Time-restricted eating alters lymphocyte counts, autophagy, and metabolite composition in chronic lymphocytic leukemia

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Background

Dietary modifications have become a promising avenue for abating cancer proliferation.[1,2]

Time-Restricted Eating (TRE)

A rhythmic eating pattern that involves limiting one's eating hours to a set window (i.e., 6-12 hours) on a daily, or near daily, basis often aligning with circadian rhythm (i.e., eating during active daytime hours)[3-6]

Metabolic Changes Resulting from TRE After 12 hours fasting:

- Fuel source: Hepatic glycogen depleted, serum glucose levels decrease by 20%[7,8] → reliance on non-hepatic glucose, fat-derived ketone bodies, and free fatty acids as energy sources[9]
- Autophagy: a survival mechanism against antitumor treatments upregulated during times of nutrient restriction[10]
- Gut microbiome: contributor in host immune factors, neoplasia, and response to therapy. TRE may modulate microbial abundance and taxa.[11-13]

Objective: To assess the feasibility of conducting a TRE study in patients with CLL and assess for a signal on tumor control, autophagy induction, and microbial-metabolite composition. Secondary outcome measures: dietary adherence and acceptability, patient safety data (changes in body weight, side effect monitoring, weekly nutrition assessments), and QOL measures.

Methods

Design: Feasibility, parallel arm trial using a pre-post design (NCT04626843)
Intervention: 16/8 TRE (limiting caloric intake to an 8-hr window, last intake by 8pm), 6 days/week, 2 weeks of 2 days off intervention permitted.
Sample: 15 patients with Rai[14] stage 0 CLL
Dates: Feb 2021- Mar 2022

Results

Enrollment (UBC-BCC REB #H19-03702)

15 participants completed the study. Median age was 60 years with 40% female, and 60% male.

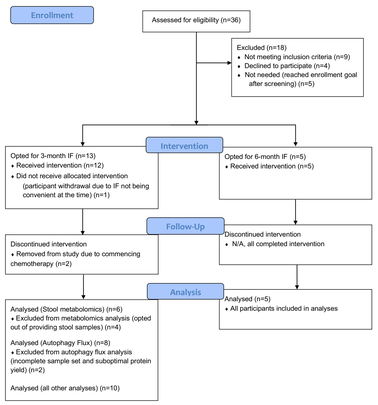

Fig 2. CONSORT diagram demonstrating the flow of participants through the study, including the three and six-month time-restricted eating parallel interventions.

Lymphocytes

Based on a linear-mixed effects model, **malignant lymphocyte counts (MLC) decreased or stabilized in 7 of 15 patients, and accumulation slowed in 5 participants (33%).** No effect was observed in 3 participants (20%).

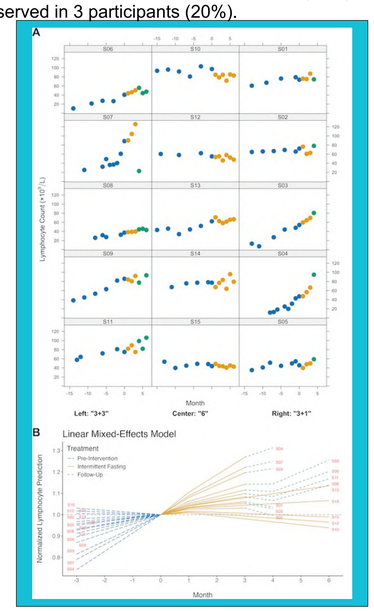

Figure 2. Plots illustrating the relationship between malignant lymphocyte count and time-restricted eating over time. (A) Scatterplot of absolute malignant lymphocyte count (MLC) over time with TRE. Blue dots represent MLC pre-intervention, gold dots during the TRE intervention, and green dots post-TRE. Left panel: three-month TRE plus three-months post-TRE; middle panel: six-months TRE; right panel: three-month TRE plus one-month post-TRE. **(B)** Linear mixed-effects model of MLCs over time with TRE.

Diet adherence, safety, weight

Adherence: 99.2%
Average eating duration: 7 hrs 28 mins (median: 7 hrs 45 mins, range: 6 hrs 10 mins - 8 hrs)
• 40% of pts did not take any permitted off-intervention days
Weight: Mean change: -0.39lb per week of TRE. Range:
• 3-month TRE: +5 to -20lbs (+3.1 to -10.5% body weight)
• 6-month TRE: -3 to -14.5lbs (-1.7 to -8.1% body weight)
Safety:
Table 1. Weekly safety scoring over duration of study, based on predefined safety criteria including weight changes, physical tolerance, thoughts/feelings (e.g. mental, social health), and concerns.

Great/ good	All pts were assessed as great/good for most of the study 7 pts (47%): entire study
Satisfactory x 1 week	3 pts: weight loss of 1.5-2% over previous week 2 pts: acute COVID-19 infection 2 pts: transient light headedness
Satisfactory x 4 weeks	1 pt: due to weight loss of 1.5-2% over previous week (non-subsequent)
Adverse event	1 cardiac event (previously undiagnosed cardiac condition, presumed unrelated)

Autophagy

TRE induces progressive reduction in tumor cell autophagy
Sharp decline in autophagy activity: between day 60 and 90, dropping below baseline levels. Despite changes within individual patients, no statistically significant differences when all data was examined in aggregate (95% CI = [-0.22, 0.04], p = .20, t_{34} = -1.31)

- 3-month TRE: a gradual decline in autophagy flux throughout all time points which continued to decline in 3 patients with longer-term follow-up data
- 6-month TRE: a small increase in autophagy flux until day 60
- Patient S12, who had the most pronounced decline in lymphocyte count, also experienced the largest reduction in autophagy activity.

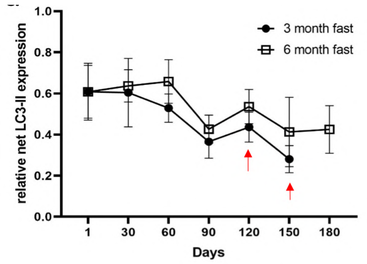

Fig 4. Autophagy in lymphocytes. LC3-II expression as marker of autophagy activation Time course of quantified LC3-II levels of all patients in the 3-month TRE group versus 6-month TRE group. Data points are expressed as the means +/- SEM of all patients in their respective cohort. Red arrows point to data points in the 3-month cohort after TRE was completed.

Quality of Life

TRE improved global health scores, which declined once returned to ad libitum eating.
Assessed through the EORTC QLQ-30

- Global health scores increased 7.73 points (95% CI = [1.54, 13.93]) from baseline of 75.27 (95% CI = [66.67, 83.87]) to 83.00 at the end of TRE, p = .022.
- Upon return to normal, ad libitum eating (n = 8): global health score returned to baseline.

Gut metabolites

Fecal metabolite abundances underwent shifts during TRE

- 3-month TRE: Glycolithocholic acid decreased while taurochenodeoxycholic acid increased during and 1 month following completion of TRE. Ursodeoxycholic acid levels were enriched at the end of the TRE but dropped beneath baseline 1 month following completion of TRE..
- 6-month TRE: 2 SCFAs, dehydrolithocholic acid and apocholic acid were the most differentially enriched.

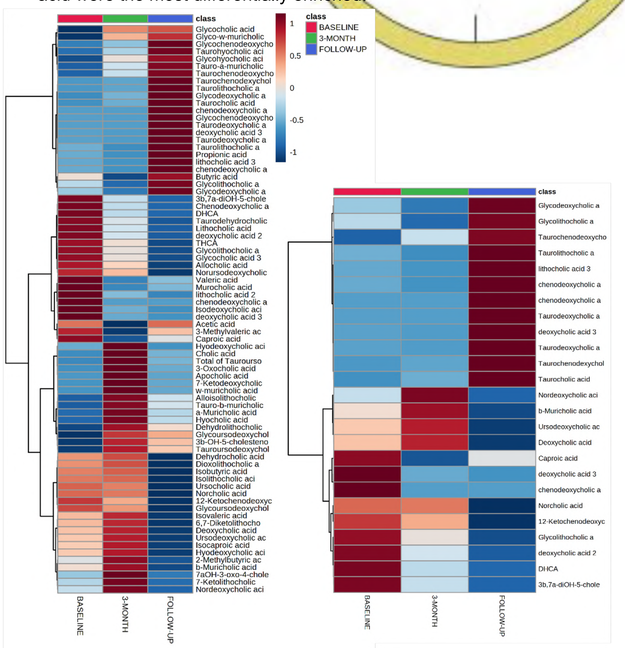

Fig 6. Heat map of metabolite abundance in the 3-month TRE group. Triplicate value calculated as the average of the duplicates. **Left:** Heat map of SCFA and BA metabolites. Dendrograms representing Ward's clustering of Euclidean distances among metabolites. **Right:** The most significant 25 SCFA and BA metabolites ranked by t-test. Dendrograms representing Ward's clustering of Euclidean distances among metabolites.

Discussion

While TRE did not consistently stabilize or reduce MLCs, **the response observed in 80% of participants remains exciting from a short term, diet intervention**. Additionally, patients reported **an improvement in sleep, digestion, energy, and overall well-being** during their weekly check-ins. The variability in weight changes aligned with patients' weight goals, demonstrating that **weight can be gained, maintained, or lost on TRE** depending on one's food choices and activity levels.

The intervention did not provide dietary guidelines, yet several participants re-evaluated their food choices to align with AICR's Cancer Prevention Recommendations,[15] commenting that this study **improved awareness of their eating habits and encouraged mindful eating**.

Given that nutrient restriction activates autophagy-mediated survival programs,[16] it was surprising that autophagy activity declined over TRE and was sustained upon return to ad libitum eating. One benefit of this scenario is that **MLCs have hindered capacity to activate protective autophagy when exposed to systemic or molecular targeted therapy, leaving them susceptible to cancer treatment**.

Lastly, **metabolite changes were apparent which have been described to affect tumor cell modulatory and immunoregulatory roles**. For example, the reduction in MLCs may also be associated with the increase in taurochenodeoxycholic and ursodeoxycholic acid which have been reported to possess anti-proliferative, apoptosis-inducing,[17] and immune-modulatory properties.[18]

Conclusion + Next Steps

This exploratory analysis demonstrates that TRE is feasible and acceptable by patients with CLL, but conclusions are limited on the effects of TRE on cancer. TRE will not be suitable for all people with cancer. Further investigation in larger cohorts is warranted to validate these initial findings. In future studies, it will be crucial to clarify the possible mechanisms of decline in MLC and autophagy from TRE. These efforts may lead to better biomarkers to stratify patients who are the most likely to benefit from metabolic interventions such as TRE.

Our team has completed a second trial (NCT05708326) duplicating this methodology but testing a different type of intermittent fasting; results are currently under analysis. With generous support of Michael Smith Health Research BC, Lotte & John Hecht Memorial Foundation, and the BC Cancer Foundation we will be recruiting for a larger trial in fall of 2024.

Want to learn more?

References + Contact information ↓

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