Abstract # 2575

Intermittent calorie restriction reverses the adverse effects of obesity and advanced age on tumor growth in a mouse model of breast cancer

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Background

- Breast cancer (BC) is the most commonly diagnosed and second deadliest cancer among women in the United States.
- Advanced age and obesity are two major risk factors for BC mortality in women. In our mouse models of BC, we have demonstrated that both advanced age and obesity accelerate mammary tumor growth.
- Mechanistically, enhanced tumor growth in mice that are obese and advanced aged can be explained, in part, by suppression of antitumor immunity-related gene expression and abundance of CD3+ and CD8+ T cells within the tumor microenvironment.
- Interventions capable of reversing the tumor-promoting effects of advanced age and obesity are needed. Calorie restrictive interventions, such as intermittent calorie restriction (ICR), attenuate aging processes and tumorigenesis and thus could offer a low-cost and accessible approach to cancer treatment in patients that are obese or older in age.

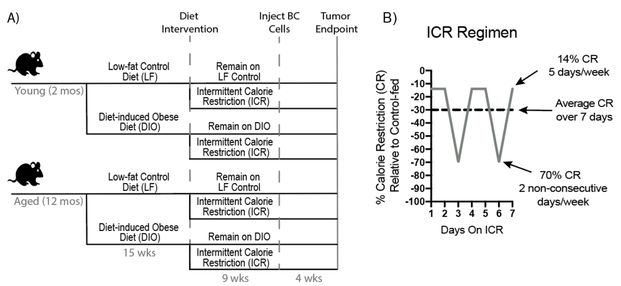
Project Aims and Hypotheses

- **Aim 1:** Determine if ICR decreases mammary tumor burden in mice that are obese, aged, or aged and obese. ***Hypothesis #1***: Weight loss by ICR in mice that are obese, aged, or aged and obese will attenuate increased tumor growth associated with obesity and advanced age.
- **Aim 2:** Assess if ICR attenuates pro-inflammatory immune signaling and tumor immunosuppression in mice that are obese, aged, or aged and obese. ***Hypothesis #2***: ICR will reduce obesity- and advanced age-related systemic pro-inflammatory immune signaling as well as increase CD8+ T cell abundance within the mammary tumor.

Animal Study Design

Figure 1. Intermittent Calorie Restriction (ICR) Study Design.

(A) Young (2 mos, n=60) and aged (12 mos, n=64) cohorts of mice were fed either a low-fat, control (control, n=30-32 per age group) or high-fat, diet-induced obesity (DIO, n=30-32 per age group) diet. After 15 weeks, mice either remained on control or DIO diets (n=15-16 per group), later referred to as baseline diet, or were placed on a 5:2 ICR regimen (n= 15-16 per group), which consisted of 14% calorie restriction (CR) 5 days/week and 70% CR 2 non-consecutive days/week (B). After 9 weeks, mice received orthotopic injection of E0771 BC cell lines and were monitored until tumor endpoint.



Methods

- Mice were weighed and serum was collected prior to tumor inoculation. At study endpoint, tumors were excised, and tumor mass/volume were measured *ex vivo*.
- Luminex multiplex chemokine assays were used to determine serum cytokine expression levels (n= 12-15 samples per group).
- Tumor samples from each group were sectioned and stained for CD8+ protein expression via immunohistochemistry. Two stained tumor sections per mouse were analyzed using QuPath software's positive cell detection algorithm, which determines percentage of positively stained cells with anti-CD8 antibody.

Results

Figure 2. ICR significantly reduces body weight in young DIO, aged control, and aged DIO mice.

Weight loss by ICR regimen was a success as shown by body weight before ICR intervention (A) compared to body weight prior to tumor inoculation, post-ICR intervention (D). Percent body fat (B) and percent lean mass (C) prior to ICR intervention show differences between groups across age and obesity statuses. Data presented as mean ± SD. Differences across groups were analyzed using one-way (A-C) or three-way (D) ANOVA. Differences in significance indicated by different letters.

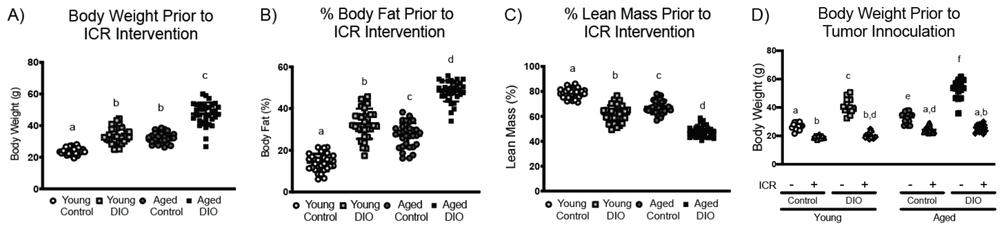


Figure 3. ICR attenuates E0771 mammary tumor growth in young DIO, aged control, and aged DIO mice.

Endpoint tumor mass (A) and tumor volume (B) were measured using a digital scale and calipers, respectively. Data presented as mean ± SD. Differences across groups analyzed using three-way ANOVA followed by Tukey multiple comparisons test. Differences in significance indicated by different letters.

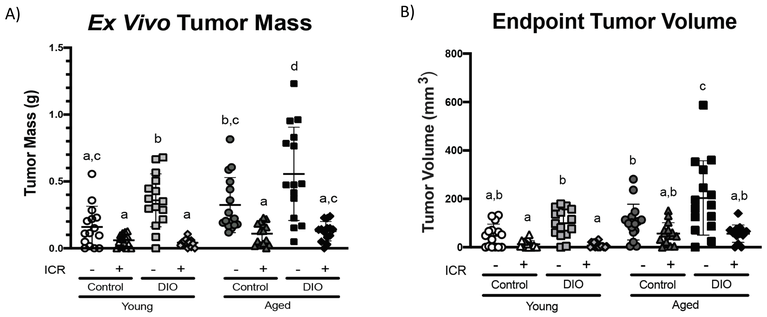
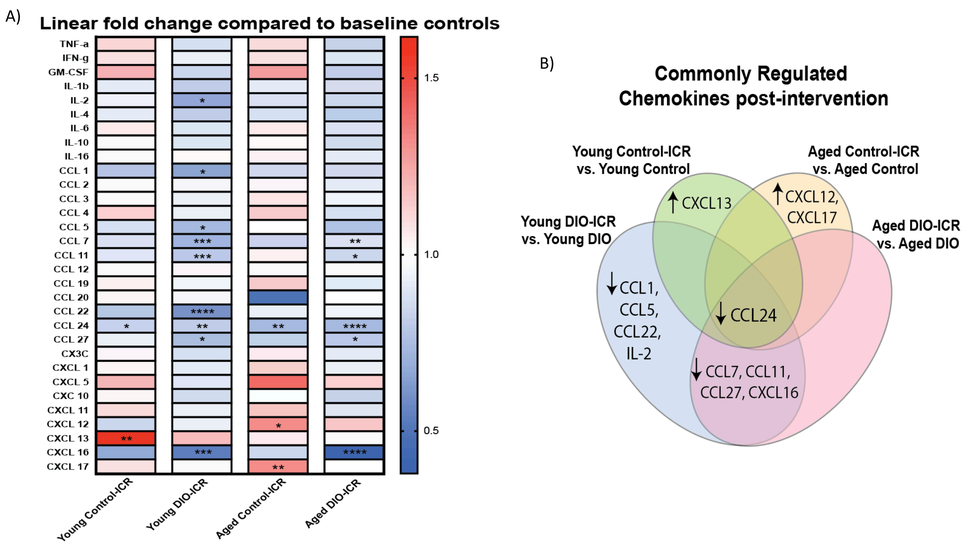


Figure 4. ICR downregulates immune-modulating cytokine expression compared to relative controls.

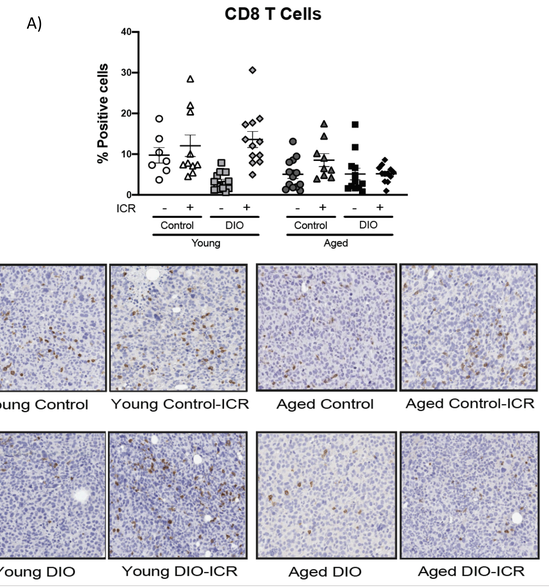
Data presented as linear fold change (A) of cytokine expression in ICR-fed mice cohorts compared to age-matched, baseline-diet-matched relative control cohorts (e.g. young control-ICR compared to young control). Venn diagram shows significantly altered cytokine expression in at least one group comparison (B). Differences across groups analyzed using three-way ANOVA followed by Tukey multiple comparisons. Asterisks indicate differences in significance.* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.



Results

Figure 5. ICR intervention in young DIO, aged control, and aged DIO mice alters CD8+ T cell abundance in E0771 tumors.

Percentage CD8 positive cells in tumor sections (A). Representative images are displayed (B). Data presented as mean ± SE. Differences across groups analyzed using three-way ANOVA. Different letters indicate significant differences.



Conclusions and Future Directions

Conclusions

- Our preclinical model demonstrates that ICR is an effective dietary intervention that attenuates accelerated tumor growth associated with advanced age and DIO.
- ICR altered overall cytokine profiles of young DIO, aged control, and aged DIO mice compared to relative controls. Downregulation of immune-modulating cytokines observed in ICR-fed mice may provide insight into how ICR reverses the adverse effects of advanced age and obesity on tumor growth.
- In align with cytokine expression, tumors from ICR-fed mice exhibited increased abundance of cytotoxic CD8+ T cells, cells vital for antitumor immunity.
- These findings suggest that ICR reduces mammary tumor burden via decreased immune-related signaling and increased CD8+ T cell abundance.

Ongoing Analysis

- Determine if CD4+ T cell abundance is altered by ICR intervention

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