



CHUN SHAN MEDICAL SCHOOL

***INTERACTION BETWEEN DIETARY PROTEIN AND
ENVIRONMENTAL TEMPERATURE IN ADIPOSE TISSUE
BROWNING***

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Introduction

- Research Background
- Research Motivation
- Research Objectives



TWO MAIN TYPES

- WAT and BAT use glucose and fatty acids to regulate energy storage and consumption.

PREVIOUS RESEARCH HAS SHOWN

- Under "transformation" conditions, WAT forms "beige" fat cells.
(Characterized by increased expression of specific markers such as UCP1.)

RECENT RESEARCH SHOWS

- Reducing the amount of dietary protein increases FGF21 expression in the liver.
- As a result, FGF21 circulation increases:
 - ① Stimulating gluconeogenesis
 - ② Fatty acid oxidation
 - ③ Accelerating fat breakdown in WAT

INTERESTINGLY

- Adrenaline stimulates FGF21 expression in BAT but not in the liver.



THE DIFFERENCES BETWEEN THESE THREE TYPES

CONTENTS	BAT (Brown Adipose Tissue)	WAT (White Adipose Tissue)	Beige Adipose Tissue
Oil droplet	Smaller	Mutiple, but not large	Huge
Mitochondria	Numerous	More	Few
UCP1	Exists	Does not exist	Does not exist
Function	Burn energy, generate heat		Store energy



UCP1 UNCOUPLING PROTEIN 1 (ALSO KNOWN AS THERMOGENIN)

01

SPECIFIC TO MAMMALS

A gene unique to mammals.

02

BAT MITOCHONDRIA

A gene unique to mammals.

03

THERMOGENIC REACTION

- Helps keep the body warm in cold environments by producing heat.
- Burns excess energy by converting fat and sugar directly into heat without making ATP, which helps prevent obesity.

04

EXPRESSION LEVEL

The amount of UCP1 gene expression and protein in the body affects heat production. A defect in the UCP1 gene can reduce energy use, potentially leading to obesity.



FGF21 (FIBROBLAST GROWTH FACTOR 21)

01

A TYPE OF LIVER ENZYME

A hormone secreted by the liver that signals through the FGF21 receptor in the paraventricular nucleus of the hypothalamus.

02

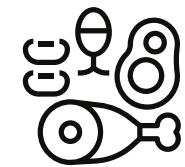
REGULATES SUGAR INTAKE

03

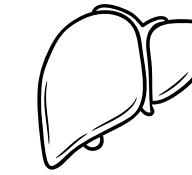
PROMOTES INSULIN SECRETION

04

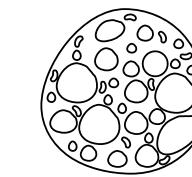
LOWERS BLOOD SUGAR/FAT



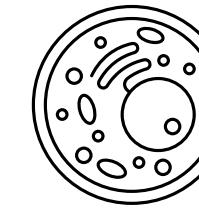
Low-protein diet



Increases FGF21
expression and
circulating levels.

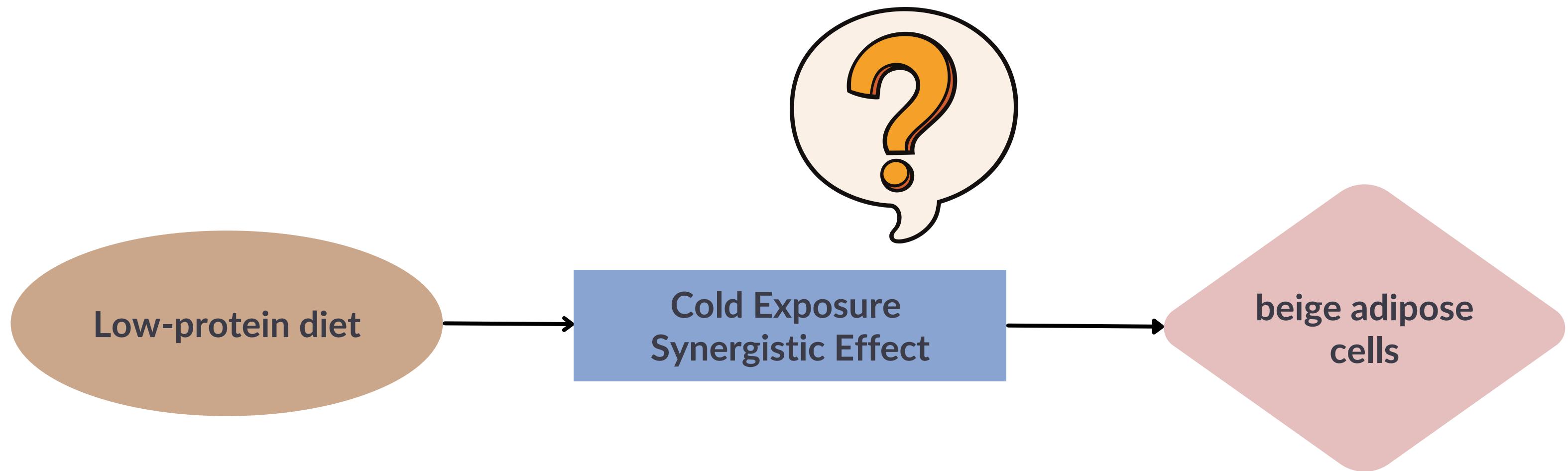


CONVERTS
white adipose tissue



INTO
beige adipose cells

This leads to an increase in the expression of PGC1 α and UCP1 mRNA.



It remains unclear whether a low-protein diet's stimulation of WAT browning can synergize with another browning stimulus, such as cold exposure.



1.

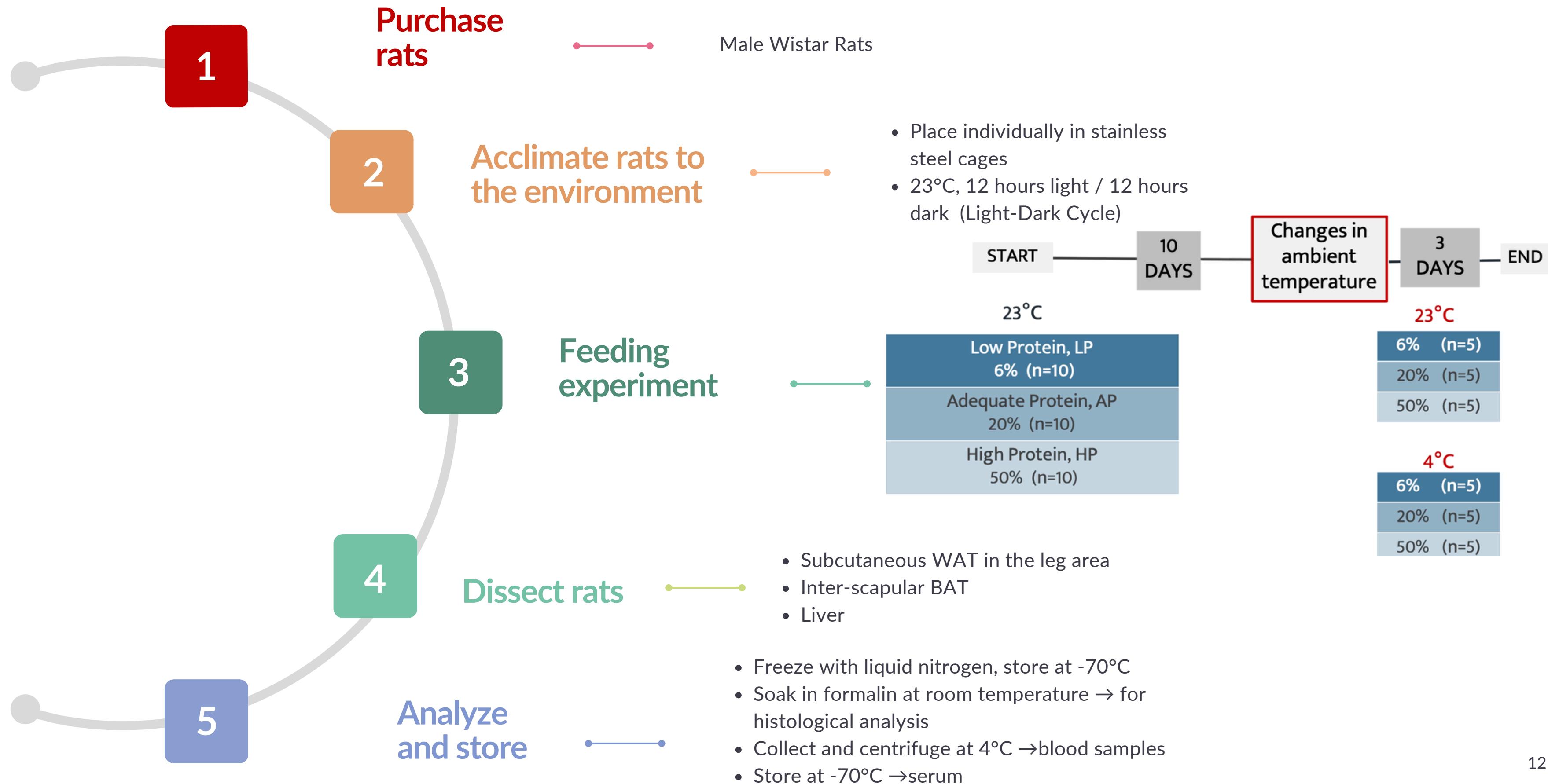
Evaluate the expression of browning markers in WAT and thermogenesis in BAT under low- or high-protein diets.

2.

Evaluate the expression of FGF21 circulation in the liver and BAT of rats at room temperature or in cold conditions.

Materials & Methods

-
- Materials
 - Methods





Body composition

- Blood glucose analyzer
- Enzymatic colorimetric assay
- Nuclear magnetic resonance

All mRNA relative quantities

Real-time quantitative PCR

Blood biochemical change

Enzyme immunoassay

WAT, BAT and relative quantities of γ -tubulin

Western blot

Measurement of BAT and subcutaneous WAT protein content

Immunoluminescence analysis

Indirect calorimetry

CLAMS metabolism measurement system

Results & Discussion

-
- Results
 - Discussion



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01

Body weight

02

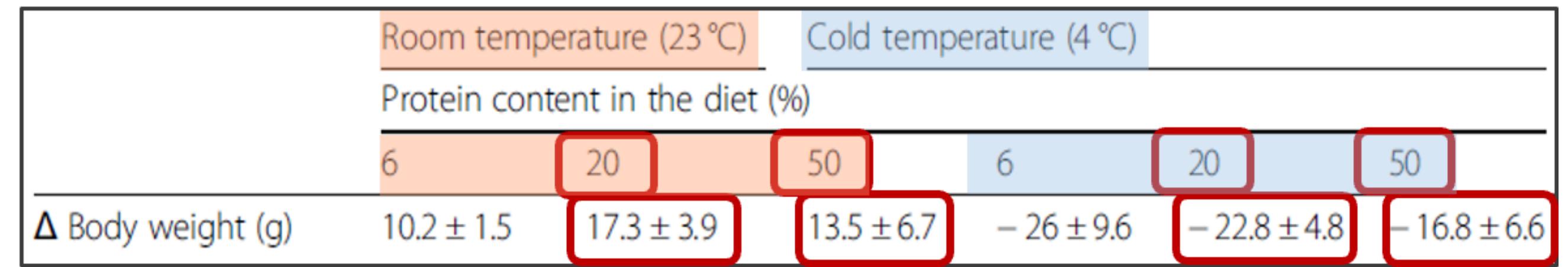
Glucose

03

Triacyglycerols

04

Fat body mass



AP and HP show greater weight gain

HP shows less reduction.

01

Body weight

02

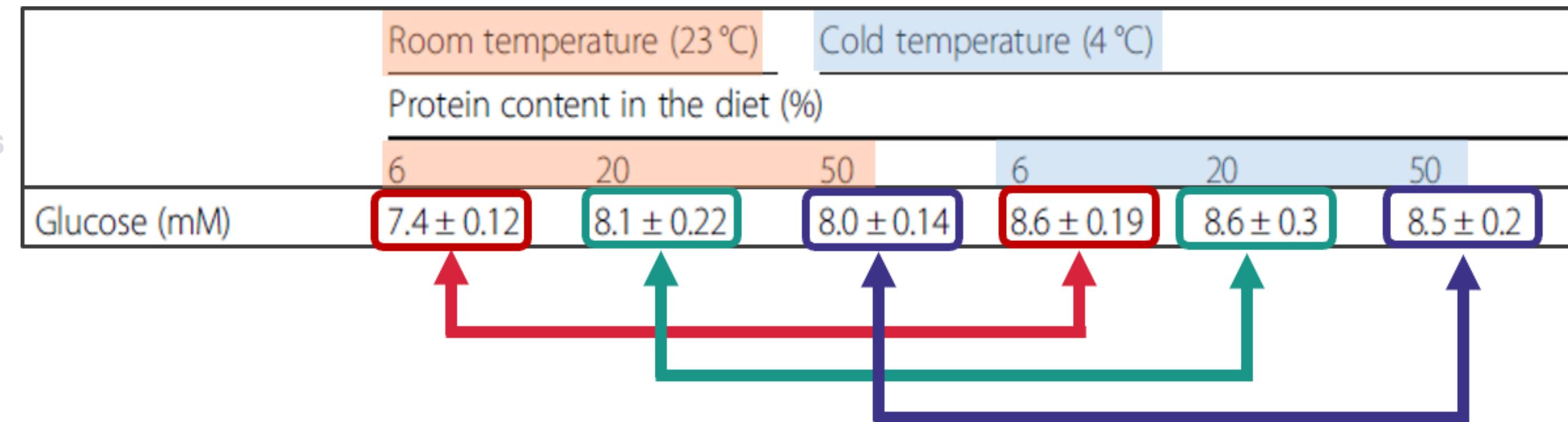
Glucose

03

Triacyglycerols

04

Fat body mass



Approximately 9% more at low temperature compared to room temperature.

01

Body weight

02

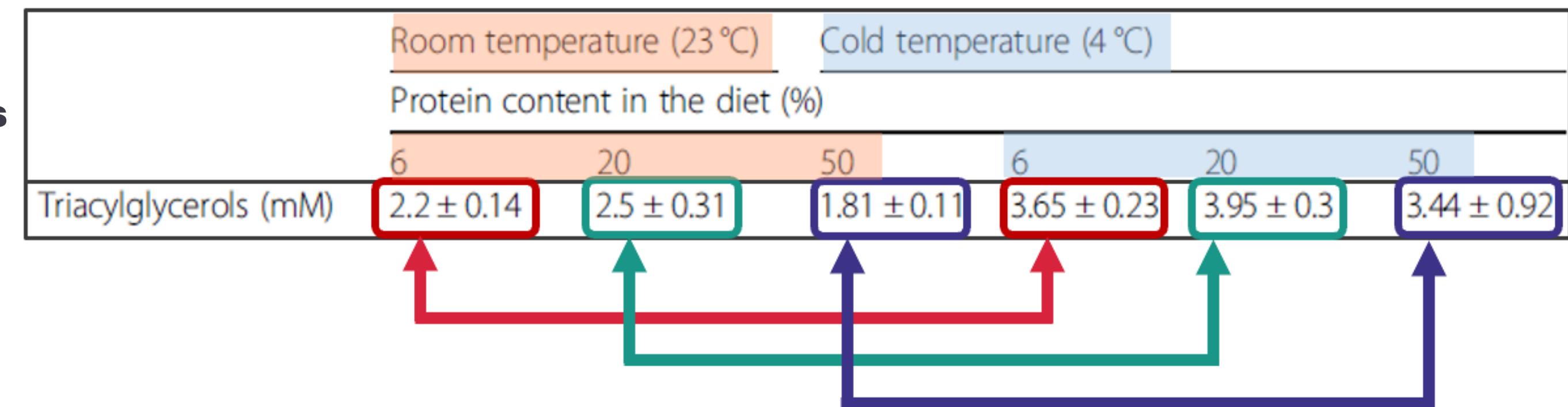
Glucose

03

Triacylglycerols

04

Fat body mass



Approximately 69% more at low temperature compared to room temperature.

01

Body weight

02

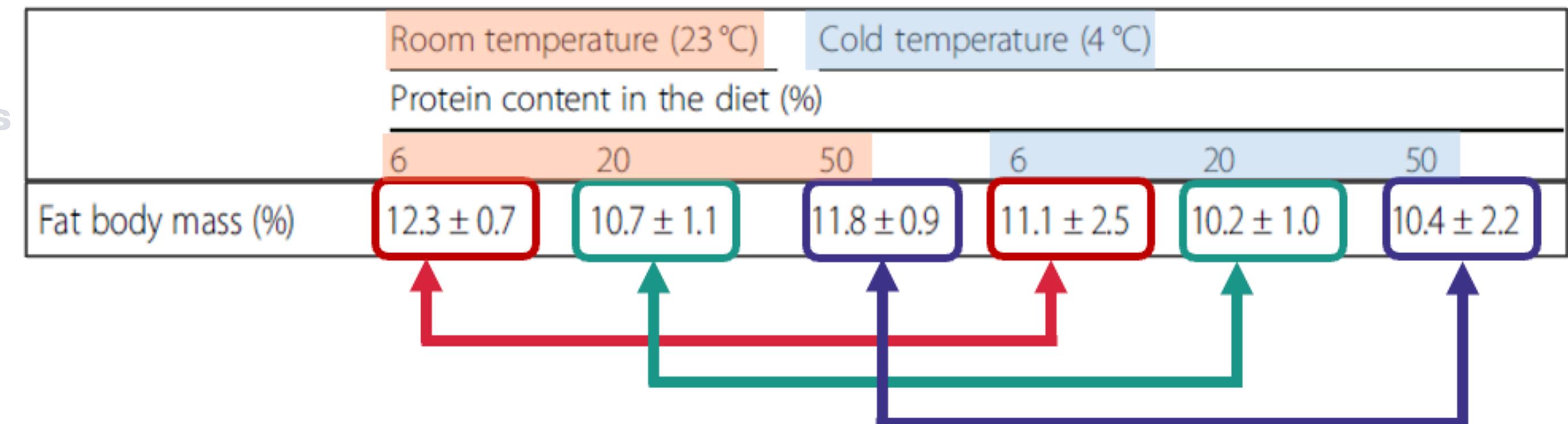
Glucose

03

Triacyglycerols

04

Fat body mass



Maintain at low temperature and room temperature.



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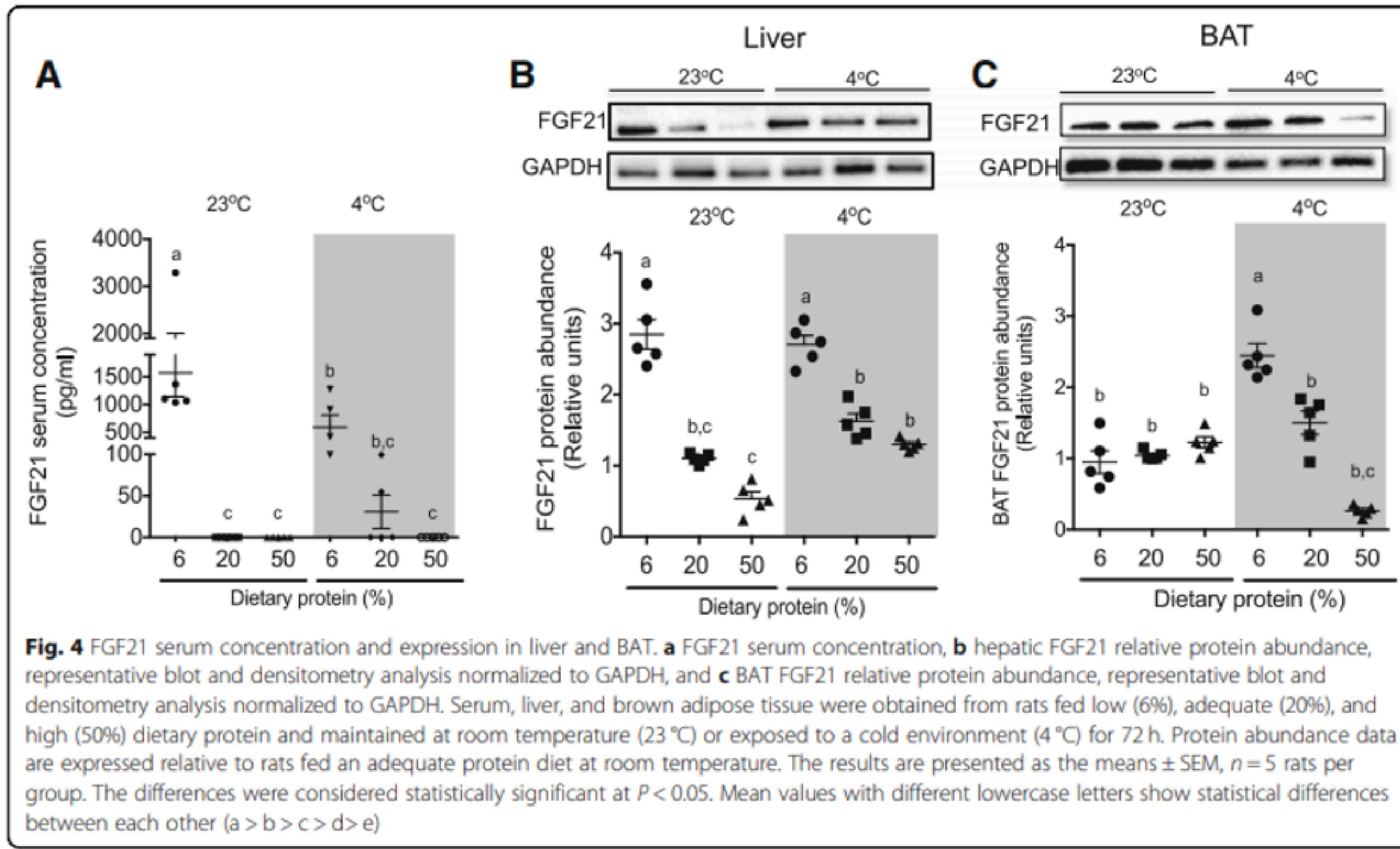
Immunoluminescence analysis

Indirect calorimetry

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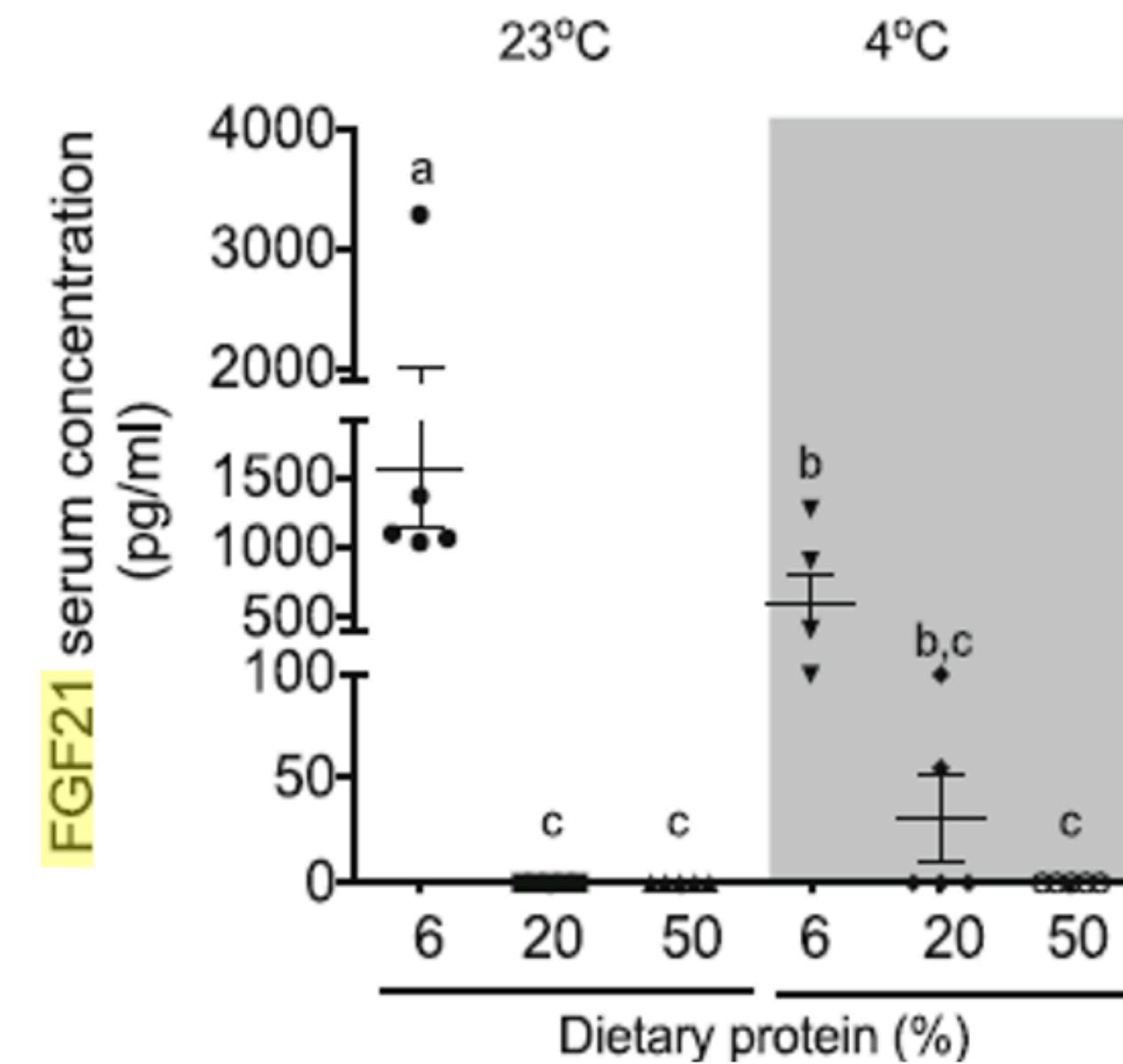


01

FGF21 serum concentration

02

Liver FGF21 protein expression levels



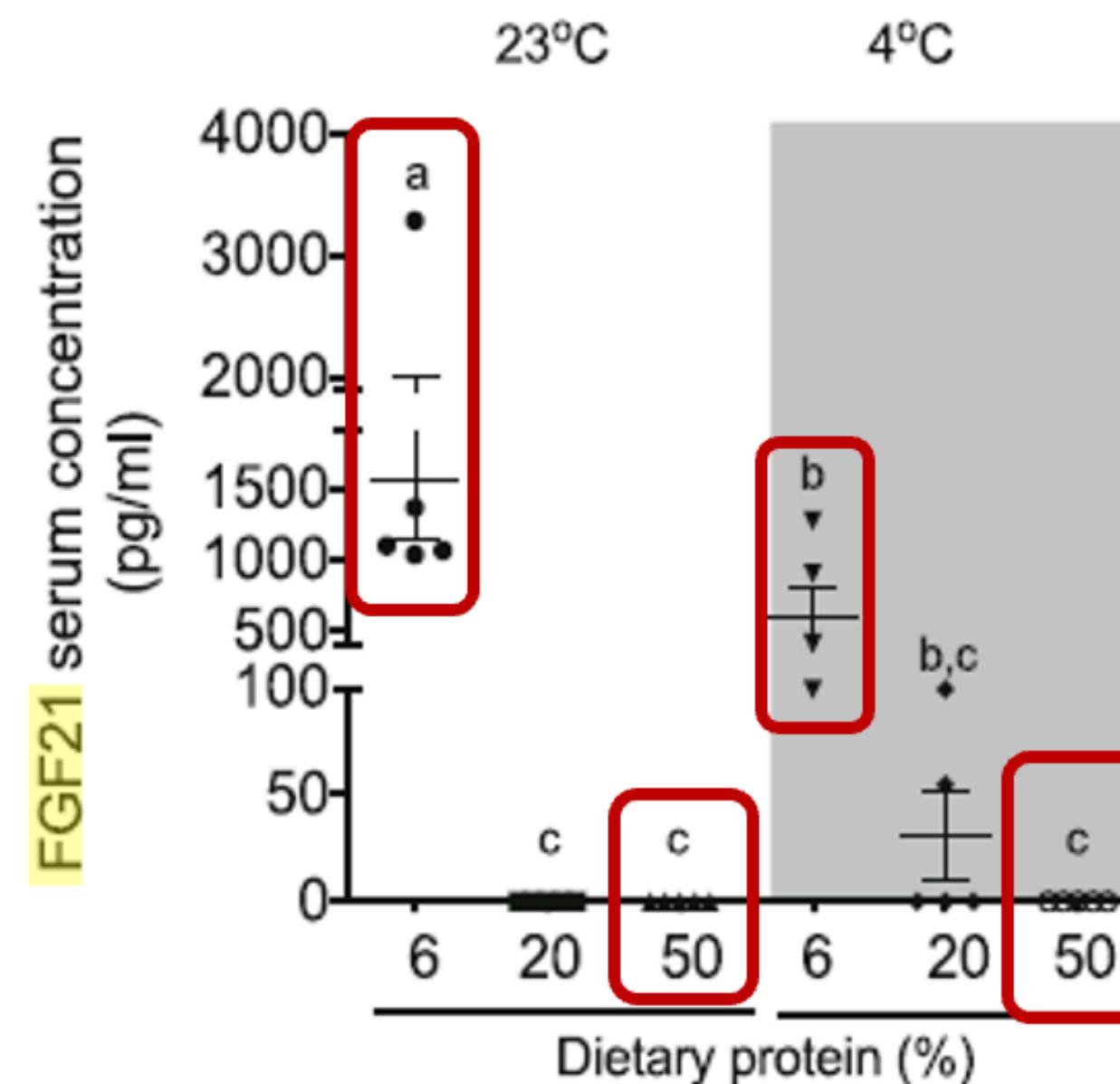
Low temperature + LP → Increased FGF21 circulation expression?

01

FGF21 serum concentration

02

Liver FGF21 protein expression levels



Under LP, FGF21 circulating levels, room temperature > low temperature.

When protein intake is \geq 20%, FGF21 circulation is strongly suppressed, with almost no response.

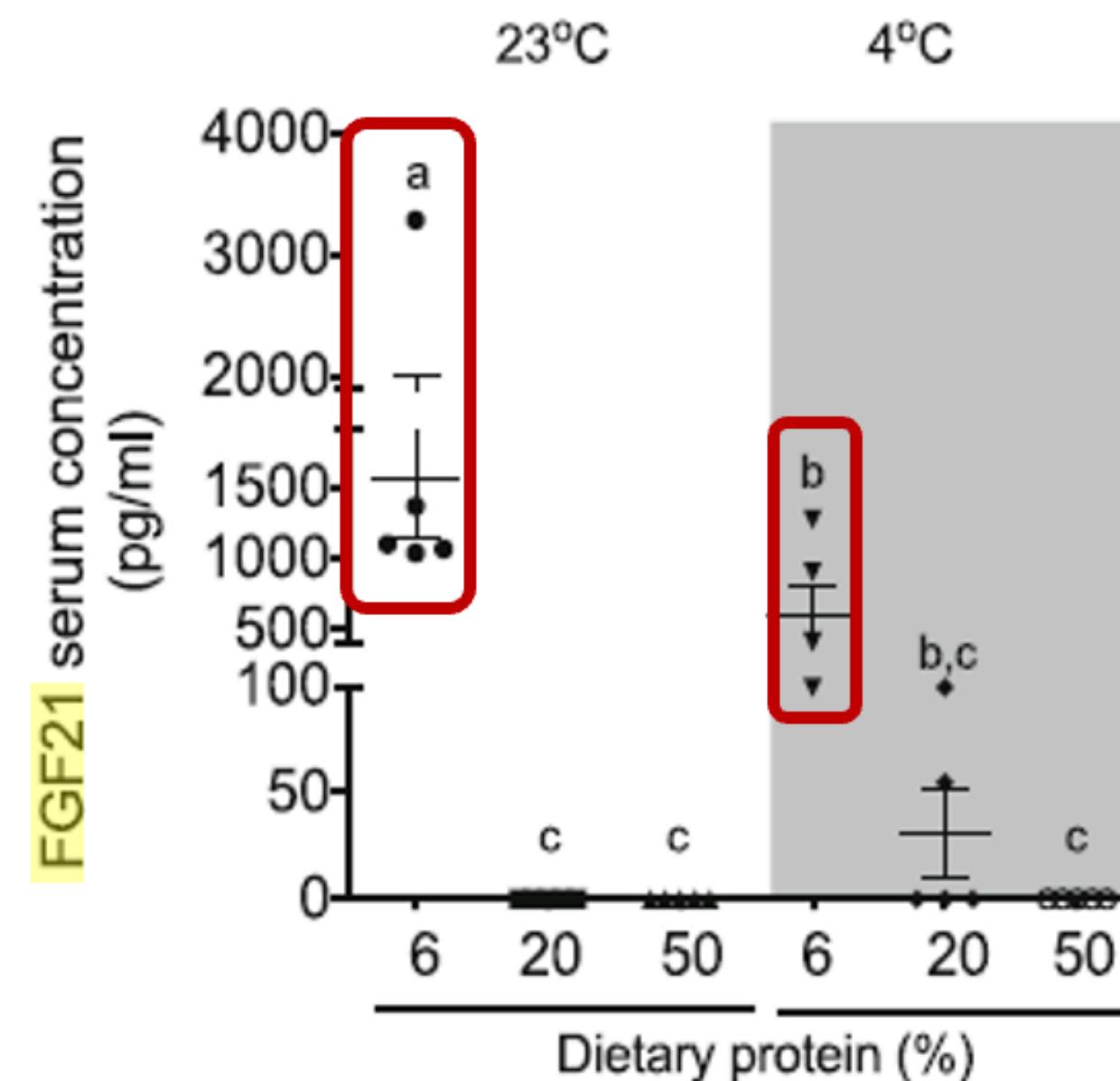
Dietary protein intake is not affected by environmental temperature.

01

FGF21 serum concentration

02

Liver FGF21 protein expression levels



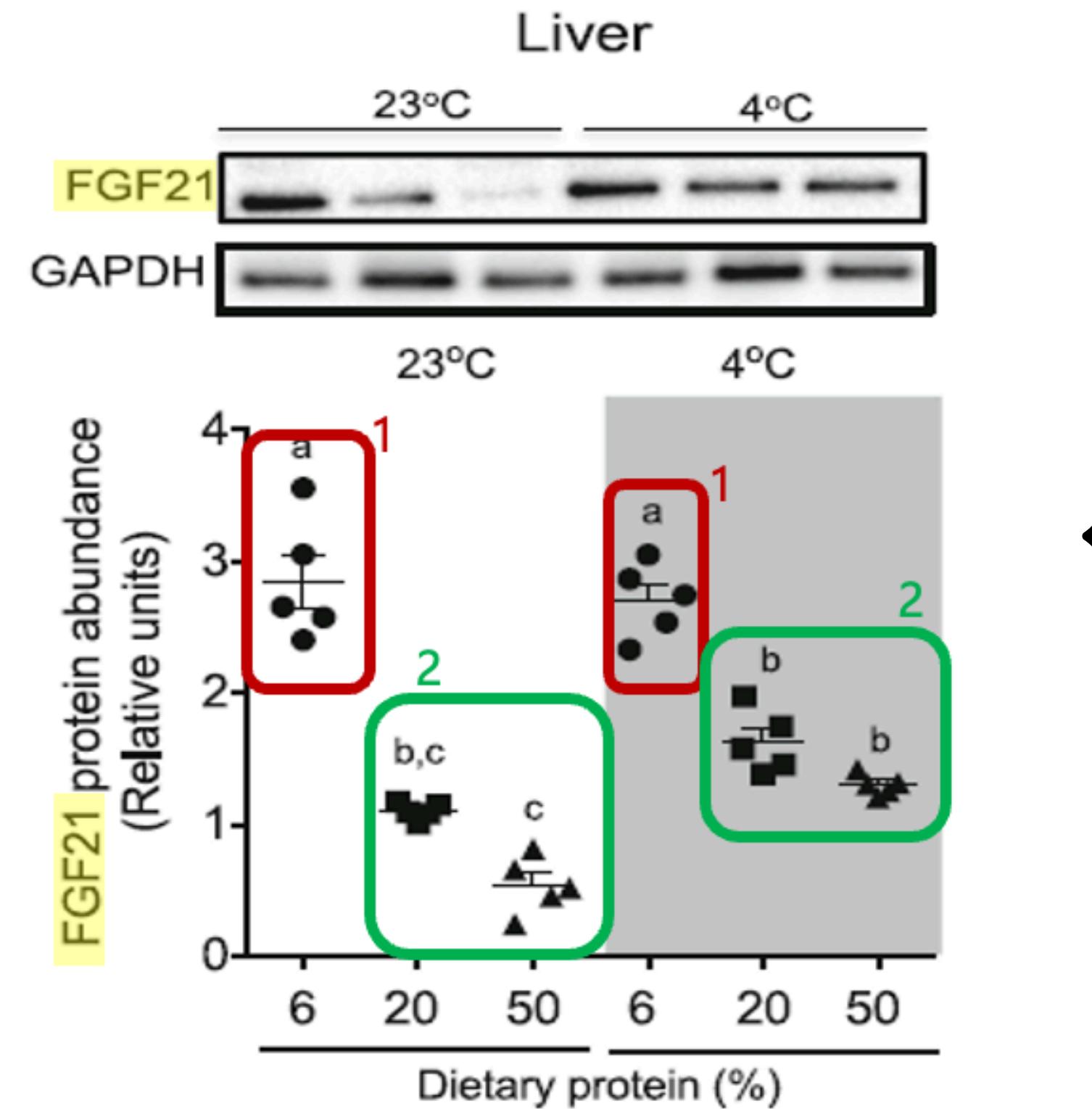
Overall, the FGF21 concentration in the serum is higher in the LP group.

01

FGF21 serum concentration

02

Liver FGF21 protein expression levels



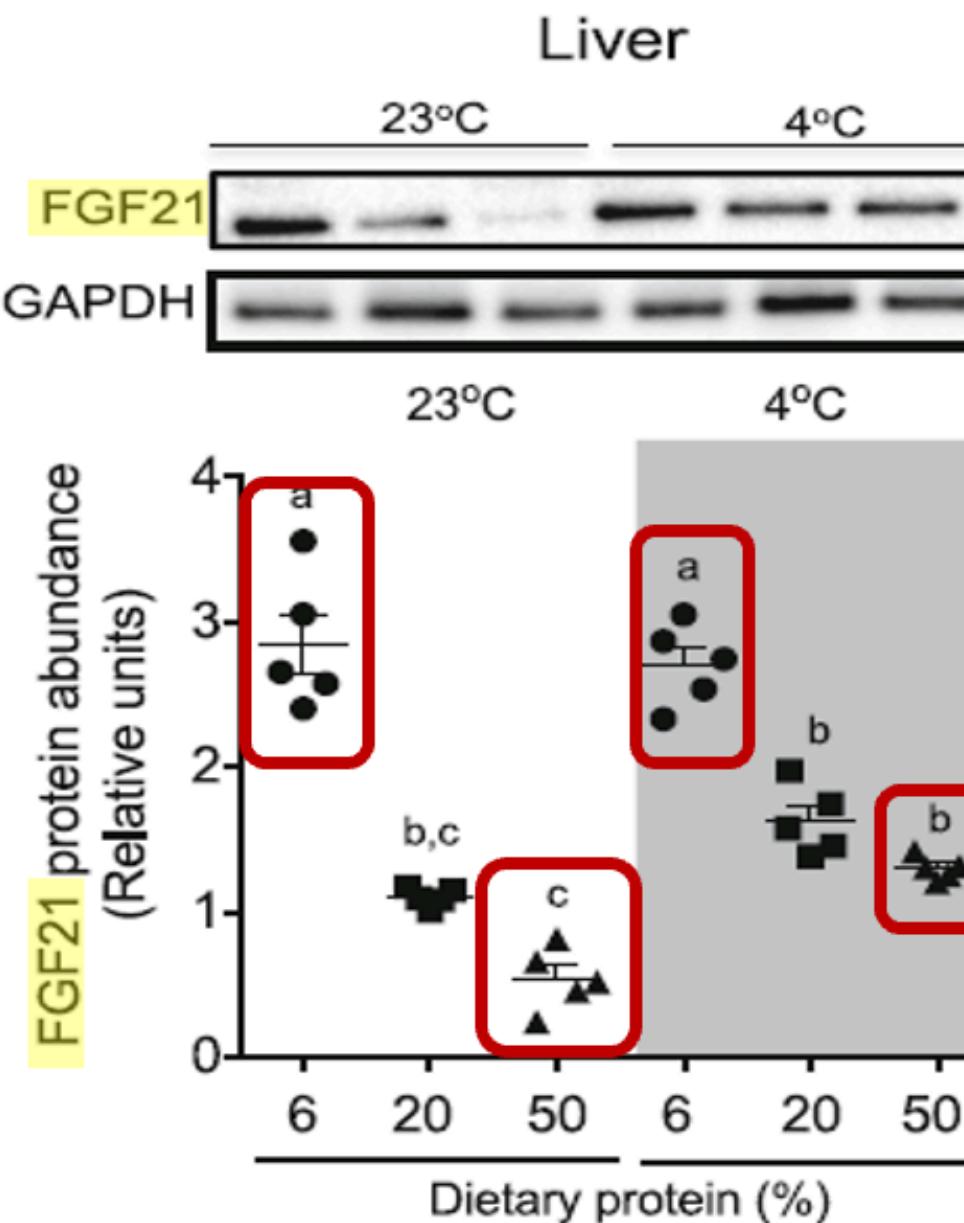
Feeding LP (1), compared to AP or HP (2), resulted in a 1.8-fold increase.

01

FGF21 serum concentration

02

Liver FGF21 protein expression levels



Feeding LP, under room temperature and low temperature conditions, showed similar expression

Feeding HP resulted in a decreased level of expression.



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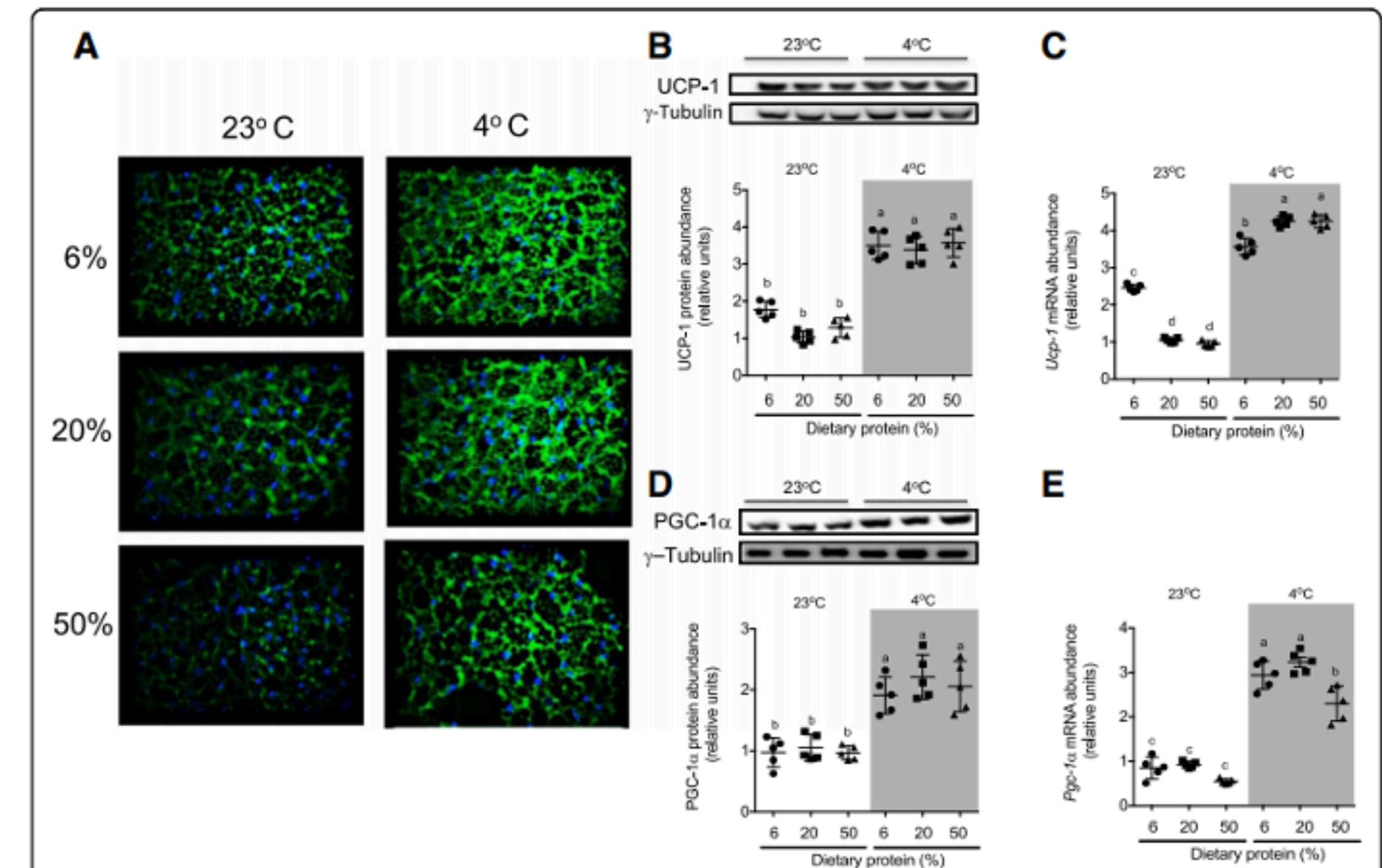
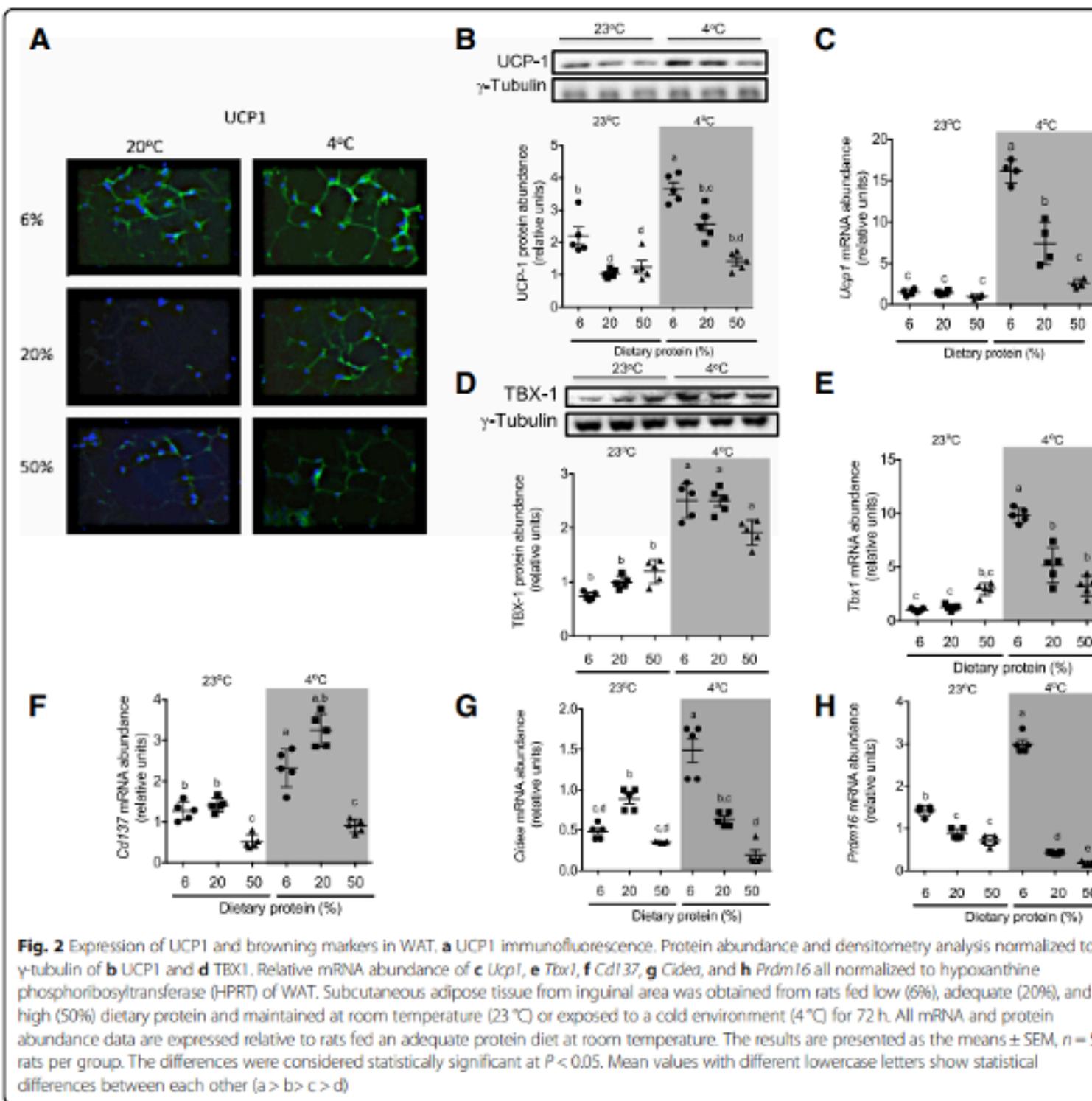
Immunoluminescence analysis

Indirect calorimetry

CLAMS metabolism measurement system

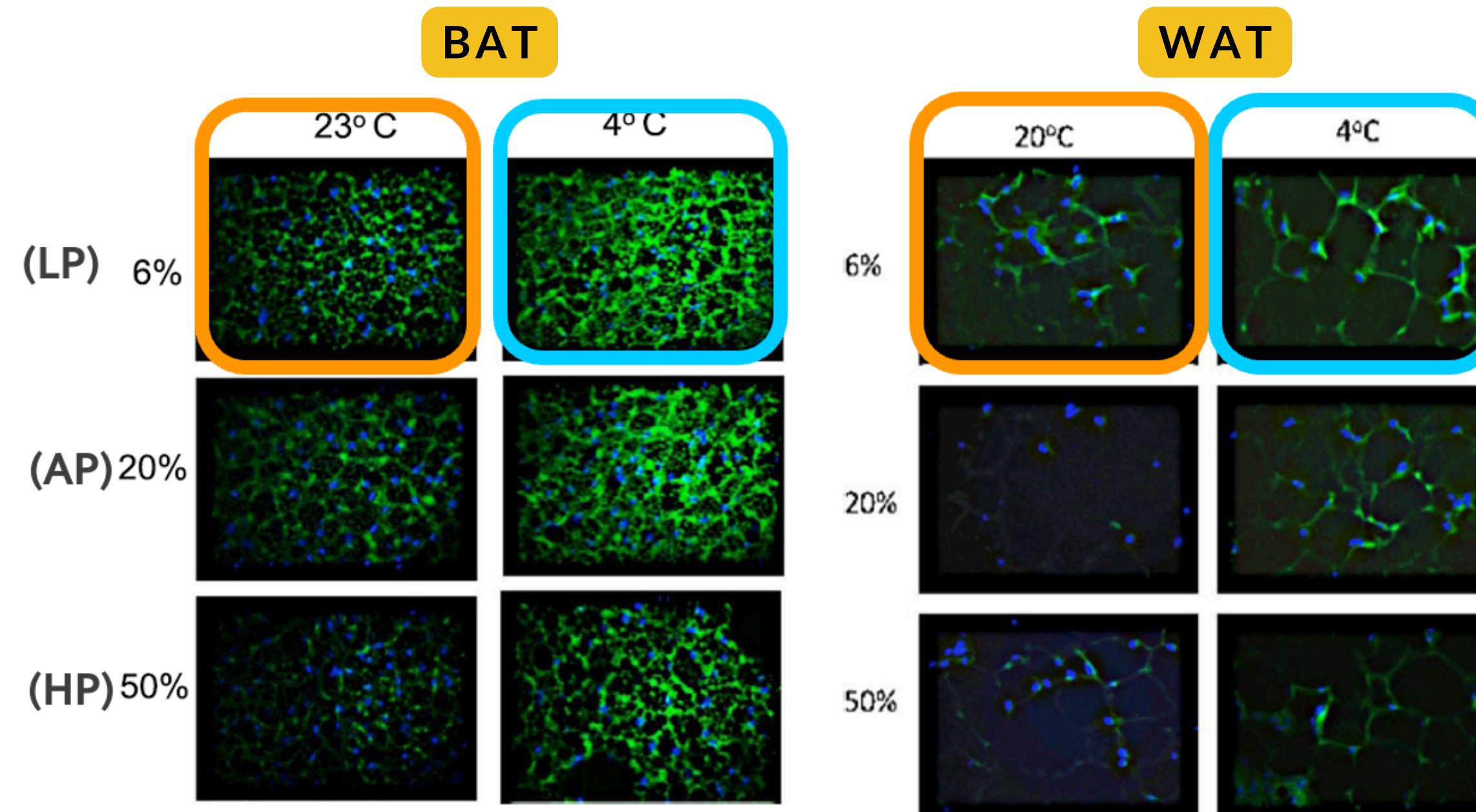
Measurement of BAT and subcutaneous WAT protein content

Immunoluminescence analysis



01

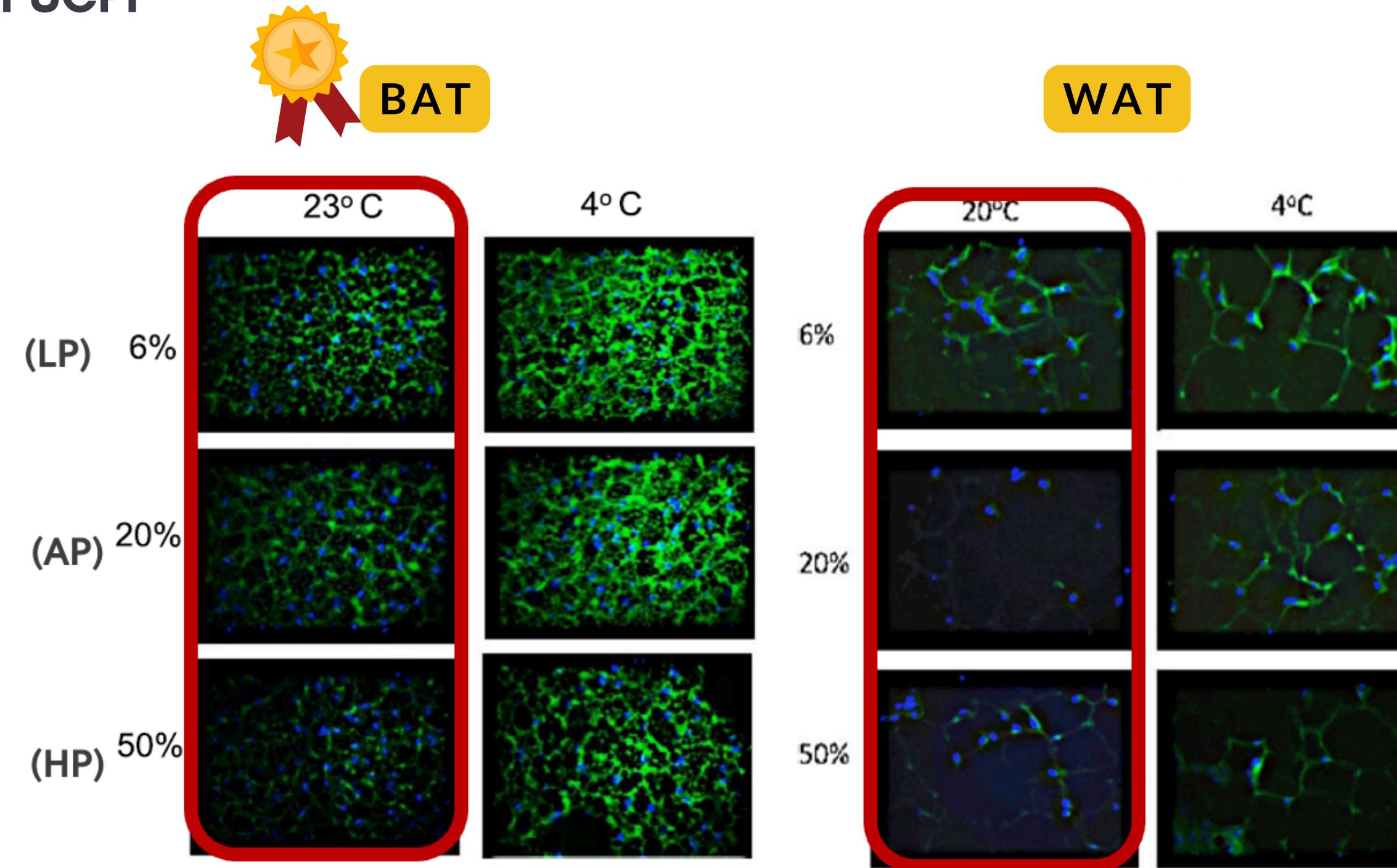
The expression level of UCP1



In LP protein, the expression level of UCP1:
Cold temperature > Room temperature

01

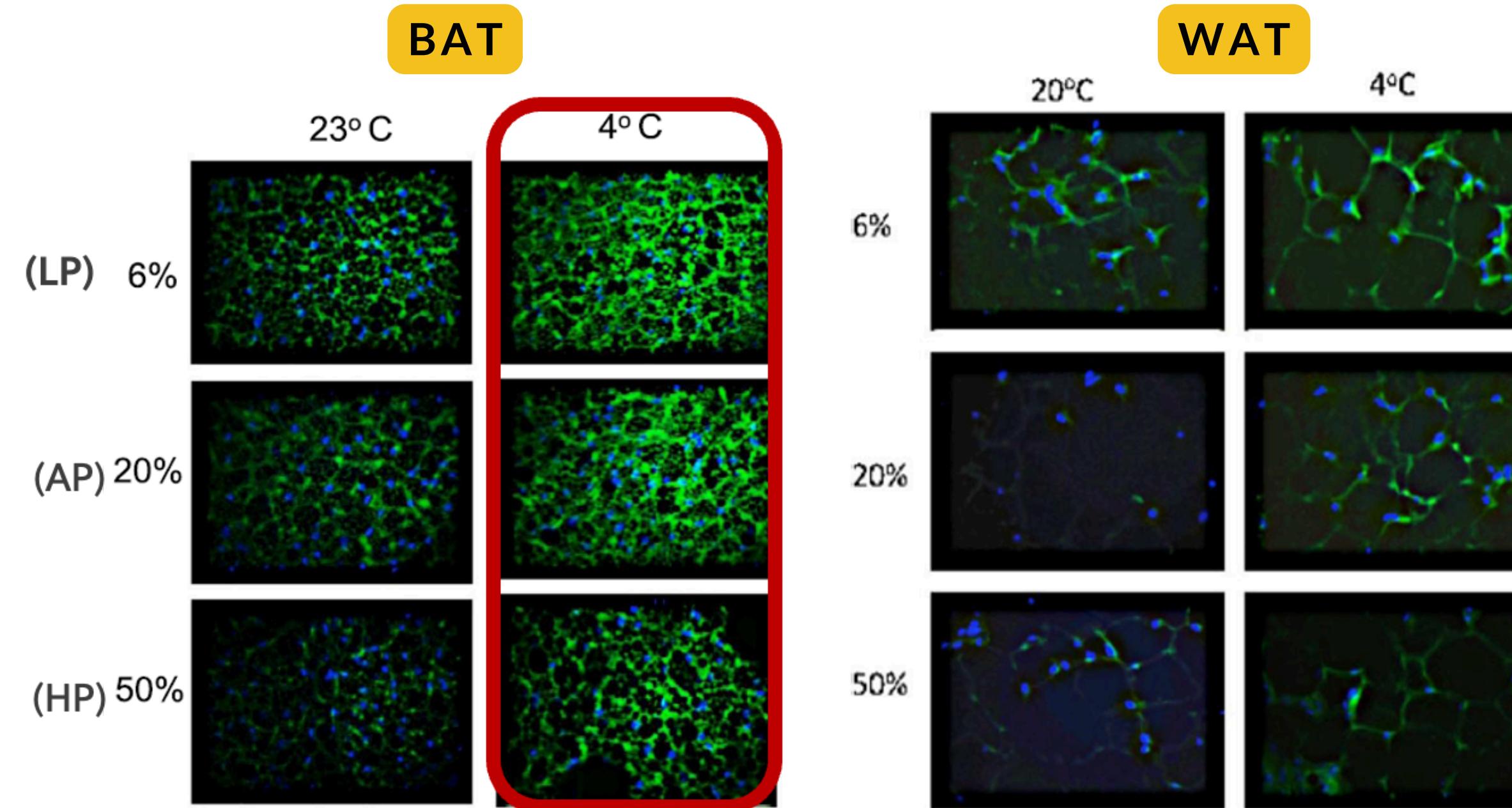
The expression level of UCP1



At room temperature, the expression level of UCP1:
BAT > WAT

01

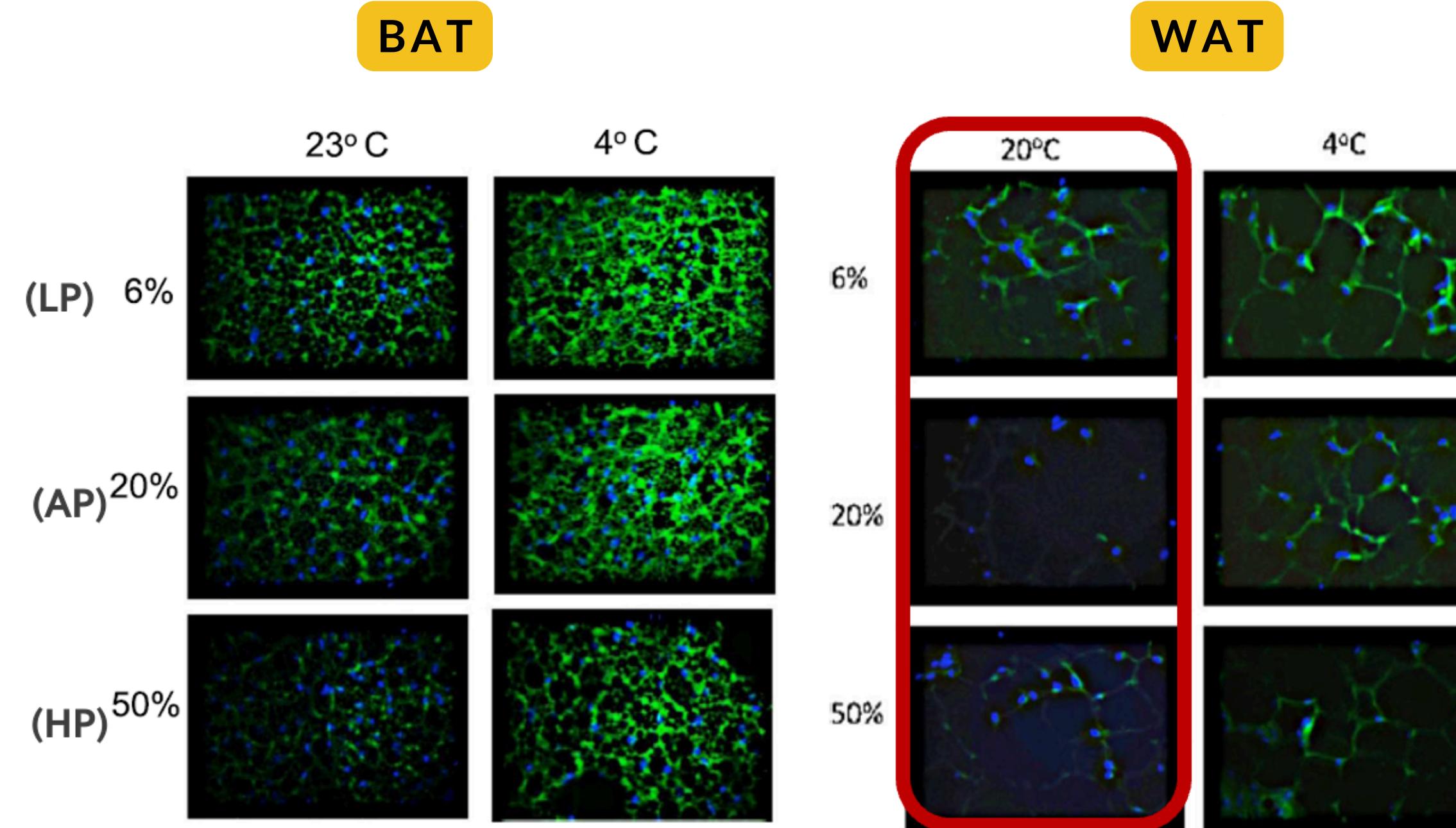
The expression level of UCP1



At low temperatures, the increase in UCP1 expression is not related to protein content.

01

The expression level of UCP1



At low temperatures, the increase in UCP1 expression is not related to protein content.



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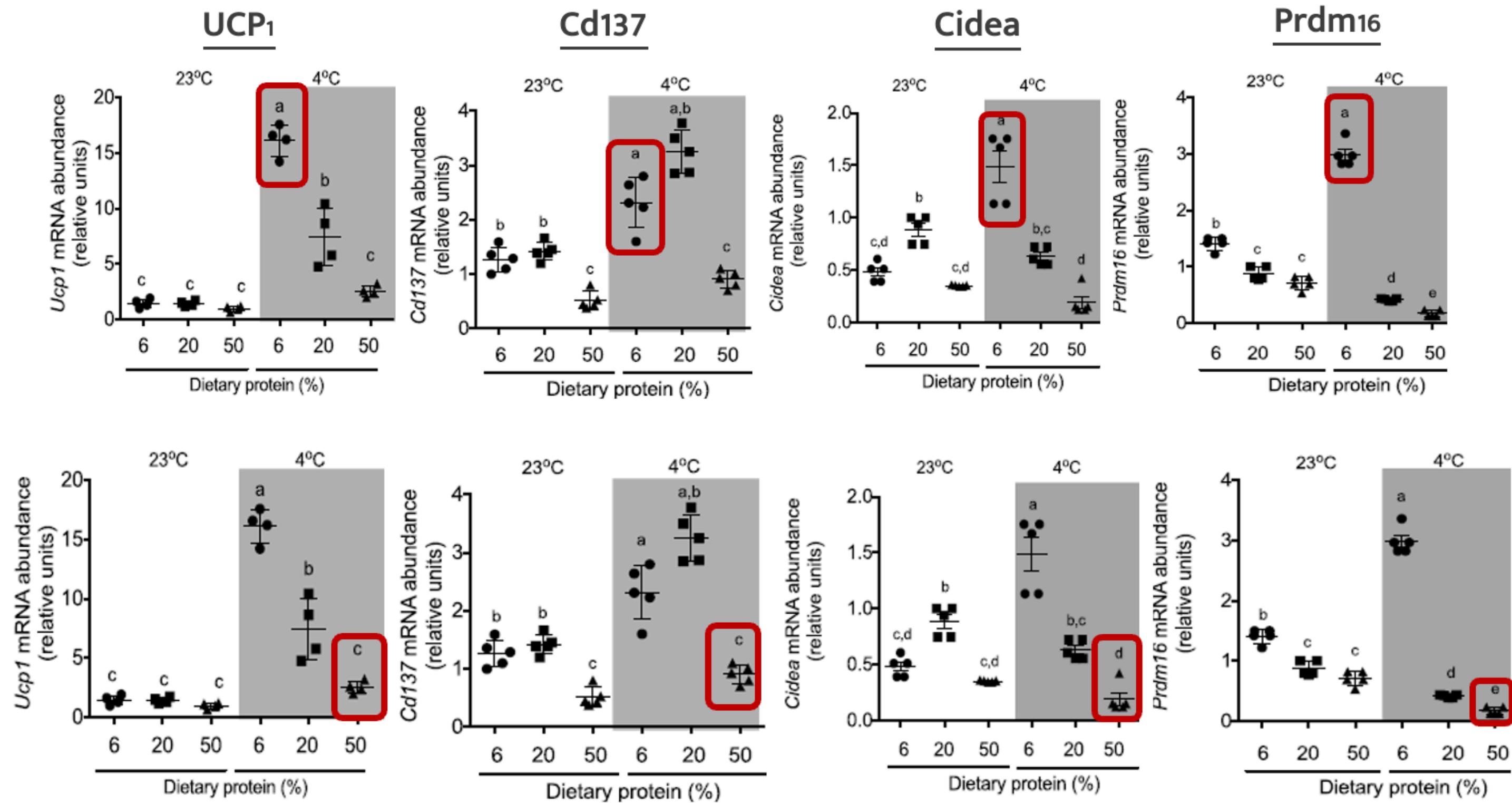
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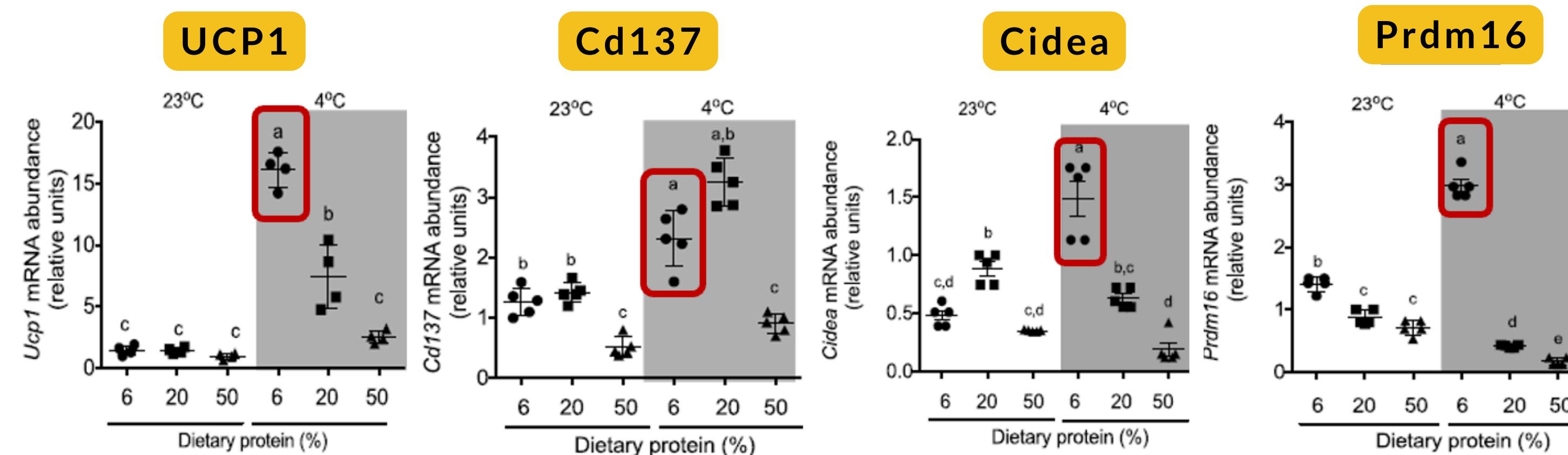
All mRNA relative quantities

Real-time quantitative PCR



WAT

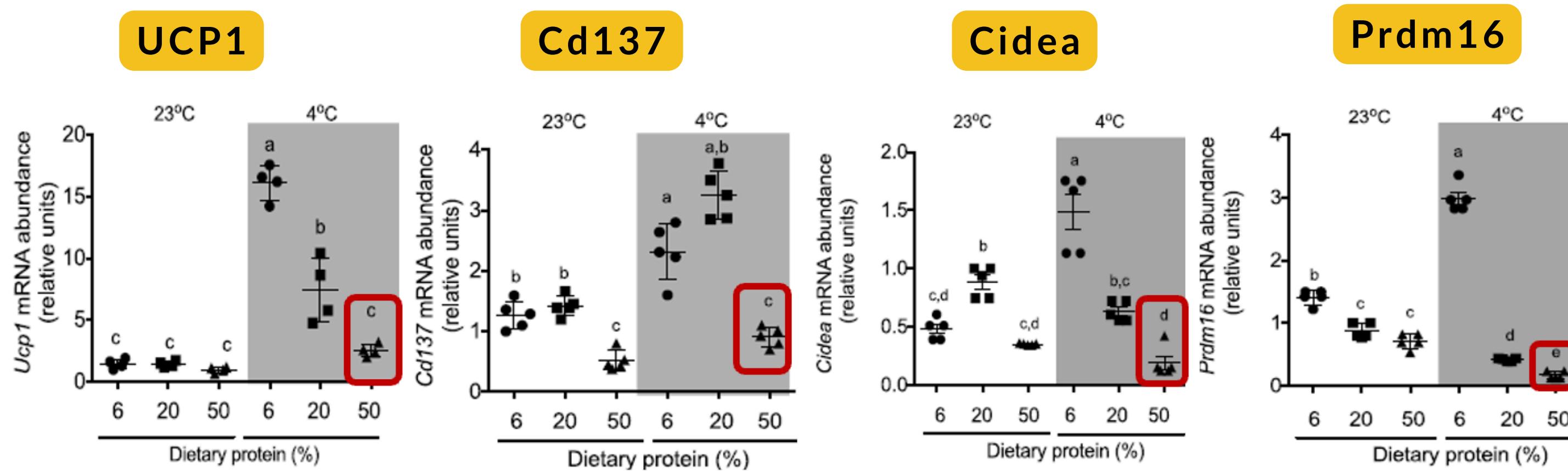
Protein expression levels and density measurement



When fed a low-protein (LP) diet in cold conditions, UCP1 expression levels increase.

WAT

Protein expression levels and density measurement



In low temperatures, feeding with HP (high-protein) highly suppresses expression levels.



Body composition

- Blood glucose analyzer
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- Nuclear magnetic resonance

All mRNA relative quantities

Real-time quantitative PCR

Blood biochemical change

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WAT, BAT and relative quantities of γ -tubulin

Western blot

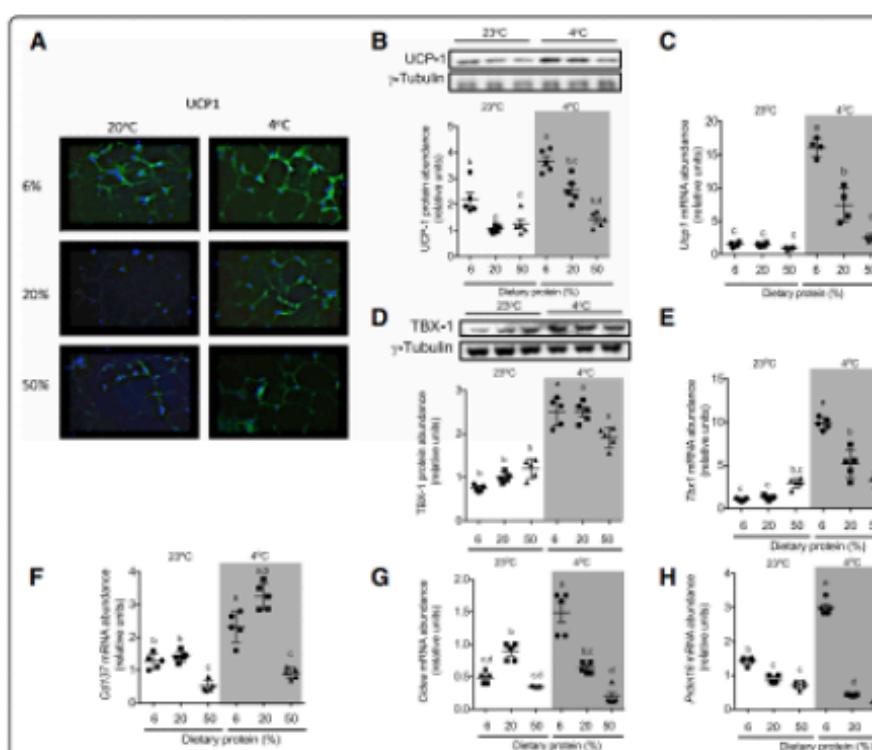
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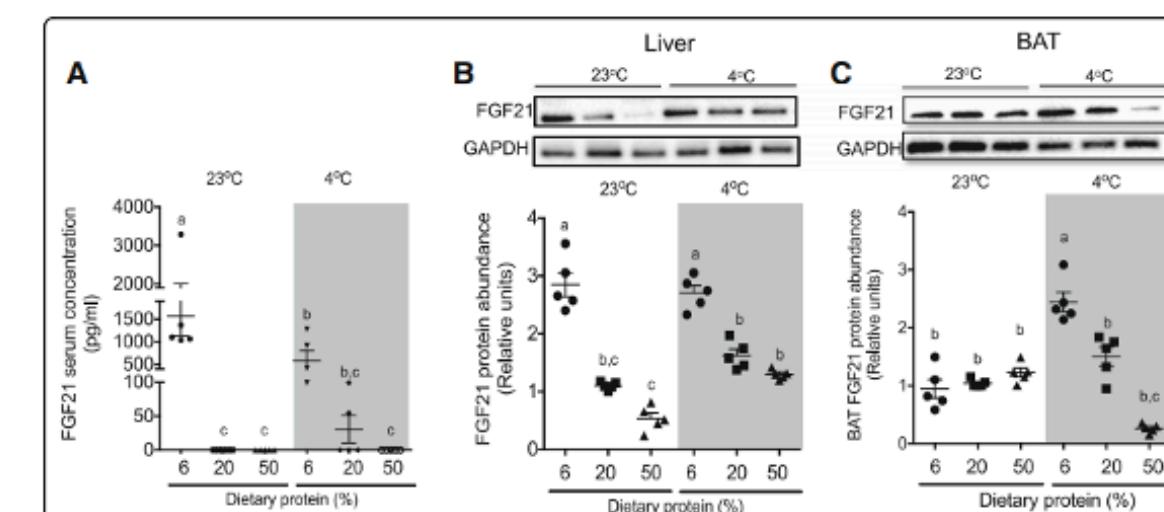
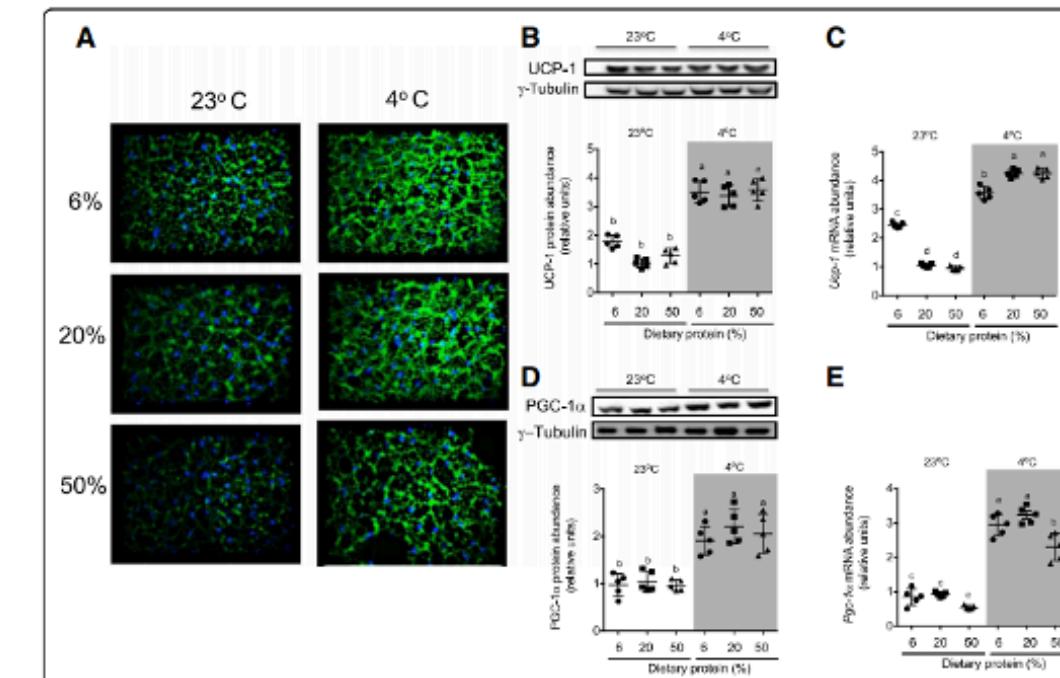
Indirect calorimetry

CLAMS metabolism measurement system

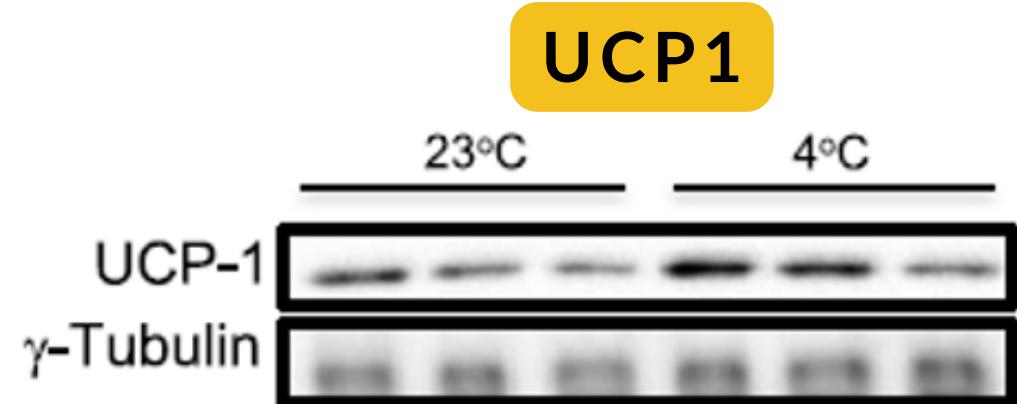
WAT, BAT and relative quantities of γ -tubulin



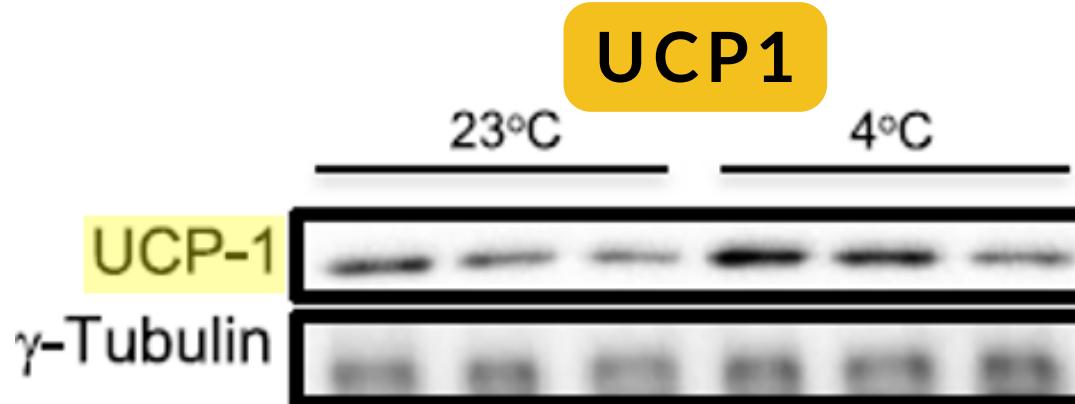
Western blot



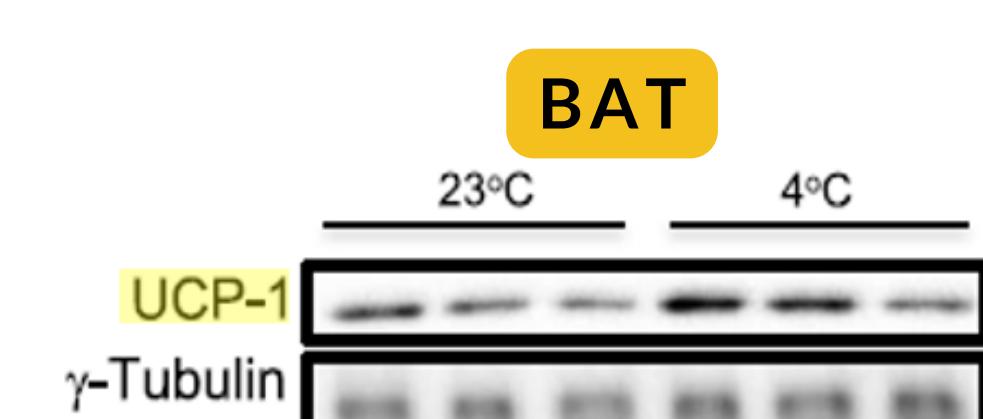
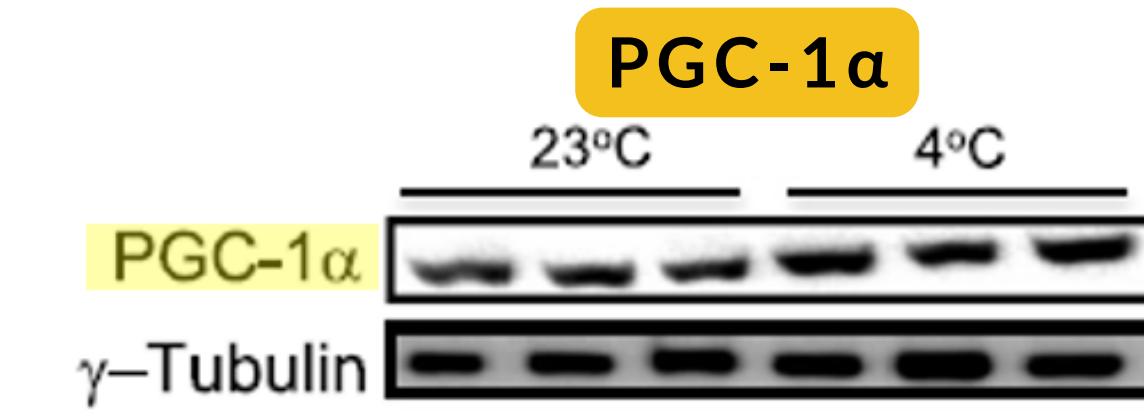
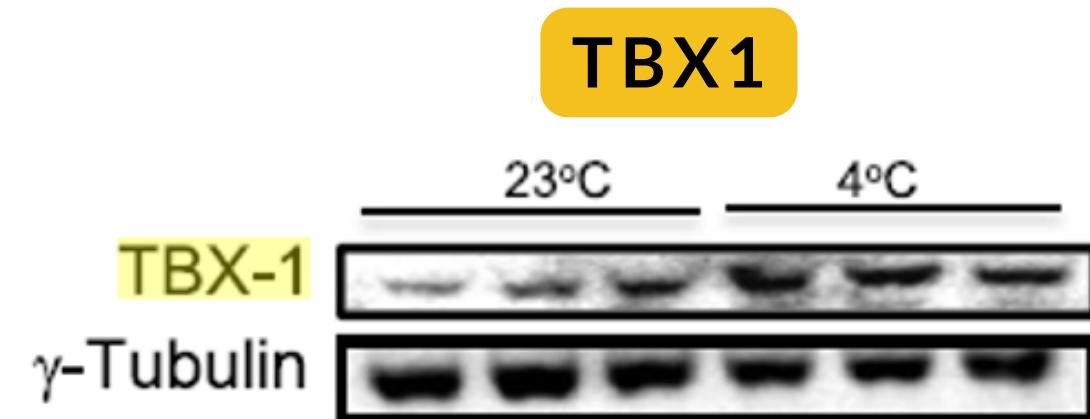
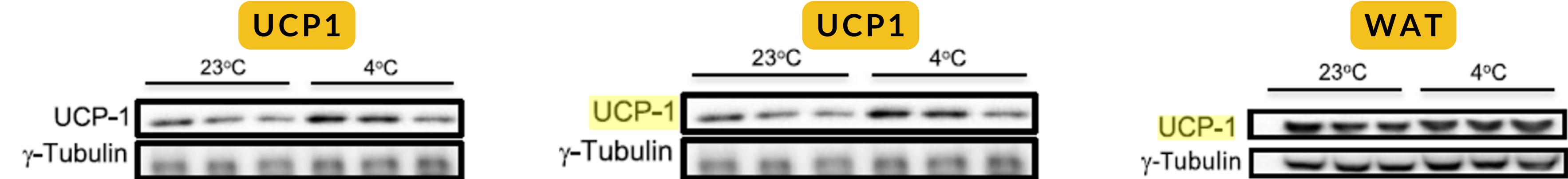
WAT
Protein expression and density measurement



BAT
Protein expression and density measurement



UCP1 Expression levels



01

WAT Protein expression and density measurement

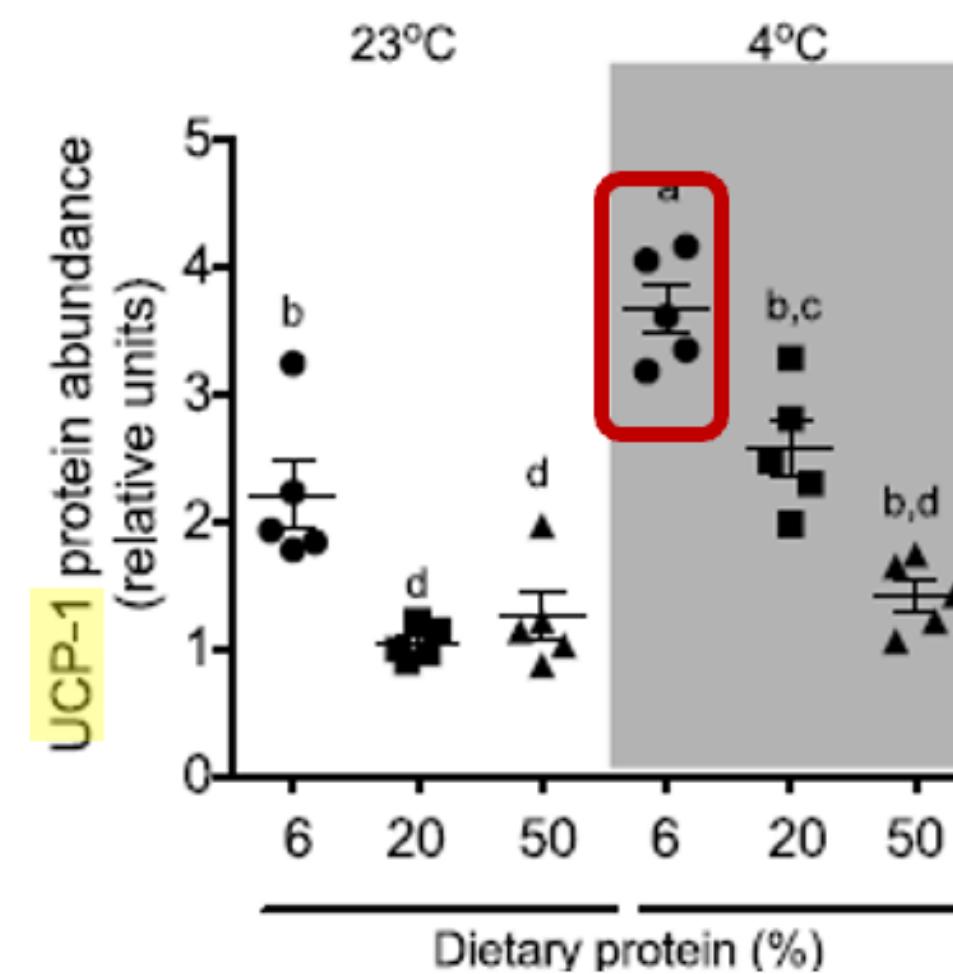
02

BAT Protein expression and density measurement

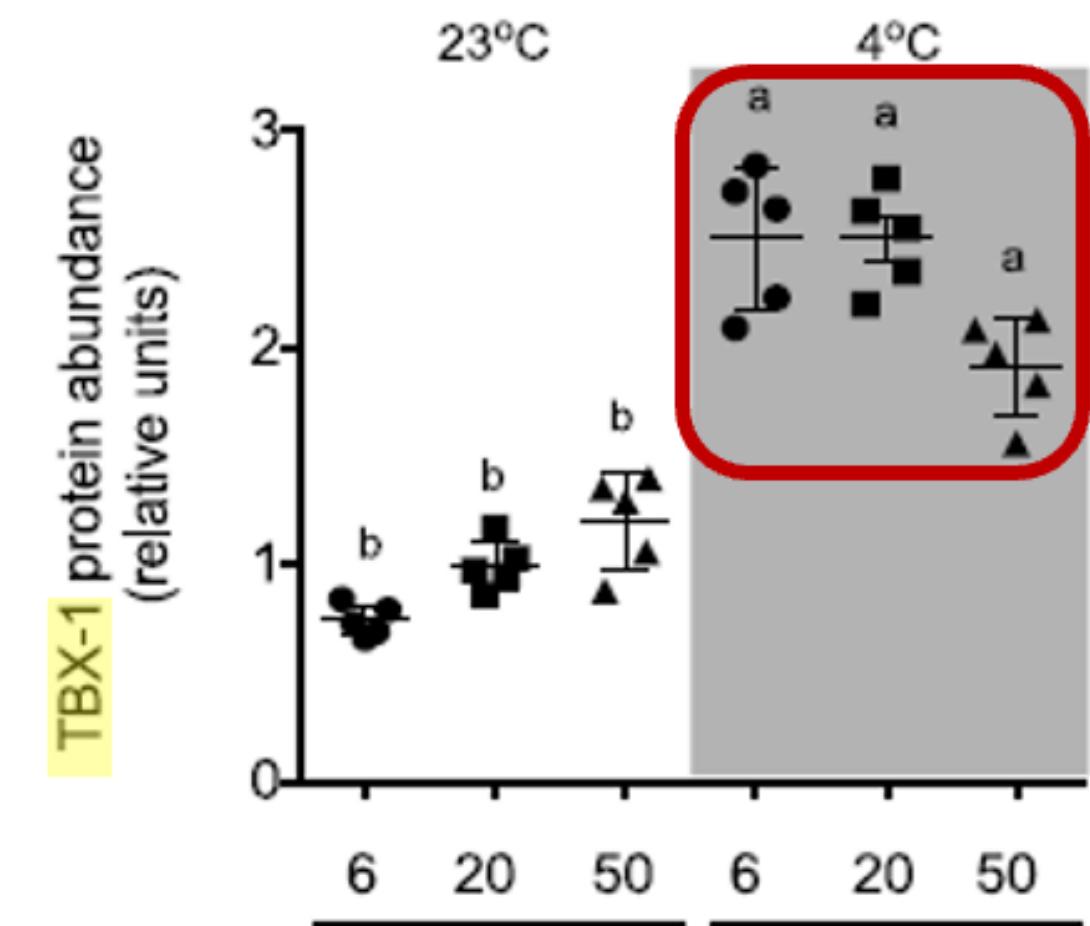
03

UCP1 Expression levels

UCP1



TBX1



At low temperatures, UCP1 expression in LP increases.

Browning marker: TBX1 increases.

01

WAT Protein expression and density measurement

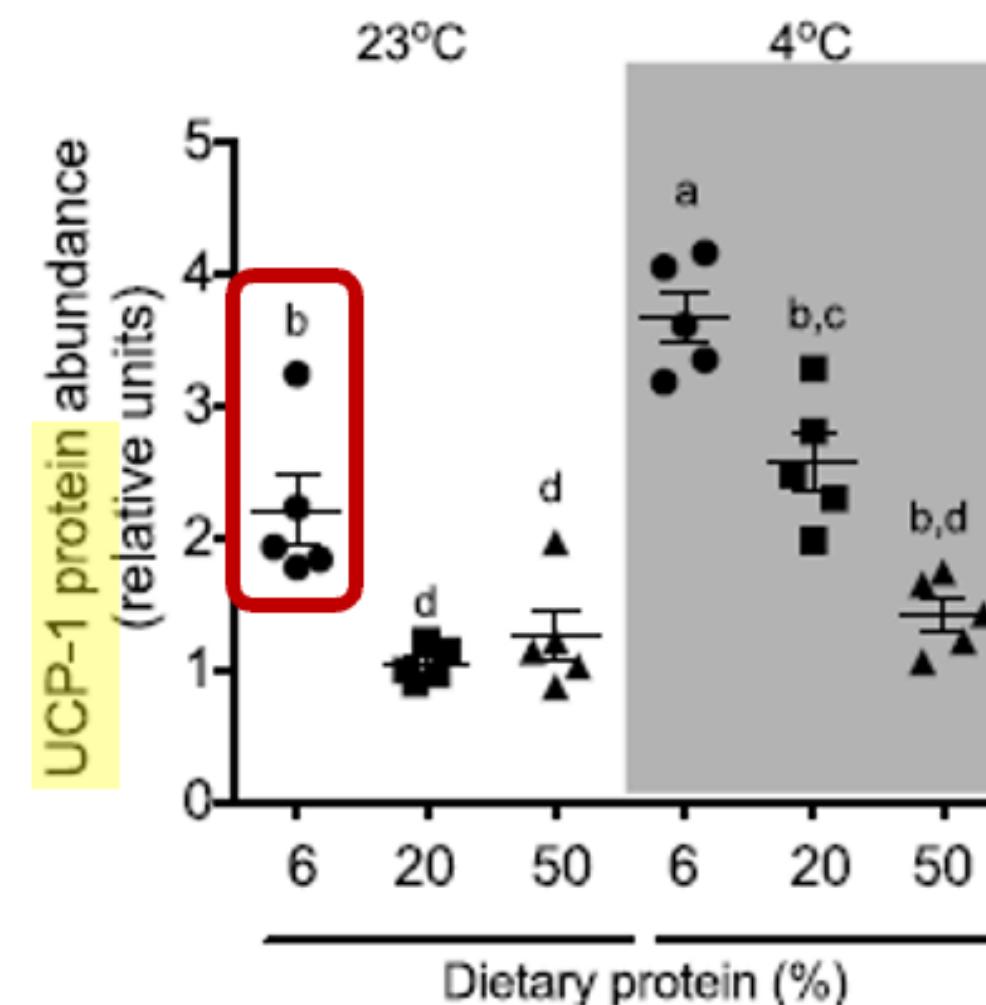
02

BAT Protein expression and density measurement

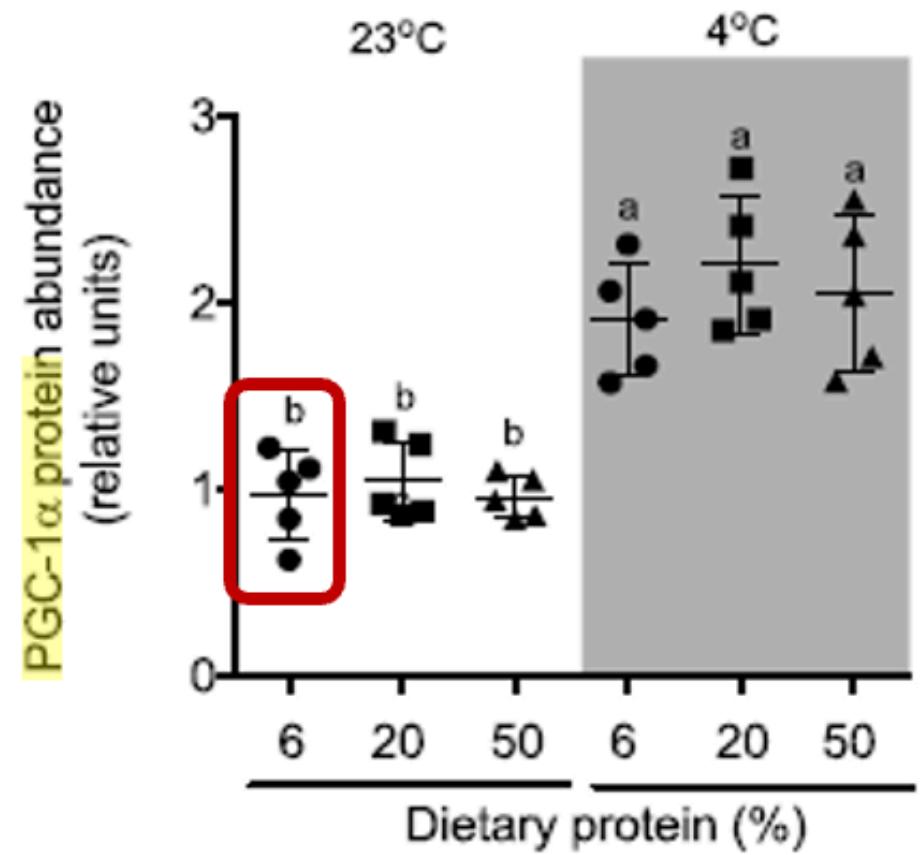
03

UCP1 Expression levels

UCP1



PGC-1 α



Not significantly increased due to LP+ at room temperature.

01

WAT
Protein expression and
density measurement

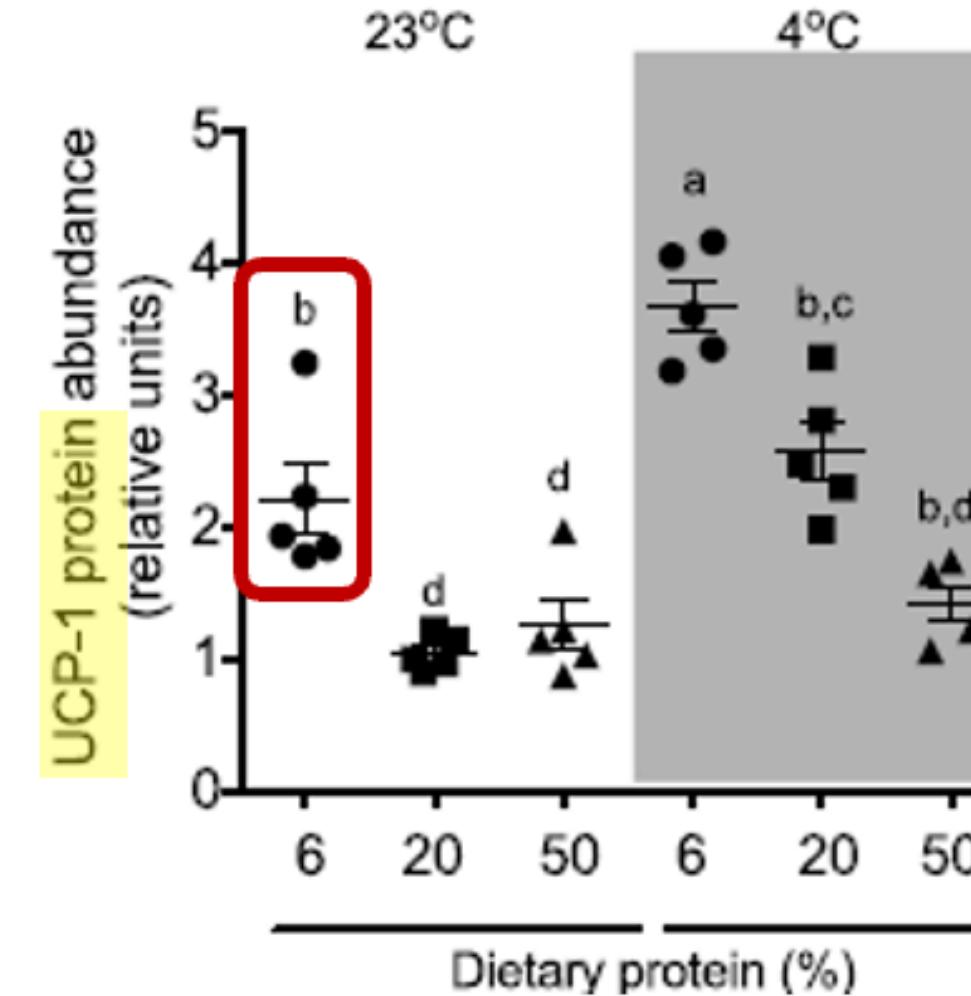
02

BAT
Protein expression and
density measurement

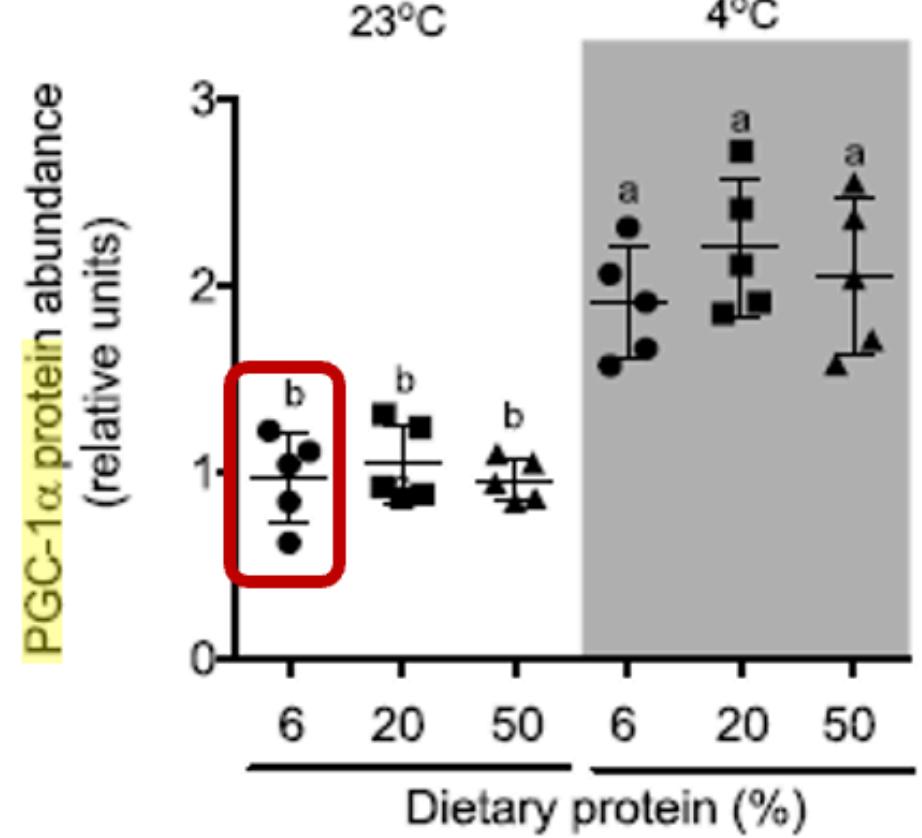
03

UCP1 Expression levels

UCP1



PGC-1 α



LP + room temperature, BAT > WAT



Body composition

- Blood glucose analyzer
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All mRNA relative quantities

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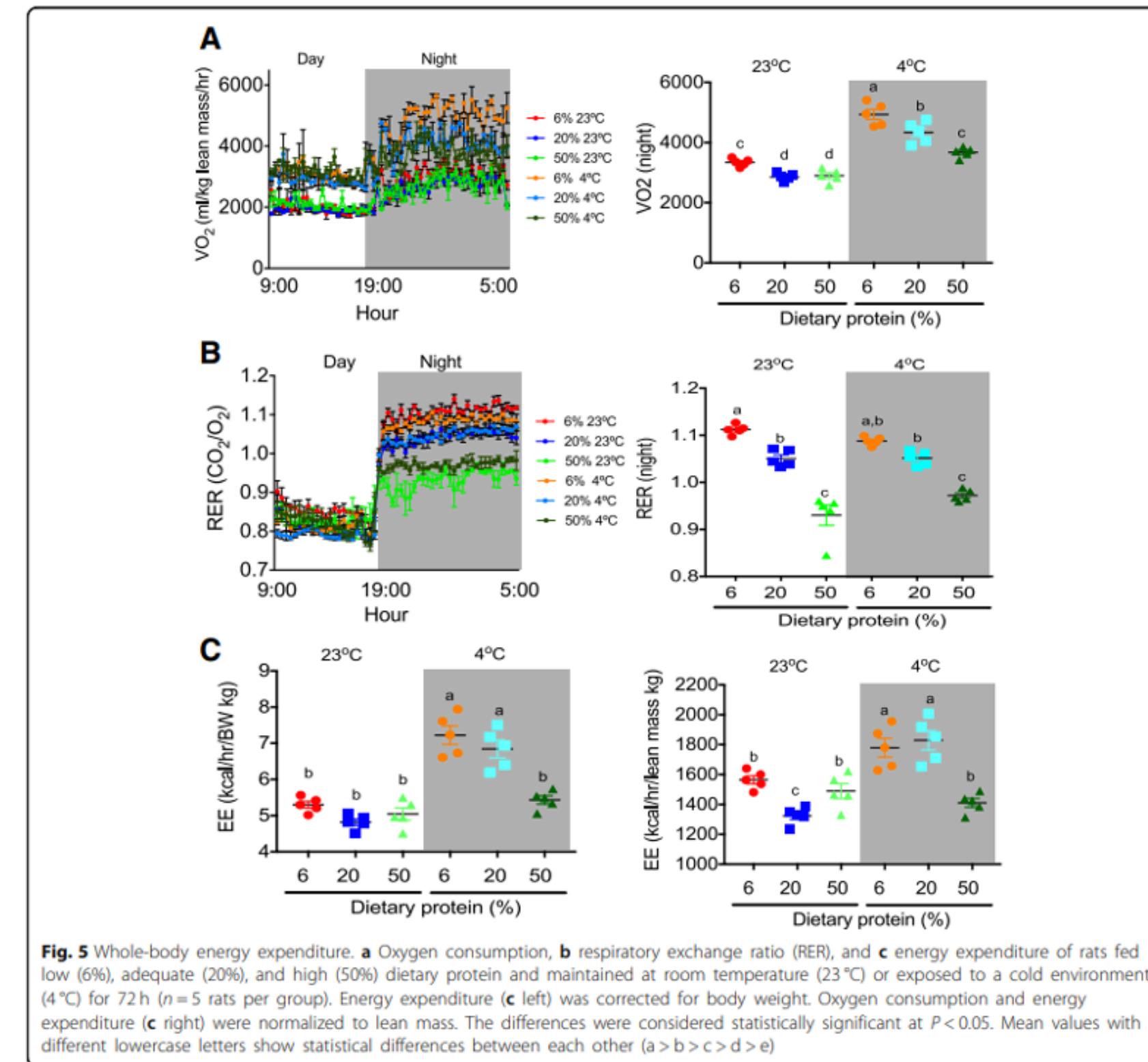
Respiratory Exchange Ratio (RER): VCO_2/VO_2

Respiratory Exchange Ratio	Primary Energy Substrate
0.7 ± 0.06	Fat
0.85 ± 0.06	Fat, Carbohydrates
≥ 1.0	Carbohydrates



Indirect calorimetry

CLAMS metabolism measurement system



01

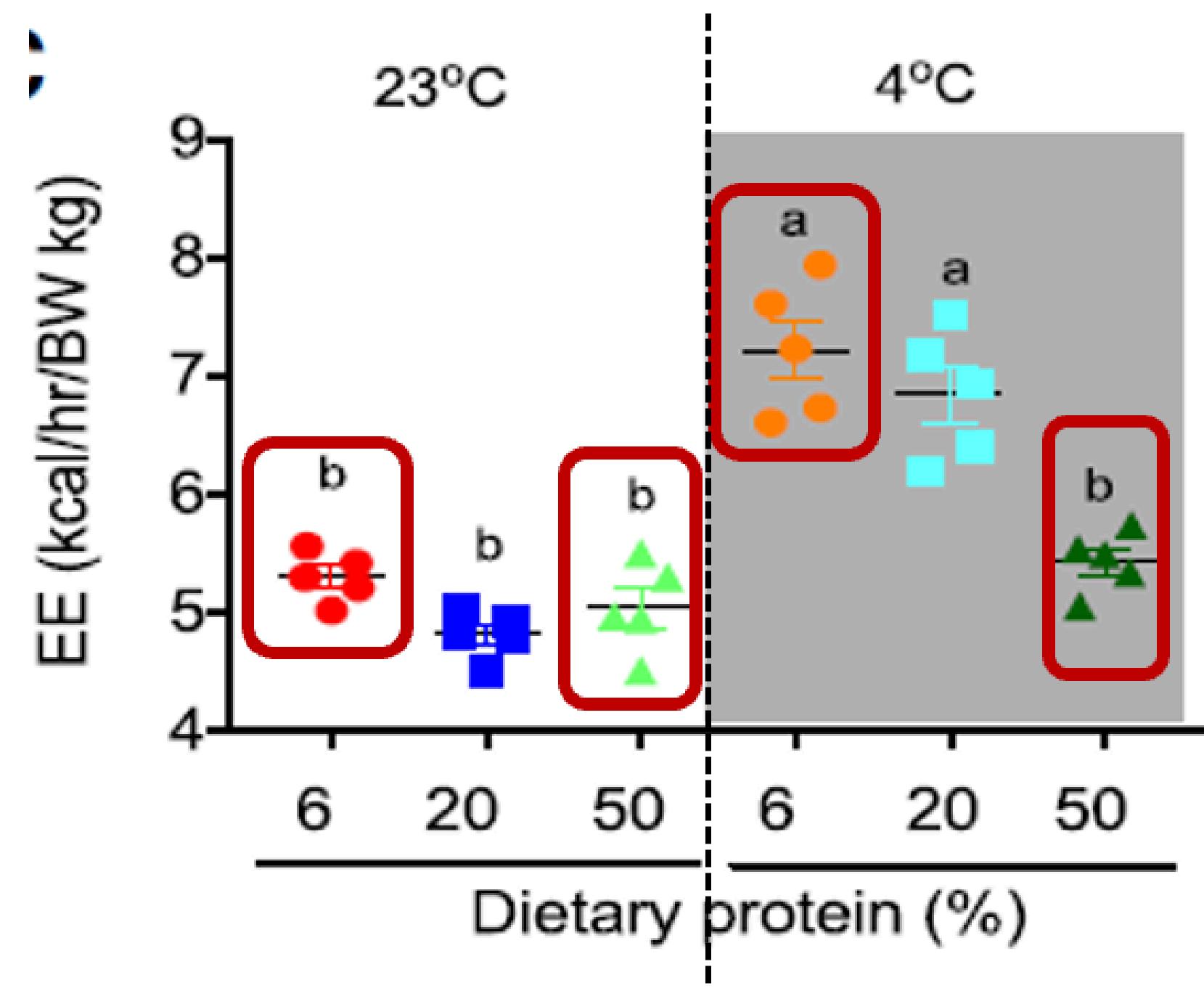
Energy expenditure

02

Respiratory exchange ratio (RER)

LP > HP

LP > HP



01

Energy expenditure

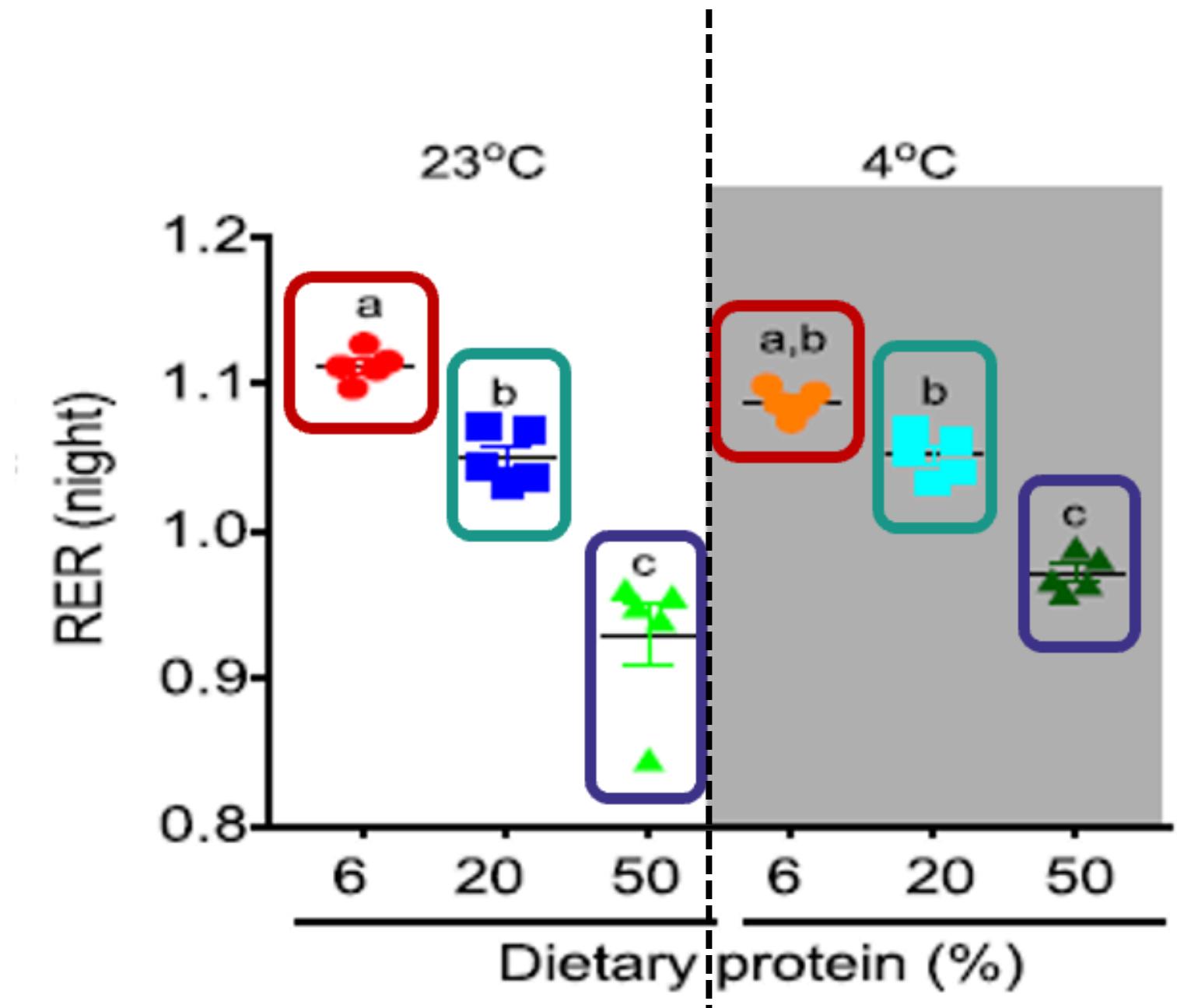
02

Respiratory exchange ratio (RER)

LP > 1 → Carbohydrates

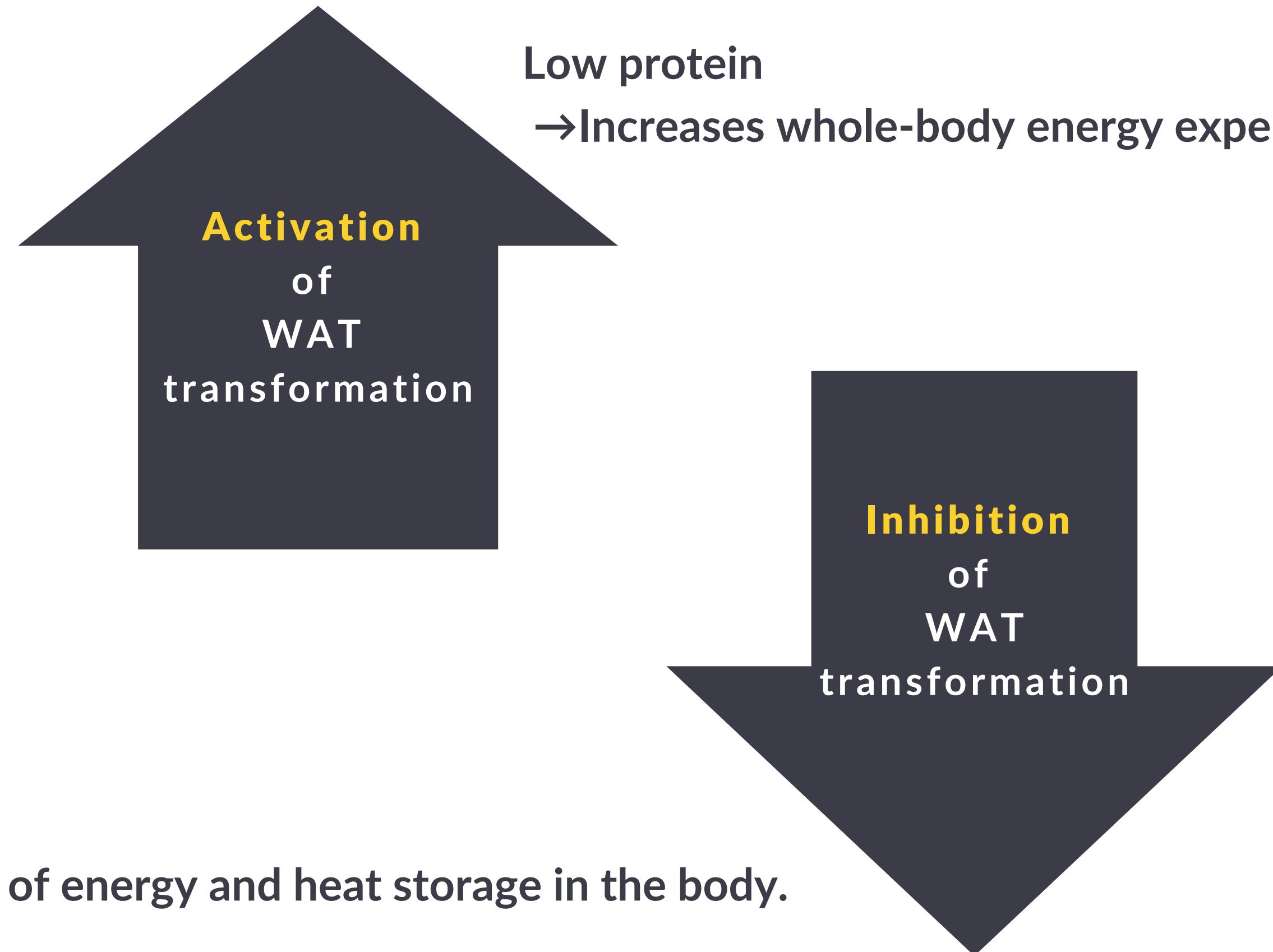
AP ≈ 1 → Carbohydrates

HP ≈ 0.9 → Fats and Carbohydrates

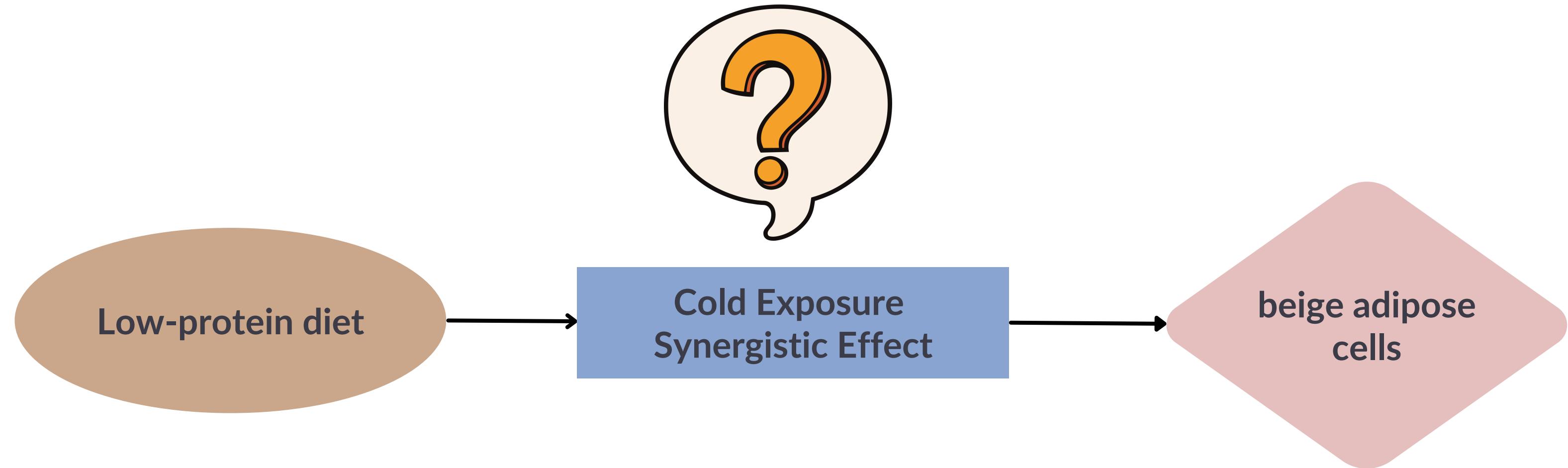


Conclusion

In summary, current results indicate:



So,



It remains **elusive** whether a low-protein diet's stimulation of WAT browning can synergize with another browning stimulus, such as cold exposure.

Future Prospects



Further research is needed to understand

The mechanism by which a **low-protein diet induces WAT browning** and this strategy in obese human subjects to increase energy expenditure and **reduce body weight and fat mass**, thereby improving the metabolic abnormalities of obesity.

References

RESEARCH

Open Access

Interaction between the amount of dietary protein and the environmental temperature on the expression of browning markers in adipose tissue of rats



Gabriela Alemán¹, Ana Laura Castro¹, Ana Vigil-Martínez¹, Iván Torre-Villalvazo¹, Andrea Díaz-Villaseñor^{1,2}, Lilia G. Noriega¹, Isabel Medina-Vera³, Guillermo Ordáz¹, Nimbe Torres¹ and Armando R. Tovar^{1*}

Abstract

Background: A low-protein diet increases the expression and circulating concentration of FGF21. FGF21 stimulates the browning process of WAT by enhancing the expression of UCP1 coupled with an increase in PGC1a. Interestingly, the consumption of a low-protein diet could stimulate WAT differentiation into beige/brite cells by increasing FGF21 expression and *Ucp1* mRNA abundance. However, whether the stimulus of a low-protein diet on WAT browning can synergistically interact with another browning stimulus, such as cold exposure, remains elusive.

Results: In the present study, rats were fed 6% (low), 20% (adequate), or 50% (high) dietary protein for 10 days and subsequently exposed to 4 °C for 72 h. Body weight, food intake, and energy expenditure were measured, as well as WAT browning and BAT thermogenesis markers and FGF21 circulating levels. The results showed that during cold exposure, the consumption of a high-protein diet reduced UCP1, TBX1, *Cidea*, *Cd137*, and *Prdm16* in WAT when compared with the consumption of a low-protein diet. In contrast, at room temperature, a low-protein diet increased the expression of UCP1, *Cidea*, and *Prdm16* associated with an increase in FGF21 expression and circulating levels when compared with a consumption of a high-protein diet. Consequently, the consumption of a low-protein diet increased energy expenditure.

Conclusions: These results indicate that in addition to the environmental temperature, WAT browning is nutritionally modulated by dietary protein, affecting whole-body energy expenditure.

Keywords: Dietary protein, Browning, Cold exposure, FGF21, Brown adipose tissue

THANK YOU

Speaker: kuo Liang-Chun

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11 May, 2021