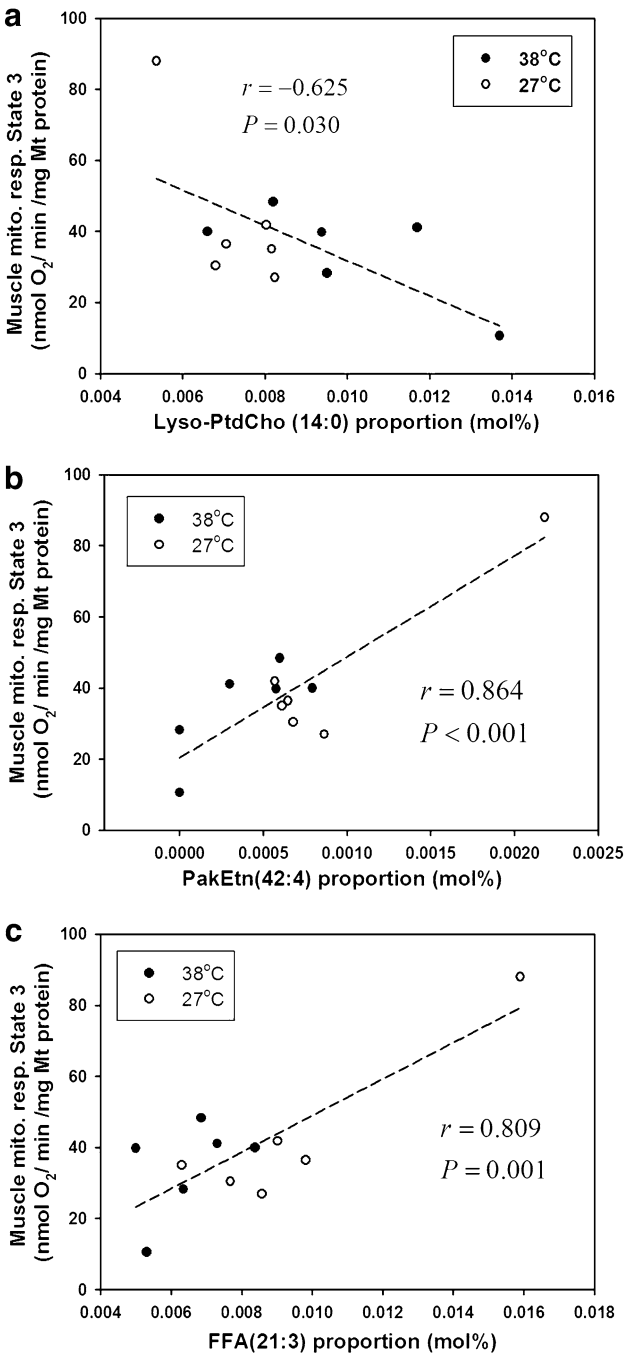

Fig. 4 Muscle mitochondrial respiration state 3 correlated significantly with **a** Lyso-PtdCho(14:0), **b** PakEtn(42:4) and **c** FFA(21:3) in muscle mitochondria, in the TNZ

Discussion

Mongolian gerbils (*Meriones unguiculatus*) have a relatively high T_{uc} (38.9 °C) and wide TNZ (26.5–38.9 °C) and were able to maintain a stable BMR when body temperature was elevated significantly (39–41 °C) at T_{uc}. Compared to a temperature near T_{lc} (27 °C), acute temperature changes to both 38 and 16 °C resulted in distinct remodeling of the membrane lipidome which is associated with acute thermoregulation. Our results, therefore, suggest an important role of mitochondrial membrane remodeling in cellular function regulation during acute thermoregulation in gerbils.

Metabolic Response to Heat Stress at T_{uc}

Using the new respirometry method based on the TSE system, our results (TNZ: 26.5–38.9 °C) confirmed previous observations (30–40 °C as in Robinson; 26–38 °C as in Wang et al.) [2, 9]. At 38.9 °C, the body temperature of gerbils was elevated in an interestingly controlled manner, and the sustaining of a low metabolic rate was important for gerbils for survival under acute heat stress, as it allows decreased heat production on one hand and conservation of water for thermoregulatory purposes on the other [7].

Gerbils switched their energy source to a larger portion of glucose as indicated by an elevated RER at T_{uc} [31, 41], which potentially gives gerbils advantages at high temperature, because glucose adds more water and energy to the system than either fat or protein when the same amount of oxygen is consumed [42, 43]. However, gerbils lost at least 2 g more water at T_{uc} than at T_{lc}, and even if gerbils consumed only glucose in the TNZ at their BMR, the amount of water they would produce in 3 h is merely 0.2 g. Therefore, the small amount of energy source adjustment did little to offset the increased water loss.

The estimation of mitochondrial protein concentration showed that an hourly time scale (3 h) was not enough for gerbils to utilize changes in mitochondria density to adjust to acute temperature changes. Although significant membrane lipid remodeling correlated to mitochondria respiration were detected in BAT and muscle, no difference were detected in mitochondrial respiration or COX activity, presumably due to large individual variations among gerbils in each of the groups.

Lipidomic Remodeling of the Mitochondrial Membrane Between 38 and 27 °C Associated with Acute Thermoregulation in the TNZ

Compare to the 27 °C group, the result of lipidomic profiling implied a distinct physiological effect on the membrane lipid environment during acute thermoregulation at 38 °C both in BAT and muscle mitochondria. Lipidomic remodeling in muscle mitochondria implied a pattern of reduced unsaturated zwitterionic phospholipid species (PtdEtn, PakEtn, PtdCho and PakCho) at 38 °C. Zwitterionic phospholipids, which make up 70–80 % of membrane phospholipids, are considered to have a major effect on membrane fluidity creating a tendency for membrane

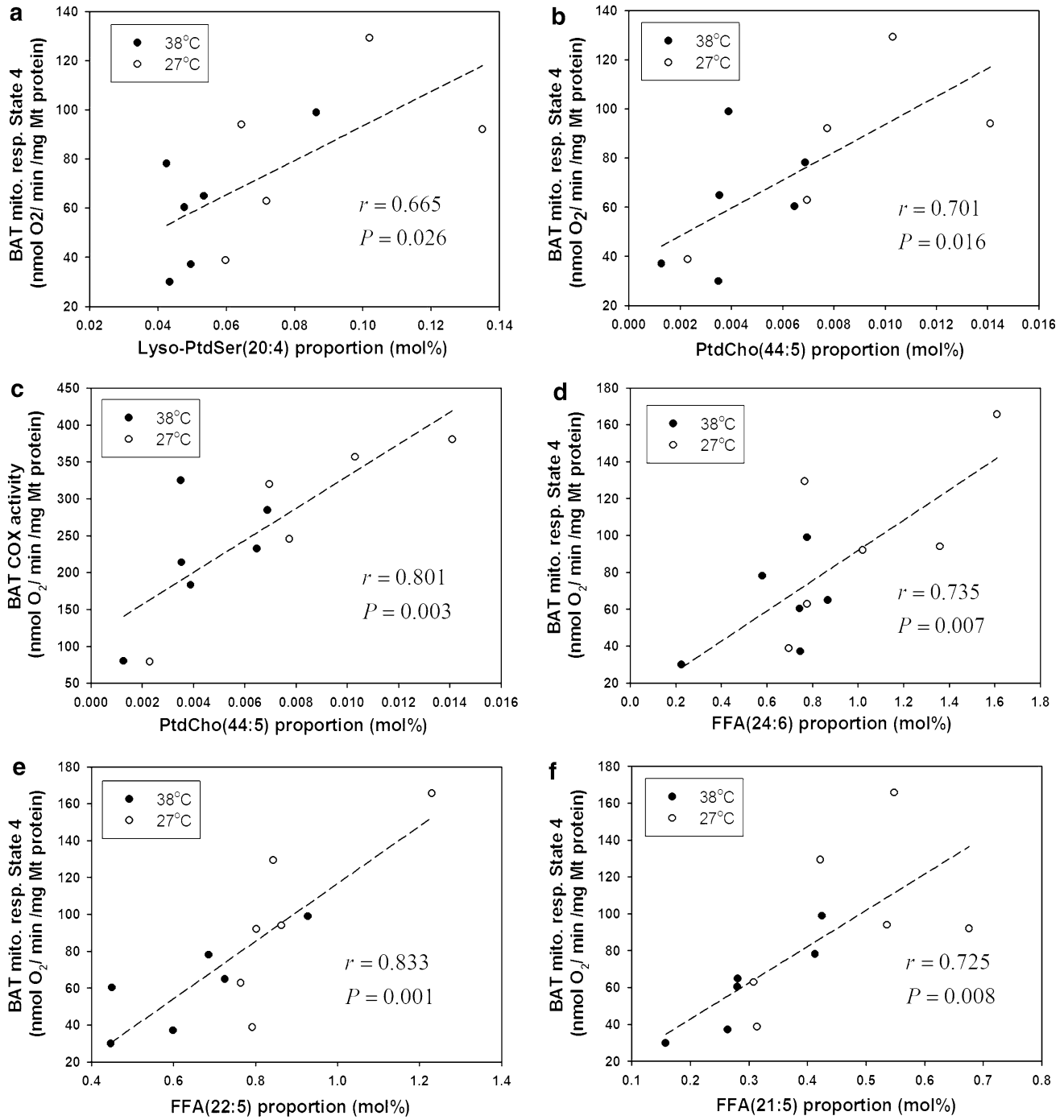

Fig. 5 BAT mitochondrial state 4 respiration correlated positively with **a** Lyso-PtdSer(20:4), **b** PtdCho(44:5), **d** FFA(24:6), **e** FFA(22:5) and **f** FFA(21:5) in BAT mitochondria, in the TNZ; BAT mitochondrial COX activity correlated significantly with **c** PtdCho(44:5)

lipids towards lateral phase separation [12, 44]. We assume this pattern might stabilize mitochondrial membrane structure in skeletal muscle and, as a result, helped gerbils offset the temperature effect when T_b was elevated at 38 °C. According to the Land's cycle [45], The opposite changes between each specific FFA and the same fatty acid conjugate in lysoglycerophospholipids indicated, that the total pool of fatty acids in mitochondria was maintained during the 3-h treatment and that each FFA changed in an opposite manner with the same fatty acyl composition in mitochondrial membrane phospholipid (FA).

The correlation between muscle mitochondrial state 3 respiration and Lyso-PtdCho(14:0) as well as PakEtn(42:4) showed that these phospholipid species may influence

Table 3 *T* values of fatty acid conjugates in lysoglycerophospholipids and FFA when comparing the 38 °C group to 27 °C groups (calculated by independent *T* tests) in muscle

Fatty acid species	*T* value	
	Fatty acid conjugates in lysoglycerophospholipid	Free fatty acid
14:1	0.424	−1.562
14:0	0.201	−1.298
15:1	0.618	−1.193
15:0	1.092	−0.961
16:2	0.980	−0.733
16:1	0.189	−2.137
16:0	0.856	−0.531
18:3	−1.570	1.080
18:2	0.416	2.033
18:1	−0.047	−0.087
18:0	0.016	−0.294
19:1	1.049	−0.721
19:0	1.021	−1.149
20:5	−0.430	0.431
20:4	−1.258	1.302
20:3	0.898	−1.239
20:2	−0.942	0.321
20:1	−0.882	0.984
20:0	0.052	−1.640
21:1	0.638	−0.505
21:0	0.743	−1.006
22:6	1.183	−0.456
22:5	1.097	−0.578
22:4	−0.837	0.558
22:3	0.373	−0.515
22:2	0.507	−0.341
22:1	−0.375	−1.282
22:0	0.144	−1.397
24:2	0.375	−0.447
24:1	0.165	−0.958
24:0	0.042	−2.022

oxidative phosphorylation in muscle mitochondria, and the direction of the potential changes in Lyso-PtdCho(14:0) and PakEtn(42:4) implied possible suppression of muscle mitochondrial respiration at T_{uc}, although the changes were not statistically significant. FFA(21:3) production, which potentially decreased at T_{uc}, correlated strongly and positively with state 3 respiration in muscle mitochondria. This result implied a possible increase in 21:3 fatty acid conjugates in the membrane might also act to suppress mitochondrial respiration in muscle.

The increased n-6 FFA production in muscle at 38 °C was partially in agreement with observations in cold acclimated mice, which were found to have a significantly increased n-6 fatty acyl composition in the mitochondria from skeletal muscle [39]. Reduction of 18:2 n-6 fatty conjugates in cardiolipin composition of mitochondrial membrane has been found to be related to the metabolic reduction in estivating snails [15]. Also, increased n-6 FA or n-6/n-3 FA ratio has been established as enhancing natural processes of the formation of new blood vessels [46]. In gerbils, we assume that more n-6 fatty acid conjugates were metabolized in the membrane which leaded to potential reduction in n-6 fatty acid conjugates and could inhibit membrane protein activity in muscle mitochondria at T_{uc}.

Mitochondrial proton leak mediated by uncoupling protein 1 in BAT mitochondria is the major source of heat production in rodent species [3]. Our data showed that n-3 FFA(22:5) and FFA(21:5) production in BAT mitochondria had strong and positive correlations with state 4 respiration. Therefore, the decreases in these two FFA species indicated a down-regulation of mitochondrial proton leak in BAT at 38 °C. Apparently, this could decrease heat production and alleviate the heat stress when body temperature was elevated at T_{uc}. Long-chain n-3 fatty acids in the membrane are considered to act as "metabolic pacemakers" [47]. Although the hypothesis has been under debate [14, 48], the increased n-3 FFA(22:5) production might result in a decrease in the n-3 22:5 fatty acid conjugate in membrane which consequently suppressed mitochondrial respiration. Other lipid species that had positive relationships with BAT mitochondrial respiration were Lyso-PtdSer(20:4), PtdCho(44:5) and FFA(24:6) production. Particularly, PtdCho(44:5) involved in regulation of both proton leak and oxidative phosphorylation in BAT mitochondria. The potential decreases of these lipid species all indicated a suppressed mitochondrial respiration and decreased heat production in BAT. The magnitude of these changes and mechanism underlying is, however, unknown.

Cardiolipin (Ptd_2Gro) is known as the signature phospholipid in the mitochondrial membrane, and has been indicated to play an essential role in thermoregulation as it interacts with a number of proteins that are involved in bioenergetic processes in the inner mitochondrial membrane, including the electron transport chain complexes and the ADP/ATP carrier [49]. During acute thermoregulation of Mongolian gerbils in the TNZ, Ptd_2Gro(72:8) correlated positively with BAT mitochondrial COX activity, while Ptd_2Gro(72:7), Ptd_2Gro(69:1) and Ptd_2Gro(67:0) correlated positively with muscle mitochondrial respiration state 3 (supplementary material, Fig. S1). However, none of the cardiolipin species that we measured showed any potential change between 38 and 27 °C (data not shown). Therefore, although our results were in agreement with the previously proved functional role of cardiolipin in mitochondrial respiration [49], it is indicated in our study that

Table 4 Molar percentage distribution of potentially changed individual FFA and related global group parameters in BAT, muscle and liver mitochondria of Mongolian gerbils at 38 and 27 °C

	38 °C	27 °C	T ($df = 10$)	P
Muscle				
FFA (n-7 16:1) (%)	1.36 ± 0.18	1.82 ± 0.10	−2.159	0.056
FFA(21:3) (%)	0.0065 ± 0.0005	0.0096 ± 0.0033	−2.078	0.064
FFA(n-6 18:2) (%)	28.2 ± 1.6	23.6 ± 1.6	2.033	0.069
n-3 FFA	0.066 ± 0.005	0.063 ± 0.004	0.445	NS
n-6 FFA	0.294 ± 0.016[a]	0.246 ± 0.015[b]	5.488[$]	**0.047**
n-3 FFA/n-6 FFA	0.229 ± 0.019	0.266 ± 0.033	−0.976	NS
BAT				
FFA(21:5)	0.304 ± 0.041[b]	0.467 ± 0.059[a]	−2.268	**0.047**
FFA(n-3 22:5)	0.639 ± 0.075[b]	0.882 ± 0.070[a]	−2.365	**0.040**
FFA(24:6)	0.66 ± 0.09	1.04 ± 0.15	−2.146	0.058
Liver				
FFA(20:2) (%)	0.287 ± 0.020[a]	0.216 ± 0.014[b]	2.745	**0.021**

Values are means ± SEM. Significant differences (highlighted in bold) is defined if $P < 0.05$ by independent-samples T test and indicated by different superscripts

[$] Effect of temperature is analyzed by group × gender two-way ANOVA, $F_{(1,8)} = 5.488$

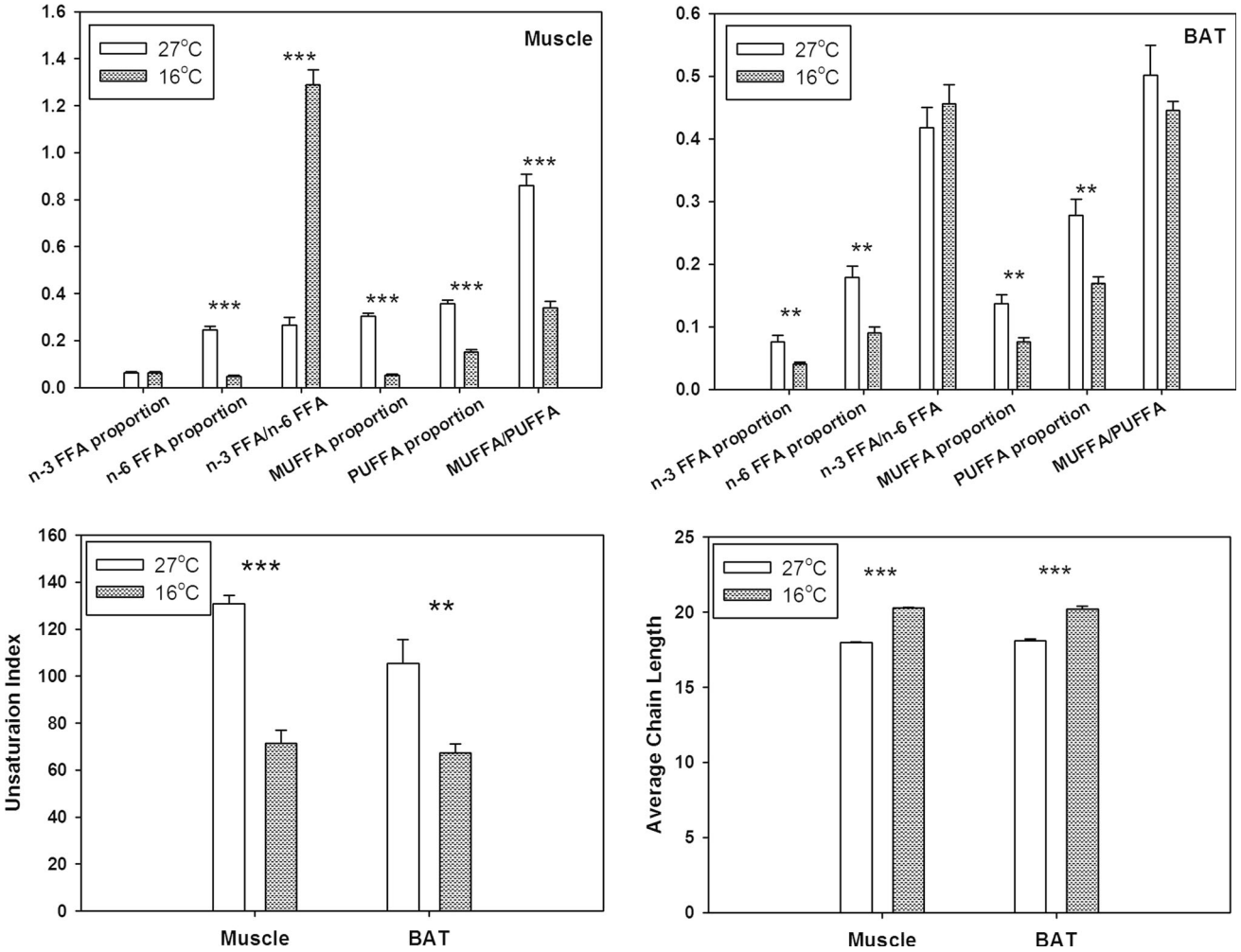

Fig. 6 Effect of acute cold stress at 16 °C on FFA production in mitochondria of BAT and muscle. The significant difference is defined if $P < 0.05$ by independent-samples T test and indicated by *different symbols*. *$0.010 < P < 0.050$, **$0.001 < P < 0.010$, ***$P < 0.001$

cardiolipin might not participate in acute thermoregulation in the TNZ of gerbils.

Changes in FFA Production Between T_{lc} and 16 °C Group Associated with Acute Thermoregulation Below TNZ

Membrane fluidity is one of the pivotal factors linked to aerobic metabolism [27]. It has been established that many ectotherms adjust the membrane composition to maintain optimum membrane fluidity for cellular functions when T_a changes [12]. Generally, membrane fluidity increases as the fatty acyl conjugates become shorter and more unsaturated. Also small changes in the extent of unsaturation of membrane phospholipids have been shown to affect bulk membrane properties and lead to specific modulation of membrane integral or associated proteins [50]. Below TNZ, significantly larger portion of saturated and long chain fatty acids, which could lower the fluidity of the membrane, were metabolized away from membrane phospholipids both in muscle and BAT. This result indicated a possible increase in mitochondrial membrane fluidity in response to acute cold exposure. Although it has been known for a long time that an increased metabolic rate for heat production during an acute cold stimulus is contributed to by activation of adaptive thermogenesis and shivering thermogenesis (reviewed in [4]), our result indicates a potential role of mitochondrial membrane remodeling as another contributory factor to elevated heat production when exposed to the cold. The magnitude of this effect however remains to be shown. Nevertheless this role may be particularly important during the initial exposure to cold conditions, as long term cold acclimation in rodents had no impact on the mitochondrial membrane saturation index or ACL [39]. We assume one possible reason for these disparate results could be that during the first hours of cold exposure, increased respiration dependent on elevated protein levels (e.g., of UCP1) were insufficient to produce enough heat, and hence membrane remodeling was activated to compensate for the lack of heat production. These compensatory changes in membrane lipids are attenuated or reversed after longer adaptation in cold exposure, when the expression of mitochondrial proteins is sufficient for the heat production.

In conclusion, with a wide TNZ and a high T_{uc}, Mongolian gerbils provide us with a useful animal model to investigate membrane remodeling during acute thermoregulation in non-hibernating endotherms. Our study suggested significant responses in the mitochondrial membrane associated with acute thermoregulation in Mongolian gerbils and also identified several lipid biomarkers that could participate in mammalian acute thermoregulation. Further study examining the interactions of these lipid biomarkers with mitochondrial membrane proteins will be required to understand the mechanism underlying acute thermoregulation in non-hibernating endotherms such as Mongolian gerbils.

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Conflict of interest The authors have declared that no conflicting interests exist.

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