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Effects of lithium orotate on cold-associated adipose tissue remodeling in young mice

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Article

Keywords: lithium orotate, cold exposure, adipose tissue remodeling, brown adipose tissue, beige adipocytes, UCP1

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11467359/v1

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Additional Declarations: There is **NO** conflict of interest to disclose

Abstract

Background/Objectives:

Cold exposure promotes thermogenic responses in brown adipose tissue (BAT) and beige changes in white adipose tissue (WAT). However, whether lithium orotate (LiOr) modifies these responses remain unclear. We examined the effects of LiOr, alone and in combination with cold exposure in mice and cultured brown adipocytes.

Subjects/Methods:

Male C57BL/6J mice received LiOr (500 μM in drinking water) or vehicle for 4 weeks, with cold exposure (6.5°C) in the final week. Body weight gain, adipose tissue mass, lipid droplet morphology, thermogenic gene expression, body temperature, and mitochondrial uncoupling protein 1 (UCP1) abundance were assessed in interscapular BAT (iBAT), epididymal and inguinal white adipose tissue (eWAT and ingWAT). Brown adipocytes were treated with 100 μM LiOr for 7 days from the onset of differentiation, for 2 days, or for 2 h; respiration was measured.

Results

Body weight gain was significantly lower in mice receiving LiOr with cold exposure than in all other groups, with reduced eWAT mass and increased iBAT mass relative to room-temperature mice; ingWAT mass was unchanged. Lipid droplet morphology was altered in all depots, and the ingWAT appeared beige. In iBAT, *Ucp1* and *Prdm16* increased with cold exposure and further with LiOr, with increased mitochondrial UCP1 protein; in ingWAT, both increased with cold exposure and further with LiOr, whereas *Pparγ* and *Pgc-1α* increased with LiOr alone. LiOr-treated mice maintained better body temperature in the cold, only 7-day LiOr exposure increased proton leak *in vitro*.

Conclusions

LiOr modified cold-associated changes in body weight gain and adipose tissue, including the beige-like phenotype in ingWAT. Prolonged LiOr exposure increased proton leak *in vitro*, indicating altered respiratory coupling. Together with better body temperature maintenance during cold exposure, these findings suggest that LiOr contributes to thermogenic function.

Introduction

Obesity is a major global health challenge and a leading risk factor for type 2 diabetes, cardiovascular diseases and other metabolic complications. Excess adiposity established early in life tends to persist with lasting alterations in adipose tissue biology and an increased risk of adverse metabolic outcomes in adulthood [1, 2]. Dietary and lifestyle interventions remain the foundation of efforts to limit weight gain however, strategies acting on energy expenditure may offer complementary opportunities to influence energy balance during early life.

Thermogenic adipose tissue dissipates chemical energy as heat rather than storing it as triglycerides and has therefore attracted attention as a determinant of energy balance [3, 4]. Brown adipose tissue (BAT) mediates non-shivering thermogenesis largely through mitochondrial uncoupling protein 1 (UCP1), which dissipates the mitochondrial proton gradient and uncouples substrate oxidation from ATP synthesis [5, 6]. White adipose tissue (WAT) is primarily an energy-storage depot however, it displays considerable plasticity, and in response to stimuli, beige adipocytes with thermogenic characteristics emerge within WAT depots [3, 4, 7]. The transcriptional regulators PRD1-BF-1-RIZ1 homologous domain containing 16 (PRDM16), peroxisome proliferator-activated receptor gamma (PPARγ), and PPARγ coactivator-1α (PGC-1α) contribute to thermogenic adipocyte identity and mitochondrial oxidative programs [4, 8, 9].

Cold exposure is the classical stimulus for thermogenic recruitment. Cold-induced sympathetic activation and norepinephrine signaling increase thermogenic gene expression in BAT and promote beige remodeling of WAT [3, 4, 10]. The responses of individual depots are not equivalent; however, they depend on age, ambient temperature and duration of exposure. Cold exposure also elicits metabolic adaptations that extend beyond BAT [10, 11]. Moreover, increased thermogenic gene expression or altered lipid droplet morphology alone do not establish an enhanced thermogenic capacity *in vivo* [3, 4, 7, 12]; thus, interventions should be assessed functionally in more than one depot.

Lithium is a monovalent cation best known for its clinical use in bipolar disorder. Low-dose lithium supplementation has been reported to promote adipose tissue browning and to alter energy expenditure, body mass and adipose tissue metabolism in rodents [13, 14], suggesting that lithium-sensitive pathways may influence thermogenesis. However, the metabolic effects of lithium are context-dependent, and the findings obtained with one lithium formulation cannot be automatically generalized to another.

Most experimental studies have used lithium chloride (LiCl) and most clinical studies use lithium carbonate (Li_2CO_3), whereas lithium orotate (LiOr) remains comparatively underexplored. Pharmacokinetic comparisons of LiOr with LiCl and Li_2CO_3 have reached inconsistent conclusions regarding differences in lithium uptake, distribution and elimination [15, 16], and there is insufficient evidence that LiOr is intrinsically more bioavailable, more effective or safer than other salts. Whether LiOr influences thermogenesis-associated responses in the adipose tissue has not been systematically assessed, and changes in adipocyte differentiation do not necessarily predict the respiratory activity of mature adipocytes.

The timing and duration of exposure are further considerations, as adipose tissue is metabolically dynamic early in life, and interventions at this stage may influence the trajectory of body weight gain and adipose tissue remodeling [2, 17]. Whether LiOr modifies cold-associated adipose tissue responses in young animals is unknown, and it is not known whether prolonged exposure during brown adipocyte differentiation affects mitochondrial respiratory parameters such as proton leak. Therefore, we examined the effects of LiOr alone and in combination with cold exposure in young male mice to assess body

weight gain, adipose tissue mass and lipid droplet morphology in interscapular BAT (iBAT), epididymal WAT (eWAT), and inguinal WAT (ingWAT), as well as thermogenic gene expression and mitochondrial UCP1 abundance. In parallel, we compared Li^+-responsive fluorescence in primary cultured brown adipocytes treated with LiOr, LiCl or Li_2CO_3 and measured mitochondrial oxygen consumption after varying durations of LiOr exposure. We hypothesized that LiOr would modify cold-associated metabolic and adipose tissue responses in young mice and that these responses would differ between brown and white depots.

Materials and Methods

Animals

Six-week-old C57BL/6J male mice were purchased from Japan SLC (Shizuoka, Japan) and housed at 22 – 24°C under a 12:12 h light:dark cycle with standard chow diet and water or 500 μM LiOr (BOC Sciences, Shirley, NY, USA) provided *ad libitum*. Body mass, food intake and water intake were recorded weekly and water bottles were refreshed weekly. At 9 weeks of age, mice were exposed to 6.5°C for one week while continuing to receive water or 500 μM LiOr. The iBAT, eWAT and ingWAT were dissected from 10-week-old mice and weighed. All procedures followed the Animal Care Guidelines of the Animal Experimental Committee of Fukuoka University (Approval No. 2513106).

Histology

Anesthetized mice were transcardially perfused with phosphate-buffered saline followed by 10% neutral-buffered formalin. iBAT, eWAT and ingWAT were dissected, post-fixed in the same fixative for 1 day at 4°C, embedded in paraffin and sectioned at 4 μm. Hematoxylin and eosin (H&E)-stained sections were imaged, the lipid droplet area was measured using a BZ-X710 microscope (Keyence, Osaka, Japan), and at least three random fields were analyzed per section.

Real-time PCR analysis

Total RNA was isolated from iBAT, eWAT and ingWAT using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), purified using an RNeasy Mini kit (QIAGEN, Venlo, Netherlands), and reverse-transcribed using the QuantiTect Reverse Transcription kit (QIAGEN). Quantitative real-time PCR was performed using a QuantiNova SYBR Green PCR kit (QIAGEN) on a StepOne Real-Time PCR System (Thermo Fisher Scientific). Primers for the genes of interest and the housekeeping gene *36B4* are listed in Supplemental Table 1.

Western blotting

Western blotting

Frozen iBAT was homogenized in 300 mM sucrose, 10 mM HEPES, 1 mM EGTA, and 1 mg/ml BSA, pH 7.4 (mitochondrial isolation medium, MIM). Homogenates were centrifuged at 4°C, 10,000 × *g* for 10 min. The supernatant was discarded and the samples were centrifuged at 4°C, 200 × *g* for 5 min twice. Each time, the supernatant was collected and centrifuged at 4°C, 10,000 × *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in MIM. The protein concentration was determined with the Pierce BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein were mixed with 4× Laemmli sample buffer. Samples (10 μg) were separated by SDS-PAGE on 12.5% polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes (Merck, Darmstadt, Germany). Membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (T-TBS) for 1 h at room temperature and incubated overnight at 4°C with rabbit anti-UCP1 antibody (1:1000, Abcam, Cambridge, UK, ab10983) or a total oxidative phosphorylation (OXPHOS) antibody cocktail (1:1000, Abcam, ab110413). After three washes with T-TBS, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:10,000; Proteintech Group, Rosemont, IL, USA, RGAR001) for 1 h at room temperature. After three washes with T-TBS, immunoreactive bands were visualized using the ECL Plus reagent (Cytiva, Marlborough, MA, USA) and detected using an LAS-4000 mini imaging system (Fujifilm, Tokyo, Japan).

Cold tolerance test

Core body temperature and locomotor activity were monitored using telemetry (TA-F10 transmitters, DSI Company, USA) implanted in the peritoneal cavity under anesthesia for at least 5 days before the test, with each cage placed on an RPC-1 receiver plate (Harvard Bioscience, Inc., Holliston, MA, USA) and analyzed using NOTOCORD-Hem (Instem, Staffordshire, UK).

Primary culture of mouse brown adipocytes

Primary brown adipocytes were obtained from male mice as previously reported with slight modifications [18]. iBAT from 3 – 5-week-old mice (4 – 5 mice per preparation) was minced and digested in Dulbecco's modified Eagle's medium (DMEM, Fujifilm Wako Pure Chemical Corp., Osaka, Japan) containing 2 mg/ml collagenase type 1 (Merck) and 2% bovine serum albumin (BSA, Merck) at 37°C for 1 h with constant agitation. The digest was filtered through 100 and 40 μm nylon screens and centrifuged twice at 800 × g for 10 min. Pellets were resuspended in DMEM containing 10% fetal bovine serum (FBS, biowest, Nuaillé, France), 100 U/ml penicillin and 100 μg/ml streptomycin (growth medium) and incubated at 37°C in 5% CO_2. After 24 h incubation, preadipocytes were washed and the growth medium replaced. Once confluent, preadipocytes were incubated for 2 days in growth medium supplemented with 500 μM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, 1 μM rosiglitazone (Merck), and 5 μg/ml human recombinant insulin (Fujifilm Wako Pure Chemical Corp.) (differentiation medium), which was then replaced with growth medium containing 5 μg/ml insulin every other day until the experiment.

Live-cell Li imaging

At the differentiation step, adipocytes were treated with 100 μM LiOr, LiCl, or Li_2CO_3 (Fujifilm Wako Pure Chemical Corp.) in differentiation medium for 2 days (Li_2CO_3 is 200 μM in terms of Li^+) and then replaced with growth medium containing 5 μg/ml insulin every other day until the experiment. Intracellular Li^+ dynamics were monitored with ILG-1 AM (ION Biosciences, San Marcos, TX, USA). Adipocytes were washed three times with bath solution (150 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.4), incubated with 5 μM ILG-1 AM containing 0.02% Pluronic F-127 for 60 min at 37°C (CO_2-free), washed three times again, and exposed to bath solution for 10 min at 37°C. Fluorescence signals were collected using an ORCA-Fusion CMOS camera (Hamamatsu Photonics, Shizuoka, Japan) and a CSU-X1 confocal scanner unit (Yokogawa Electric Corp., Tokyo, Japan) at 2 s intervals and analyzed with NIS-Elements software (Nikon Corp., Tokyo, Japan).

Oxygen consumption measurements

Primary brown preadipocytes were plated in Seahorse 24-well microplates (Agilent, Santa Clara, CA, USA) and treated with 100 μM LiOr for 7 days from the start of differentiation, for 2 days or 2 hours before the measurement. Adipocytes were incubated in differentiation medium for 2 days and then replaced with growth medium containing 5 μg/ml insulin every other day. Growth medium containing insulin was replaced with Seahorse XF DMEM medium (Agilent) containing 5.6 mM glucose, 1 mM pyruvate, and 2 mM glutamate, pH 7.4, and cells were incubated for 60 min at 37°C (CO_2-free) before loading into an XFe24 extracellular flux analyzer (Agilent). After steady basal consumption rates were obtained, norepinephrine (Merck) was injected to a final concentration of 1 μM, followed by oligomycin (2 μM), carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, 2 μM) and finally rotenone plus antimycin A (1 μM each; Agilent, 103015-100) to obtain the non-mitochondrial respiration fraction.

Statistical analysis

All data are presented as means ± SEM. Comparisons were made using two-way ANOVA, and statistical significance was set at $P < 0.05$. Data with only two groups were analyzed using two-tailed Student's *t* test. Data were analyzed using Origin 2019 software (Origin Lab, Northampton, MA, USA).

Results

LiOr enhances Li^+-responsive fluorescence in primary brown adipocytes

To compare cellular Li^+ responses to different lithium salts, primary brown adipocytes were treated with LiOr, LiCl, or Li_2CO_3 (100 μM each) before the induction of differentiation, and fluorescence imaging was performed on day 6 when differentiation was complete (Fig. 1A). Li^+ imaging using ILG-1 AM revealed fluorescent responses to all three compounds (Fig. 1B). Quantification showed that LiOr and Li_2CO_3 significantly increased fluorescence intensity relative to the vehicle control, whereas LiCl produced the

weakest response and LiOr elicited the highest intensity of the three salts (Fig. 1C). Therefore, LiOr elicits the strongest response to the three salts under the conditions examined.

Combined LiOr treatment and cold exposure alter body weight gain and adipose tissue mass in young mice

Six-week-old mice received drinking water containing 500 μM LiOr *ad libitum* for 4 weeks and were exposed to cold (6.5°C) during the final week (Fig. 2A). Body weight gain was significantly lower in mice receiving LiOr together with cold exposure than that in the other three groups (vehicle at room temperature, vehicle with cold exposure and LiOr at room temperature) (Fig. 2B). Food intake was higher in the cold-exposed mice than in the room temperature mice (Fig. 2C), whereas water intake tended to be higher in the LiOr-treated, cold-exposed group, without reaching statistical significance (Fig. 2D). At the end of the experiment, adipose and skeletal muscles were collected and weighed (Fig. 2E). The eWAT mass was significantly lower in the combined-treatment group than in the comparison groups, whereas ingWAT mass did not differ significantly among groups; macroscopic inspection revealed a conspicuously more beige appearance of ingWAT in the combined-treatment group. The iBAT mass was significantly higher in the combined-treatment group than in the vehicle- and LiOr-treated groups maintained at room temperature (Fig. 2F), and skeletal muscle mass did not differ among the groups. Thus, LiOr combined with cold exposure attenuated body weight gain in young mice fed a standard diet, with reduced eWAT mass and increased iBAT mass but no significant changes in the ingWAT or non-adipose tissues examined.

Combined LiOr treatment and cold exposure alter lipid droplet morphology in adipose tissues

To determine whether these changes were accompanied by histological alterations, H&E-stained sections of iBAT, eWAT and ingWAT were examined (Fig. 3A). Lipid droplet morphology differed notably in all three adipose depots in the combined-treatment group, and quantification showed a significant increase in the number of small lipid droplets relative to the analyzed lipid droplet area in iBAT, eWAT, and ingWAT (Fig. 3B). Conspicuous changes in the ingWAT lipid droplet morphology were also observed after LiOr treatment alone. Therefore, the combined treatment altered lipid droplet morphology across brown and white adipose tissues, and the changes in ingWAT after LiOr treatment alone suggest that this response may not depend entirely on cold exposure.

LiOr enhances UCP1 and PRDM16 expression in brown adipose tissue under cold exposure

Thermogenesis-related and adipocyte regulatory factors were examined in iBAT. Cold exposure increased *Ucp1* and *Prdm16* mRNA expression, with further increases after LiOr treatment, whereas *Pparγ* and *Pgc-1α* mRNA expression did not change significantly (Fig. 4A). Western blotting of isolated mitochondrial fractions showed that UCP1 protein abundance increased with cold exposure and further

increased with LiOr treatment, while OXPHOS complex V protein increased in the LiOr-treated group (Fig. 4B). LiOr treatment under cold-exposure conditions is therefore associated with increased *Ucp1* and *Prdm16* mRNA expression and increased mitochondrial UCP1 abundance in brown adipose tissue, without detectable changes in *Pparγ* or *Pgc-1α* mRNA.

Combined LiOr treatment and cold exposure increase thermogenesis-related gene expression in inguinal white adipose tissue

Ucp1, *Prdm16*, *Pparγ*, and *Pgc-1α* mRNA expression was assessed in ingWAT. Cold exposure increased *Ucp1* and *Prdm16* expression, with further increases after LiOr treatment, while *Pparγ* and *Pgc-1α* expression was significantly increased not only by combined treatment but also by LiOr alone (Fig. 4C). Therefore, the combined treatment increased the expression of the four thermogenic genes in ingWAT, alongside the lipid droplet changes described above (Fig. 3A, B).

LiOr enhances thermogenic function under cold environment

Body temperature was measured to assess whether an increase in thermogenic gene expression was accompanied by a functional effect. Mice treated with LiOr maintained their body temperature better than control mice under cold exposure (Fig. 4D), whereas locomotor activity did not differ between the groups (Fig. 4E). These findings suggest that LiOr enhances the maintenance of body temperature in cold environments.

Prolonged LiOr exposure increases mitochondrial proton leak in primary brown adipocytes

The oxygen consumption rate (OCR) was measured in primary cultured mouse brown adipocytes using an XFe24 flux analyzer. Cells were exposed to 100 μM LiOr from the onset of differentiation induction for 7 days, for 2 days after the completion of differentiation, or for 2 h (Fig. 5A), and basal respiration, norepinephrine-stimulated respiration, maximal respiration and proton leak were assessed (Fig. 5B). A significant increase in proton leak relative to the vehicle was observed only in cells treated from the onset of differentiation for 7 days (Fig. 5C), consistent with an alteration in mitochondrial respiration associated with proton leak, whereas maximal respiration did not differ among the four groups.

Discussion

This study examined whether LiOr, alone or in combination with cold exposure, modifies body weight gain, adipose tissue characteristics, body temperature and mitochondrial respiration in young mice and cultured brown adipocytes. LiOr modified cold-associated responses in a depot-specific and context-dependent manner, with the most consistent effects observed when LiOr was combined with cold

exposure. Together with better body temperature maintenance during cold exposure, these findings suggest that LiOr contributes to thermogenic functions either directly or indirectly, although energy expenditure was not measured.

The attenuation of body weight gain was confined to mice receiving LiOr with cold exposure, suggesting that the effect on body weight depends on the environmental context rather than being a strong effect under standard conditions. Previous studies have indicated that low-dose lithium supplementation can influence body mass and adipose tissue metabolism, although the responses vary with experimental conditions and sex [13, 14]. Cold exposure elicits broad metabolic adaptations involving BAT and several other tissues that cannot be attributed exclusively to brown adipose thermogenesis [10, 11]. Therefore, LiOr may modify the metabolic response to cold rather than act as a body-weight-lowering agent.

This interpretation is limited by two caveats. Cold-exposed mice ate more than mice at room temperature and food intake did not match. Therefore, since energy expenditure was not measured, the contributions of intake and expenditure to the difference in body weight gain cannot be separated. Changes in tissue mass cannot be distinguished from altered lipid storage, adipocyte size, adipocyte number and tissue remodeling. The divergent behavior of the three depots—reduced eWAT mass, unchanged ingWAT mass, and increased iBAT mass relative to room temperature groups—indicated that the response was not a uniform reduction in adipose tissue mass, and increased iBAT mass should not be equated with increased thermogenic capacity.

Interpretations must also consider the animals used in this study. Early life exposure can influence adipose tissue development and later metabolic physiology [2, 17]; however, the present study used young animals fed a standard chow diet over a relatively short period and did not employ an established model of diet-induced obesity. Therefore, the attenuation of body weight gain should not be interpreted as evidence that LiOr prevents obesity or elicits a sustained effect on body weight.

In iBAT, cold exposure increased *Ucp1* and *Prdm16* mRNA expression and these responses were further enhanced by the combined treatment, whereas *Pparγ* and *Pgc-1α* mRNA expression showed little change. UCP1 is the central mediator of mitochondrial uncoupling in classical brown adipocytes, and PRDM16 contributes to thermogenic adipocyte identity [4, 5, 8, 9]. Thus, the parallel increase in UCP1 protein levels in the mitochondrial fraction is consistent with a shift in the thermogenesis-related molecular profile of iBAT. The response was selective, being restricted to *Ucp1* and *Prdm16* among the transcripts examined, although transcript abundance does not necessarily reflect protein abundance or tissue function [3–5]; the change in oxidative phosphorylation complex V abundance requires confirmation. A greater iBAT mass does not necessarily indicate greater thermogenic capacity, as tissue enlargement may reflect changes in lipid content, cellularity, or vascularization, and BAT thermogenesis is not obligatorily coupled to *de novo* lipogenesis [19]. Respiration measurements in isolated iBAT and *in vivo* assessments of the thermogenic activity are required to determine whether these changes translate into increased heat production [5, 6].

The most conspicuous observation was the beige-like appearance of the ingWAT in mice receiving LiOr with cold exposure, which was accompanied by altered lipid droplet morphology and increased *Ucp1* and *Prdm16* expression. *Pparγ* and *Pgc-1α* mRNA levels were also increased in this group. These convergent macroscopic, histological, and molecular findings support a beige adipocyte-associated phenotype in this depot. Notably, *Pparγ* and *Pgc-1α* expression increased not only with the combined treatment but also with LiOr alone, suggesting that part of this response may not depend entirely on cold exposure. White adipose tissue browning is regulated by multiple pathways, including AMPK/PGC-1α signaling, myostatin–miR-34a signaling, the leptin–STAT3–PRDM16 axis, and adipocyte mitochondrial quality control [20–23], and pharmacological browning has been induced through melanocortin signaling [24]; the present data do not identify which of these pathways, if any, mediates the effect of LiOr.

These two aspects of the ingWAT phenotype warrant caution. Macroscopic color is qualitative and may reflect factors other than beige adipocyte abundance or activity. Neither UCP1 nor PRDM16 expression, even with altered lipid droplet morphology, is sufficient to demonstrate UCP1-dependent heat production [3, 4, 7, 12]. The dissociation between these changes and the absence of a change in ingWAT mass further suggests a shift in the tissue phenotype rather than a reduction in depot size. Quantification of beige adipocytes, measurement of UCP1 protein and mitochondrial function within the ingWAT, and direct evaluation of tissue thermogenic activity are required to establish their functional significance [12, 25].

Consistent with these molecular findings, mice treated with LiOr maintained their body temperature better than the control mice during cold exposure, whereas locomotor activity did not differ. As this group also gained less weight, the better thermal performance is unlikely to be explained by body size alone, as a smaller body mass implies greater rather than lesser heat loss. However, because weight gain was suppressed despite equal or higher food intake, this was not inconsistent with increased energy expenditure. Together with the increased *Ucp1* and *Prdm16* expression, mitochondrial UCP1 abundance, and proton leak in cultured brown adipocytes, this suggests that LiOr contributes to thermogenic function during cold exposure, directly through its action on thermogenic adipocytes or indirectly through the remodeling of iBAT and ingWAT and cold-induced adaptations beyond BAT [11]. This suggestion remains provisional because body temperature was recorded in only a few animals and energy expenditure was not assessed directly.

In primary cultured brown adipocytes, LiOr exposure from the onset of differentiation for seven days significantly increased proton leak, whereas treatment for two days after differentiation or two hours did not alter the measured respiratory endpoints. Therefore, the effect on respiratory coupling appears to depend on the duration of exposure, and possibly on the differentiation stage of the cells, consistent with the role of UCP1-mediated proton conductance in adaptive thermogenesis [5, 6]. Proton leak is not synonymous with UCP1-dependent heat production because proton conductance can arise from other processes. The absence of significant changes in the other endpoints should not be read as evidence that these processes were unaffected. Studies combining UCP1 inhibition or deletion with measurements of cellular heat production are required to define the mechanism. The two systems also

differ in exposure route, tissue environment and regulation; therefore, the cultured-cell finding provides complementary evidence that LiOr can alter respiratory behavior under defined conditions, but does not establish that the same mechanism accounts for the changes *in vivo*.

With a lithium-responsive fluorescent probe, LiOr and Li_2CO_3 increased fluorescence relative to the vehicle control, whereas LiCl produced the weakest response, and LiOr the largest. Fluorescence intensity cannot be equated directly to intracellular lithium concentration unless the probe has been calibrated and the effects on probe uptake, retention, and fluorescence are excluded. Although previous studies have provided a rationale for examining LiOr in cold-associated adipose tissue remodeling [13, 14], the present study does not establish whether LiOr is metabolically superior to other lithium salts, nor does it distinguish between the effects of lithium ions, the orotate component and differences in cellular exposure. Comparisons at matched lithium concentrations with appropriate counterion controls and with measurements of lithium in plasma and tissues are required to resolve these possibilities [15, 16].

This study had several limitations. The animal experiments used young male mice over a relatively short treatment period; therefore, the findings cannot be generalized to females, older animals, established obesity, or long-term outcomes. Adipose tissue remodeling was assessed based on tissue mass, histological appearance, selected transcript measurements and UCP1 protein abundance, while whole-body energy expenditure, body composition, and energy intake were not controlled for or measured. Finally, neither the safety nor the long-term consequences of LiOr administration were addressed.

In conclusion, LiOr modified cold-associated changes in body weight gain and adipose tissue characteristics in young male mice, and the effects were depot-specific: eWAT mass was reduced, iBAT showed increased *Ucp1* and *Prdm16* expression with increased mitochondrial UCP1 abundance, and ingWAT displayed a beige-like phenotype without a change in mass. Prolonged LiOr exposure during brown adipocyte differentiation increases mitochondrial proton leak, indicating that LiOr can influence respiratory coupling *in vitro*. Future studies should measure whole-body energy expenditure and body composition, assess respiration in freshly isolated adipose tissue and thermogenic activity *in vivo*, directly quantify beige adipocytes and UCP1-dependent proton conductance, and compare LiOr with other lithium salts at matched lithium concentrations to determine whether these changes reflect increased functional thermogenesis and whether they are attributable to the lithium ion, the orotate moiety, or their combination.

Declarations

Conflict of Interest

The authors declare no conflicts of interest.

Funding Information

This work was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS) [Grant Numbers 25K10173 to Tomo Kita, 23K14448 to Yasuharu Shinoda and 25K10195 to Takayuki Nemoto]. This work was also supported by research and experimental training funds provided by Fukuoka University to the participating departments and research units.

Author contributions

T. Kita. was responsible for the funding acquisition, conceptualization, methodology, investigation, formal analysis, data curation, visualization, and writing of the original draft. Y. Shinoda contributed to the funding acquisition, investigation, methodology, and data curation. T. Komatsu contributed to investigation, methodology, and data curation. T. Tachibana contributed to investigation, methodology, and data curation. S. Ochiai. contributed to the investigation and methodology. Y. Uehara. contributed to the supervision, project administration, writing, reviewing, and editing. T. Nemoto contributed to conceptualization, supervision, project administration, funding acquisition, writing, reviewing, and editing. All authors contributed to the interpretation of results, critically reviewed the manuscript and approved the final version.

Acknowledgements

The authors sincerely thank Kumi Fujitsu, Education and Technical Staff, Department of Pharmacology, Faculty of Medicine, Fukuoka University, for her invaluable technical assistance and dedicated support throughout all the experimental procedures in this study.

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

1. Palacios-Marin I, Serra D, Jiménez-Chillarón JC, Herrero L, Todorčević M. Childhood obesity: implications on adipose tissue dynamics and metabolic health. Obes Rev. 2023;24:e13627.
2. Donato J Jr. Programming of metabolism by adipokines during development. Nat Rev Endocrinol. 2023;19:385–397.
3. Shamsi F, Wang CH, Tseng YH. The evolving view of thermogenic adipocytes—ontogeny, niche and function. Nat Rev Endocrinol. 2021;17:726–744.
4. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nat Med. 2013;19:1252–1263.

5. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004;84:277–359.
6. Fedorenko A, Lishko PV, Kirichok Y. Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. Cell. 2012;151:400–413.
7. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell. 2012;150:366–376.
8. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, et al. PRDM16 controls a brown fat/skeletal muscle switch. Nature. 2008;454:961–967.
9. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, et al. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest. 2011;121:96–105.
10. Richard D, Carpentier AC, Doré G, Ouellet V, Picard F. Determinants of brown adipocyte development and thermogenesis. Int J Obes. 2010;34:S59–S66.
11. van Beek S, Hashim D, Bengtsson T, Hoeks J. Physiological and molecular mechanisms of cold-induced improvements in glucose homeostasis in humans beyond brown adipose tissue. Int J Obes. 2023;47:338–347.
12. Lee P, Werner CD, Kebebew E, Celi FS. Functional thermogenic beige adipogenesis is inducible in human neck fat. Int J Obes. 2014;38:170–176.
13. Geromella MS, Ryan CR, Braun JL, Finch MS, Maddalena LA, Bagshaw O, et al. Low-dose lithium supplementation promotes adipose tissue browning and sarco(endo)plasmic reticulum Ca^{2+} ATPase uncoupling in muscle. J Biol Chem. 2022;298:102568.
14. Finch MS, Gardner GL, Braun JL, Geromella MS, Murphy J, Colonna K, et al. Creatine and low-dose lithium supplementation separately alter energy expenditure, body mass, and adipose metabolism for the promotion of thermogenesis. iScience. 2024;27:109468.
15. Smith DF. Lithium orotate, carbonate and chloride: polydipsia and polyuria in rats. Br J Pharmacol. 1976;56:399–402.
16. Pacholko AG, Bekar LK. Different pharmacokinetics of lithium orotate inform why it is more potent, effective, and less toxic than lithium carbonate in a mouse model of mania. J Psychiatr Res 2023;164:192–201.
17. Hill KB, Mullen GP, Nagareddy PR, Zimmerman KA, Rudolph MC. Key questions and gaps in understanding adipose tissue macrophages and early-life metabolic programming. Am J Physiol Endocrinol Metab. 2024;327:E478–E497.
18. Sun W, Uchida K, Suzuki Y, Zhou Y, Kim M, Takayama Y, et al. Lack of TRPV2 impairs thermogenesis in mouse brown adipose tissue. EMBO Rep. 2016;17:383–99.
19. Wei C, Wang P, Dong Q, Ma XH, Lu M, Qi S, et al. ChREBP-regulated lipogenesis is not required for the thermogenesis of brown adipose tissue. Int J Obes. 2022;46:1068–1075.

20. Zhang X, Zhang QX, Wang X, Zhang L, Qu W, Bao B, et al. Dietary luteolin activates browning and thermogenesis in mice through an AMPK/PGC1α pathway-mediated mechanism. Int J Obes. 2016;40:1841–1849.
21. Ge X, Sathiakumar D, Lua BJG, Kukreti H, Lee M, McFarlane C. Myostatin signals through miR-34a to regulate Fndc5 expression and browning of white adipocytes. Int J Obes. 2017;41:137–148.
22. Gan L, Liu Z, Feng F, Wu T, Luo D, Hu C, et al. Foxc2 coordinates inflammation and browning of white adipose by leptin-STAT3-PRDM16 signal in mice. Int J Obes. 2018;42:252–259.
23. Chen S, Yuan W, Huang Q, Xiong X, Wang C, Zeng W, et al. Asprosin contributes to pathogenesis of obesity by adipocyte mitophagy induction to inhibit white adipose browning in mice. Int J Obes. 2024;48:913–922.
24. Rodrigues AR, Salazar MJ, Rocha-Rodrigues S, Gonçalves IO, Cruz C, Neves D, et al. Peripherally administered melanocortins induce mice fat browning and prevent obesity. Int J Obes. 2019;43:1058–1069.
25. Ma Y, Zhang Z, Ramos ET, Martin E, Gunukula R, Sprabary K, et al. Optimizing adipogenic cocktail composition to enhance beige adipogenesis and evaluate thermogenic potential in primary mouse subcutaneous fat cell cultures. Int J Obes. 2026;50:386–396.

Figures

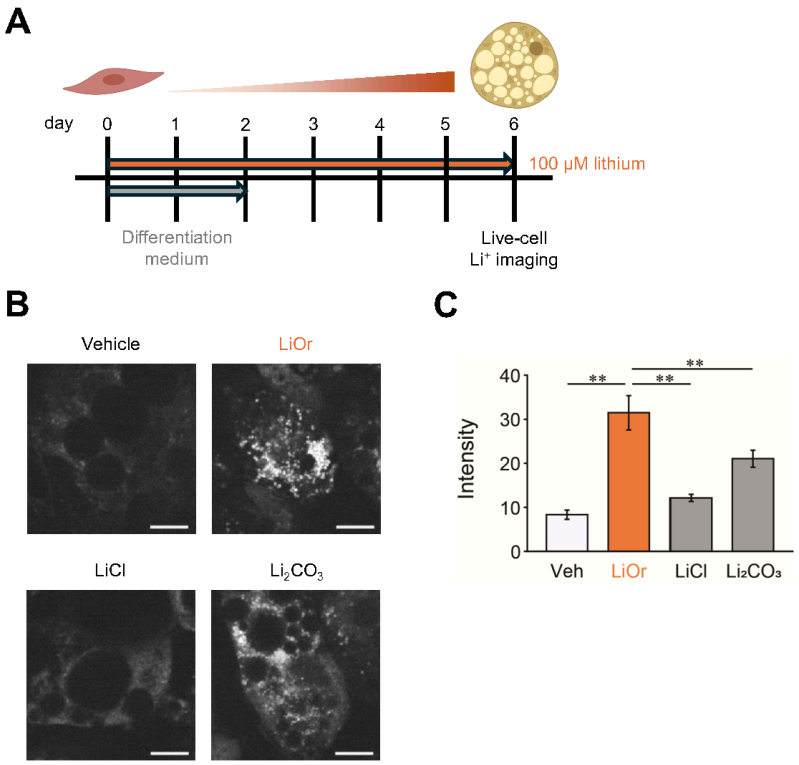


Figure 1.

Figure 1

Comparison of Li^+-responsive fluorescence in primary brown adipocytes treated with different lithium salts.

A Experimental protocol showing treatment with lithium orotate (LiOr), LiCl, or Li_2CO_3 at 100 μM before the induction of differentiation, with fluorescence imaging on day 6. **B** Representative time-lapse

fluorescence images of primary brown adipocytes loaded with the Li-responsive probe, ILG-1 AM, after treatment with the indicated lithium salts. Scale bars, 20 μm. **C** Quantification of fluorescence intensity in cells treated with the indicated lithium salts. Data are presented as mean ± SEM (*n*= 9–18). *P < 0.05, **P < 0.01.

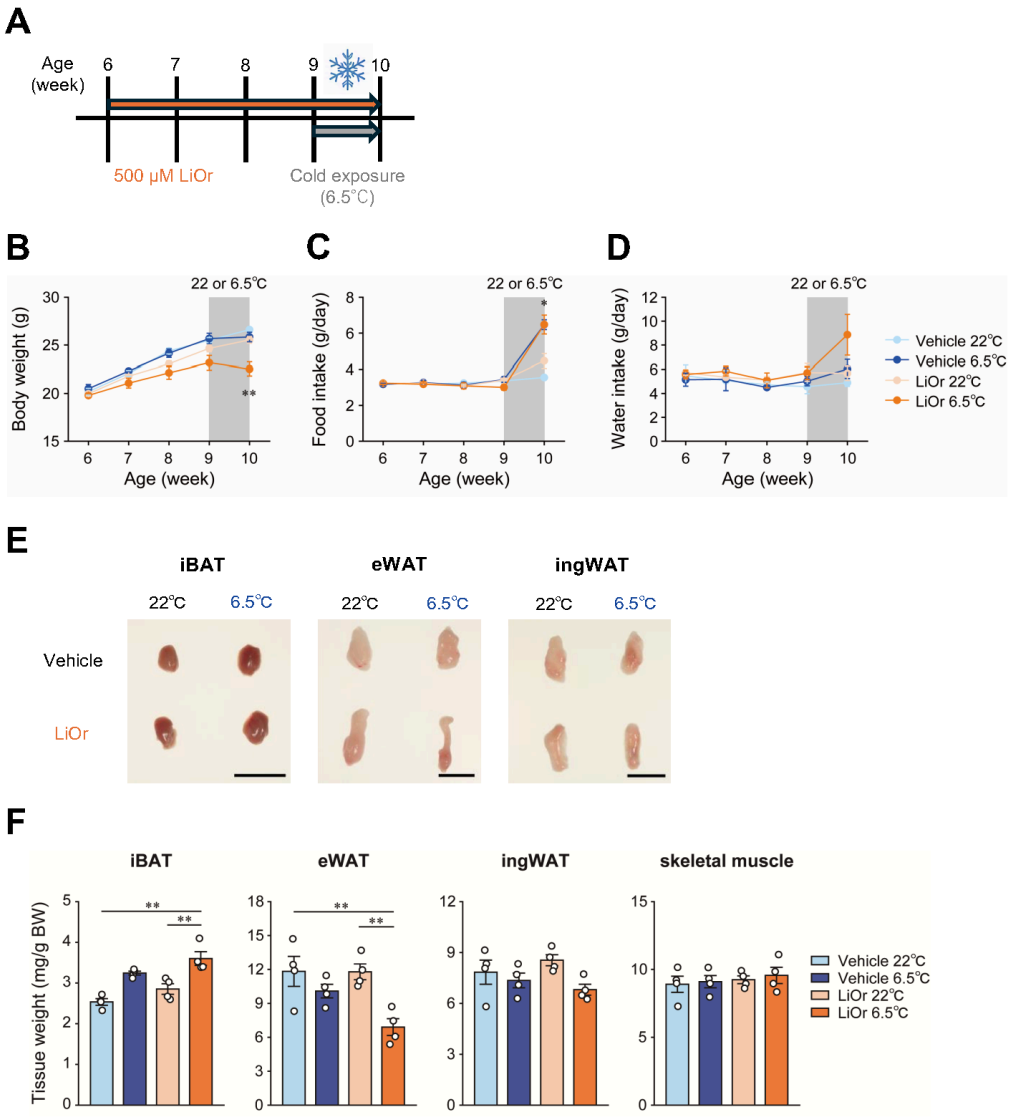


Figure 2.

Figure 2

Effects of LiOr treatment and cold exposure on body weight gain and tissue mass in young mice.

A Experimental protocol. Six-week-old mice received drinking water containing 500 μM LiOr or vehicle *ad libitum* for 4 weeks and were maintained at room temperature or exposed to cold (6.5°C) during the final week. Body weight gain (**B**), food intake (**C**), and water intake (**D**) over a 4-week experimental period. **E** Overview of tissue collection at the end of experiment. **F** Masses of interscapular brown adipose tissue (iBAT), epididymal white adipose tissue (eWAT), inguinal white adipose tissue (ingWAT) and skeletal muscle. Data are presented as mean ± SEM (n = 5–7). *P < 0.05, **P < 0.01.

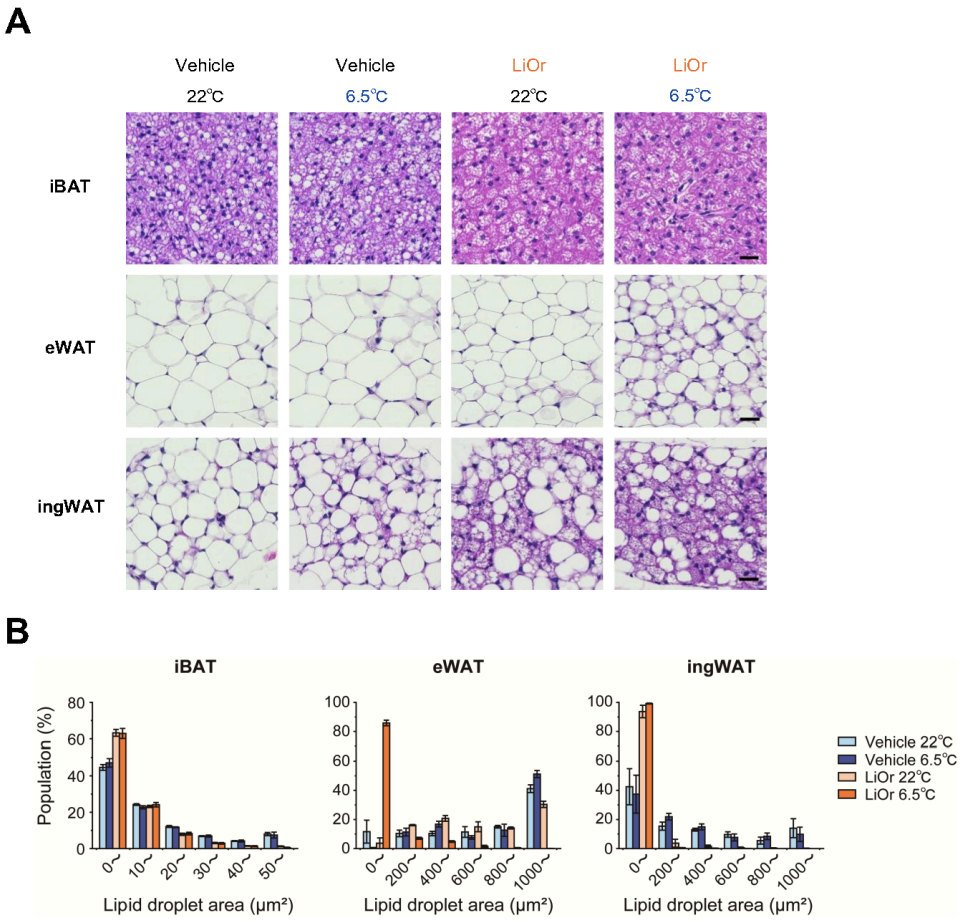


Figure 3.

Figure 3

Effects of LiOr treatment and cold exposure on lipid droplet morphology in brown and white adipose tissues.

A Representative hematoxylin and eosin (H&E)-stained sections of iBAT, eWAT, and ingWAT from mice receiving vehicle or LiOr treatment under normal or cold conditions. Scale bars indicate 20 μm. **B**

Quantification of the lipid droplet number relative to the analyzed lipid droplet area in iBAT, eWAT, and ingWAT. Data are presented as SEM images (*n* = 3).

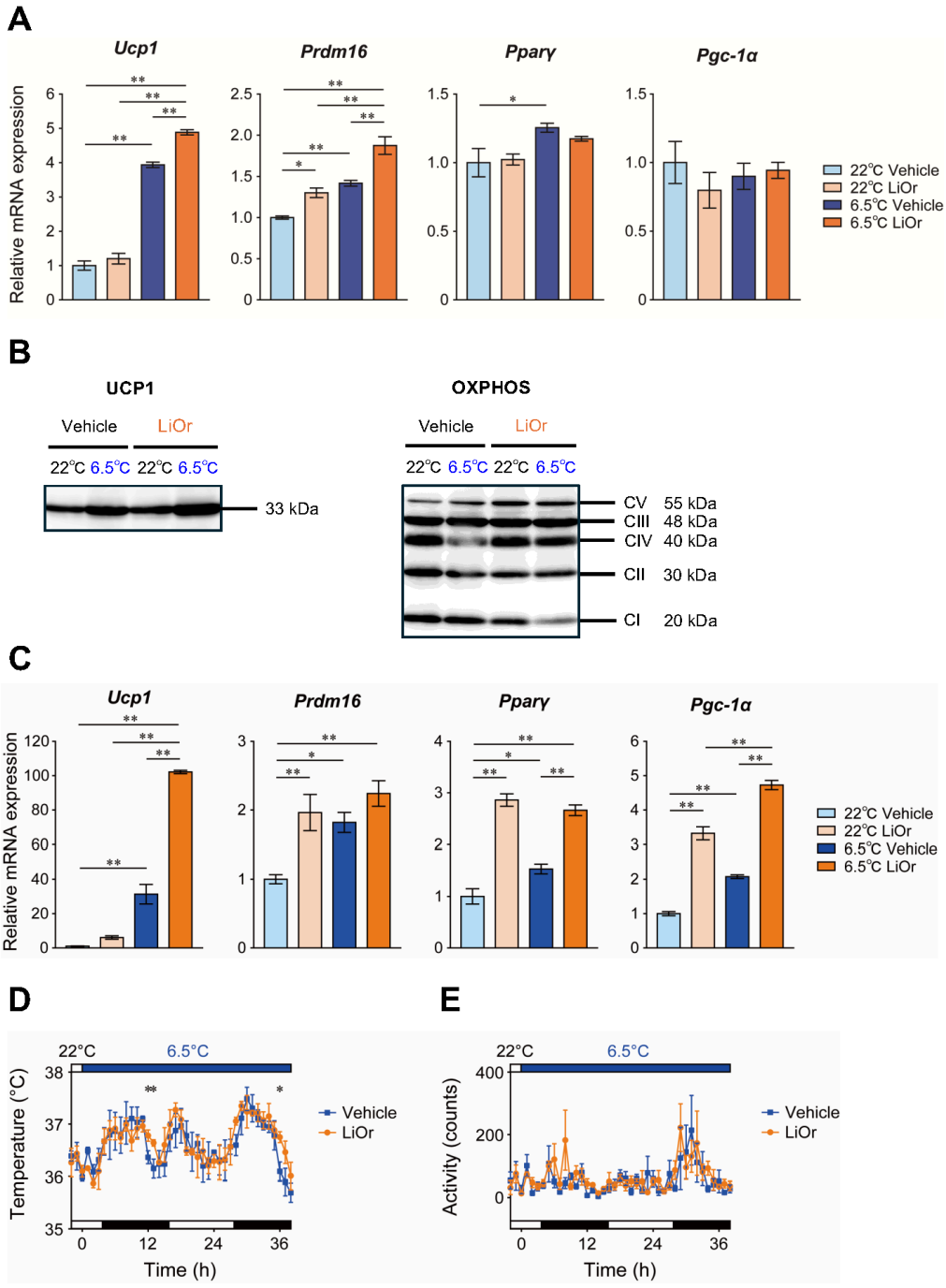


Figure 4.

Figure 4

Effects of LiOr treatment and cold exposure on thermogenic gene expression, mitochondrial protein abundance, and thermogenic function.

A Relative mRNA expression of *Ucp1*, *Prdm16*, *Pparγ*, and *Pgc-1α* in iBAT, normalized to *36B4*. Data are presented as mean ± SEM (*n* = 6). **B** Representative western blots of UCP1 and oxidative phosphorylation (OXPHOS) complexes I–V in the mitochondrial fractions isolated from iBAT. **C** Relative mRNA expression of *Ucp1*, *Prdm16*, *Pparγ*, and *Pgc-1α* in ingWAT. Data are presented as mean ± SEM (*n*= 6). Body temperature (**D**) and locomotor activity (**E**). Data are presented as mean ± SEM (*n* = 4).

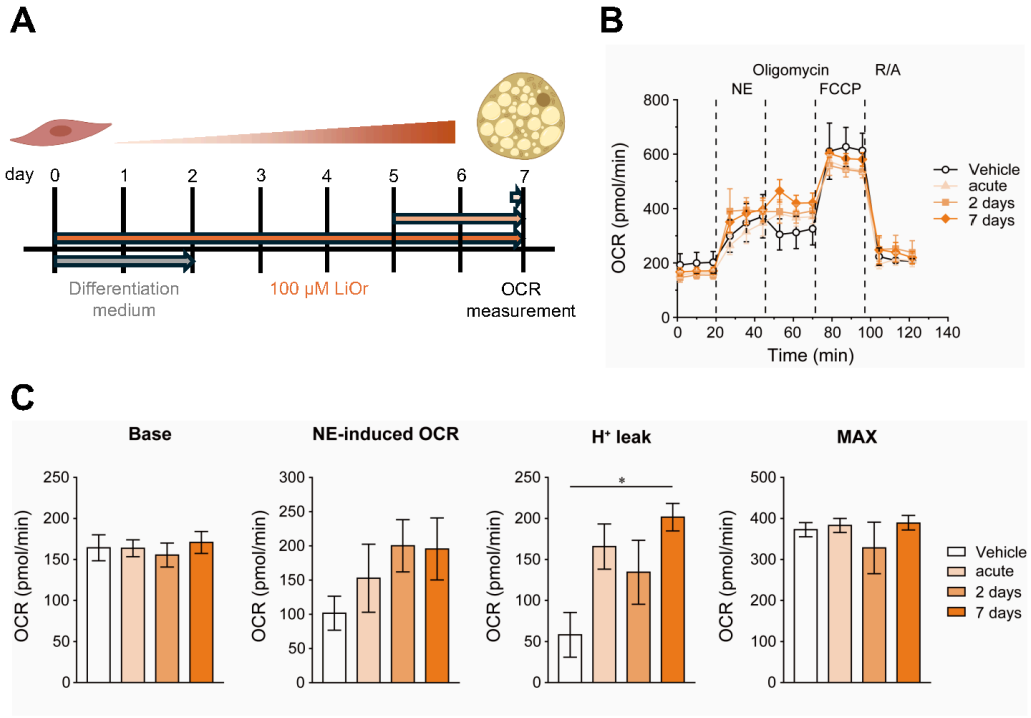


Figure 5.

Figure 5

Effects of LiOr exposure on mitochondrial respiration in primary cultured brown adipocytes.

A Schematic representation of LiOr treatment schedule. **B** Oxygen consumption rate (OCR) profiles measured using a flux analyzer. **C** Quantification of basal respiration, norepinephrine-stimulated respiration, maximal respiration and proton leak under the indicated treatment conditions. Data are presented as mean ± SEM (*n*= 4–5). *P < 0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.docx