Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism
(Fiscal Year 2025)

Date：2026/03/20

To Director of Institute for Molecular and Cellular Regulation, Gunma University

Principal Applicant	
Institution	Academia Sinica, Taiwan
Position	Associate Research Fellow
Name	Yi-Ching Lee

We report on the results of joint research in fiscal year 2025 as below.

（Program No. 24005）

1． Research Title	Regulation and interplay between iron and ascorbic acid in epigenetic rewriting during adipocyte differentiation
2． Purpose and Significance of the research project	This research extends our 2024 supported project to investigate the dynamic relationship between ascorbic acid and iron in regulating epigenetic modifications during adipocyte differentiation. Adipogenesis is essential for metabolic health, with both ascorbic acid and iron playing critical roles in adipogenesis and the pathogenesis of obesity. However, the co-regulatory mechanisms remain unclear. Our recent findings suggest that ascorbic acid has a more significant impact on brown adipocyte differentiation than on white adipogenesis. Brown adipose tissue (BAT) is vital for energy homeostasis, thermogenesis, and metabolism, actively dissipating energy as heat and contributing to overall metabolic regulation. This collaborative study aims to explore how ascorbic acid and iron interact at the epigenetic level during brown adipocyte differentiation, uncovering key regulatory factors. By understanding the molecular mechanisms of BAT differentiation, this research aims to provide insights into metabolic disorders like obesity and diabetes, potentially leading to novel therapeutic strategies that enhance BAT activity to improve energy expenditure and combat metabolic dysfunction.
3． Period of The Program	April 1, 2025～ March 31, 2026

4． Project Members

Name	Age	Sex	Affiliation	Position	Role
（Principal Applicant） Yi-Ching Lee	54	F	Institute of Cellular and Organismic Biology, Academia Sinica	Position：Associate Research Fellow Degree：PhD Acquisition date：2003.01.03	Principal investigator
（Research Collaborators） Chung-Lin Jiang	36	M	Institute of Cellular and Organismic Biology	Postdoctoral Research Fellow	Experimental Planning and Analysis

※If additional space is required, please attach a separate sheet.

5. Collaborating Researcher of IMCR	Name of Laboratory	Epigenetics and Metabolism	Name	Dr. Inagaki Takeshi

6. Research Plans

This collaboration will employ state-of-the-art techniques to explore novel molecular mechanisms with the potential to inform therapeutic interventions for metabolic diseases. In current proposal includes the following objectives:

Investigation of Ascorbic Acid Effects in Brown Adipocyte Differentiation

We will assess the dose- and time-dependent effects of ascorbic acid on brown adipocyte differentiation using stromal vascular fraction (SVF) cells isolated from brown adipose tissues. This will involve testing various concentrations and exposure times of ascorbic acid to evaluate its impact on cell differentiation. Subsequently, we will explore how ascorbic acid influences cellular iron homeostasis to cooperative regulate adipogenesis.

Ascorbic Acid and Iron Modulation

To investigate the effects of ascorbic acid on iron homeostasis, which may affect epigenetic modification. The cellular and nuclear ascorbic acid content will be assayed using an Ascorbic Acid Assay Kit (Abcam, Cambridge, UK). The cellular and nuclear iron status will be assessed by measuring transferrin receptor transcript levels and protein accumulation, which serve as indicators of cellular iron availability.

DNA Methylation

We will analyze DNA demethylation by performing whole-genome bisulfite sequencing (WGBS). CpG methylation levels within 1 kb upstream of the transcription start site (TSS) will be evaluated, following protocols established in Dr. Inagaki's recent publication. This will provide insights into how ascorbic acid influences DNA methylation patterns during brown adipocyte differentiation.

Histone Modification Profiling

Histone modifications will be assessed using the Global Histone H3 Multiplex Assay Kit (Abcam). This will allow us to examine key histone marks, offering a detailed understanding of how ascorbic acid and iron influence epigenetic regulation during brown adipocyte differentiation.

Examine the epigenetic mechanisms underlying brown adipocyte differentiation

Gene Expression Analysis: To elucidate the functional impact of these epigenetic changes on brown adipocyte differentiation, we will analyze the gene expression levels using RT-PCR. This analysis will focus on genes associated with regions showing differential DNA methylation and histone modifications, aiming to determine whether their expression is directly regulated by these epigenetic changes.

Functional Validation: To validate the role of identified epigenetic modifications, we will utilize CRISPR-based epigenetic editing tools. For example, dCas9 fused with demethylase or histone acetyltransferase domains will be used to specifically modify DNA methylation or histone marks at target loci. Effects on brown adipocyte differentiation will be assessed using established differentiation assays.

7. Research results:

Please describe the details of the contribution of the joint research with IMCR in obtaining the results.

Ascorbic Acid Regulates Brown Adipocyte Differentiation

We demonstrated that ascorbic acid (AA, vitamin C) can reprogram stromal vascular fraction (SVF) by pretreating SVFs for 2 days with or without AA 2 days before inducing brown adipocyte differentiation. SVFs pretreated under vitamin C insufficient conditions showed significantly reduced brown adipogenesis. Although we do not directly measure intracellular iron levels, we observed that vitamin C insufficient increased of alpha-ketoglutarate levels and reduced succinate levels in SVFs, suggesting metabolic changes that may affect by vitamin C and iron-dependent enzymes activities and epigenetic regulation.

Ascorbic Acid Impacts Histone Modification

We next examined how ascorbic acid affects the epigenetic landscape during brown adipocyte differentiation. Through buck RNA sequencing and pathway analysis, we found that cultured SVFs isolated from

brown adipose tissue of postnatal day 7 mice and cultured under vitamin C-insufficient conditions showed significantly downregulation of histone demethylation pathways and upregulation of histone methylation pathways. These findings suggest that vitamin C strongly influences histone modification in SVFs. We further profiled the histone modifications by western blotting and identified specific histone demethylation mark that was significantly reduced under vitamin C-insufficient conditions. This modification was previously been implicated in the regulation of gene expression and stem cell differentiation. Together, these results indicate that ascorbic acid affects histone methylation status and thereby influences brown adipogenesis.

Ascorbic Acid Affects Histone Modification and Gene Expression to Regulate Brown Adipogenesis in vivo

Key genes involved in thermogenic brown fat function were further validated by RT-PCR and Western blots analysis in intrascapular brown adipose tissue (iBAT) from offspring of maternal vitamin C-sufficient and vitamin C-deficient mice. The expression of genes involved in mitochondrial biogenesis, oxidative metabolism, and UCP1, was significantly downregulated in the iBAT of offspring from vitamin C-deficient dams. Notably, a master regulator required for activation of the thermogenic program was also significantly downregulated under maternal vitamin C deficiency. To investigate the underlying epigenetic mechanism, we then examine histone modification status at the UCP1 promoter in vitamin C-deficient SVFs, where predicted binding sites for this regulator are present. We found that a specific histone trimethylation mark was significantly enriched at the regulator binding sites and near the transcription start site of the Ucp1 promoter. Importantly, this enrichment was already detectable in undifferentiated SVFs that do not yet express Ucp1, indicating early epigenetic priming at the progenitor stage.

Conclusion

Collectively, this joint research with IMCR shows that ascorbic acid is an important regulator of brown adipocyte differentiation through metabolic and epigenetic mechanisms. Vitamin C insufficiency impaired brown adipogenesis, altered metabolic intermediates, and shifted the epigenetic landscape toward reduced histone demethylation and increased histone methylation, leading to suppression of thermogenic genes such as **Ucp1**. Because many histone demethylases are iron- and α-ketoglutarate–dependent enzymes, these findings suggest that vitamin C may influence brown adipogenesis partly through iron-dependent epigenetic regulation.

8． Present status of academic conference presentations and research papers associated with the results of the joint research, and exchange of information on the joint research with the collaborating researcher at IMCR.
(As much as possible, please state papers that include the names of the collaborating researcher at IMCR or papers stating that the research was supported by the Joint Research Program with IMCR.
Regarding papers, please send a PDF file together with the report to the email address of the general affairs section of the Institute.) Office of General Affairs: kk-msomu4@jimu.gunma-u.ac.jp

① Please list the publications that include the name of the collaborating researcher from IMCR and send a reprint of each publication to IMCR.

Although no joint publications have been finalized at this stage, the ongoing analyses are progressing well and will form the basis for future co-authored submissions.

② Please list the publications that include a description that the research was supported by the Joint Research Program with IMCR and send a reprint of each publication to IMCR.

No publications have been submitted or published yet that formally achnowledge support from the IMCR Joint Research Program. However, a manuscript is currently in preparation and will include appropriate acknowledgment upon submission.

③ List up to 3 conferences (name of conference, date of conference, and title of the presentation).

We have presented the results at the following meetings under the title: **“**Maternal Vitamin C Programs Thermogenic Fat Development and Metabolic Health.”

(1) Visit to the laboratory of LEM, Institute for Molecular and Cellular Regulation (IMCR), Gunma Uni-

versity, March15-18, 2016
(2) Interesting group (IG) meeting, Academia Sinica, January 28, 2026
(3) PI progress reports, ICOB, Academia Sinica, December 11, 2025

These presentations provided valuable comments and feedback that helped refine the interpretation of the results and the direction of the ongoing study.

④ Exchange of information exchange with collaborating researcher from IMCR (please list main points of communication).

We maintained active and productive communication with Dr. Takeshi Inagaki throughout the project period, regularly updating him on our progress and discussing experimental strategies and interpretations. The main points of communication included the following:

(1) Careful interpretation of early epigenetic priming at the progenitor stage.
(2) Direct assessment of the impact of vitamin C on intracellular iron levels using the iron reporter developed by Dr. Takeshi Inagaki team, together with assessment of potential mechainsm through measurement of transferrin receptor transcript levels and protein accumulation, which serve as indicators of cellular iron availability.
(3) Testing whether the effects of vitamin C deficiency are mediated through its antioxidant activity by treating vitamin C-deficiency SVFs with antioxidants, such as N-acetylcysteine or mitoTEMPO, and examining whether these treatments rescue the observed defects.
(4) Genome-wild profile of the specific histone mark occupancy and chromatin accessibility in SVFs under vitamin C-sufficient and vitamin C-insufficient conditions, and correlation of these profiles with gene expression changes.
(5) Determination of whether vitamin C enhance mitochondrial function in preadipocytes.
(6) Evaluation of the transgenerational impact of vitamin C–dependent epigenetic imprinting on the F2 generation.