EXPLORING THE LYMPHATIC CONTINUUM • VIRTUAL MEETING

LYMPHATIC FORUM 2021

Role and therapeutic potential of dietary ketone bodies in lymph vessel growth

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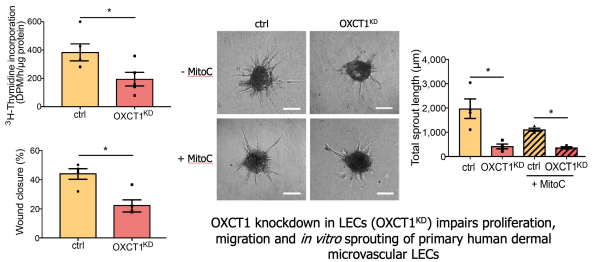
INTRODUCTION

Lymphatic vessels (LVs), lined by lymphatic endothelial cells (LECs), are indispensable for life. However, the role of metabolism in LECs has been incompletely elucidated. In the present study, we reported that LEC-specific loss of OXCT1, a key enzyme of ketone body oxidation (KBO), reduces LEC proliferation, migration and vessel sprouting *in vitro* and impairs lymphangiogenesis in Prox1$^{\Delta OXCT1}$ mice. Mechanistically, OXCT1 silencing lowers acetyl-CoA levels, TCA metabolite pools, nucleotide precursor and dNTP levels required for LEC proliferation. Ketone body supplementation to LECs induces the opposite effects. Notably, elevation of lymph ketone body levels by a high-fat, low-carbohydrate ketogenic diet increases lymphangiogenesis after corneal injury and improves LV function and growth in a mouse model of microsurgical ablation of LVs in the tail, which recapitulates the features of acquired lymphedema in humans.

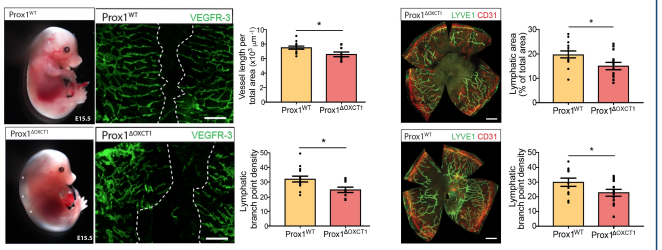
RESULTS

1. KBO IS REQUIRED FOR LEC PROLIFERATION, MIGRATION AND SPROUTING



OXCT1 knockdown in LECs (OXCT1^{KD}) impairs proliferation, migration and *in vitro* sprouting of primary human dermal microvascular LECs

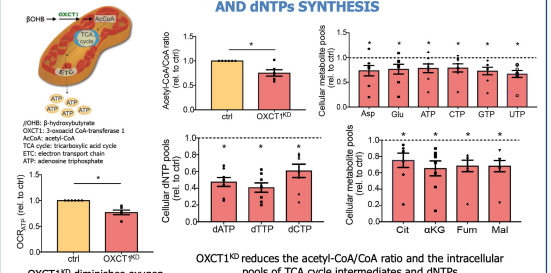
2. *IN VIVO* ROLE OF KBO IN LYMPHANGIOGENESIS



Loss of OXCT1 in Prox1$^{\Delta OXCT1}$ mice impairs developmental lymphangiogenesis and lymphatic growth after cornea injury

Prox1^{WT}: *Cre-negative x OXCT1$^{lox/lox}$*
Prox1$^{\Delta OXCT1}$: *Prox1-Cre^{ERT2} x OXCT1$^{lox/lox}$*

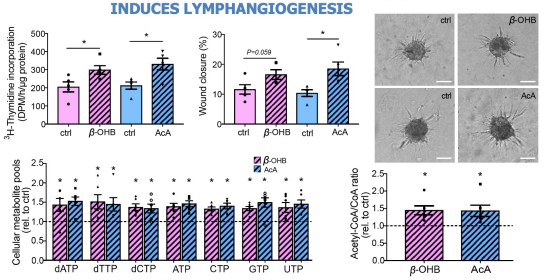
3. KBO IS REQUIRED FOR MITOCHONDRIAL FUNCTION AND dNTPs SYNTHESIS



OXCT1^{KD} diminishes oxygen consumption linked to ATP synthesis

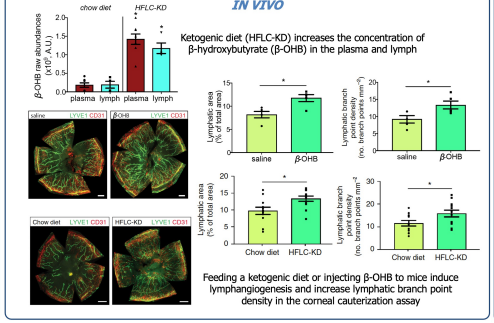
OXCT1^{KD} reduces the acetyl-CoA/CoA ratio and the intracellular pools of TCA cycle intermediates and dNTPs

4. IN VITRO KETONE BODY SUPPLEMENTATION INDUCES LYMPHANGIOGENESIS



Ketone bodies stimulate proliferation, migration and sprouting of primary human LECs, and increase the intracellular pools of dNTPs and the acetyl-CoA/CoA ratio

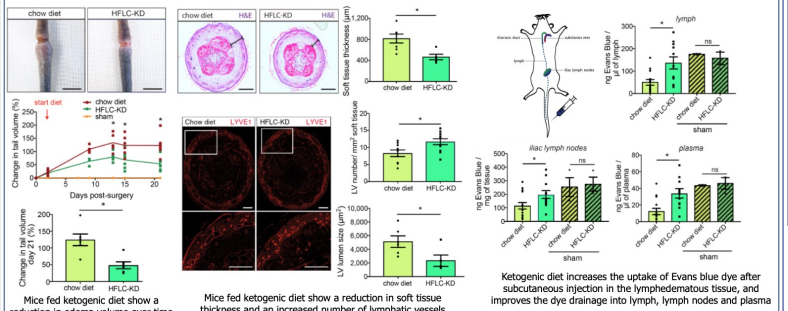
5. KETONE BODY SUPPLEMENTATION INDUCES LYMPHANGIOGENESIS *IN VIVO*



Ketogenic diet (HFLC-KD) increases the concentration of β-hydroxybutyrate (β-OHB) in the plasma and lymph

Feeding a ketogenic diet or injecting β-OHB to mice induce lymphangiogenesis and increase lymphatic branch point density in the corneal cauterization assay

6. KETOGENIC DIET DECREASES TAIL LYMPHEDEMA AND IMPROVES LYMPHATIC UPTAKE AND DRAINAGE CAPABILITY



Mice fed ketogenic diet show a reduction in edema volume over time

Mice fed ketogenic diet show a reduction in soft tissue thickness and an increased number of lymphatic vessels

Ketogenic diet increases the uptake of Evans blue dye after subcutaneous injection in the lymphedematous tissue, and improves the dye drainage into lymph, lymph nodes and plasma

CONCLUSIONS

- ✓ KBO regulates proliferation, migration and sprouting *in vitro*
- ✓ OXCT1 specific deletion in LEC impairs lymphangiogenesis *in vivo*
- ✓ LECs utilize ketone bodies to produce ATP
- ✓ KBO is necessary to maintain adequate availability of dNTPs in LECs
- ✓ Ketone body supplementation stimulates proliferation, migration and sprouting *in vitro*
- ✓ Ketogenic diet and β-OHB enhances lymphangiogenesis after cornea injury
- ✓ Ketogenic diet decreases tail lymphedema and improves the functionality of lymphatic vessels

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Reference: García-Caballero M et al. 2019, Nature Metabolism, 1, 666-675; Melissa@uma.es