Tirzepatide attenuates mammary tumor progression in diet-induced obese mice

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Running head: Tirzepatide and mammary tumor growth

Disclosures: The authors have no conflicts of interest.

Keywords: Breast cancer, obesity, incretin therapeutics, tirzepatide

Abstract

We report for the first time an anticancer benefit of tirzepatide—a dual glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide receptor agonist—in a model of obesity and breast cancer in female mice. Long-term tirzepatide treatment induced weight loss, mitigated obesity-driven changes in circulating metabolic hormone levels, and suppressed orthotopic E0771 mammary tumor growth. Relative to tirzepatide, chronic calorie restriction, an established anticancer intervention in preclinical models, promoted even greater weight loss, systemic hormonal regulation, and tumor suppression. We conclude that tirzepatide represents a promising pharmacologic approach for mitigating the procancer effects of obesity. Moreover, strategies promoting greater weight loss than achieved with tirzepatide alone may augment the anticancer benefits of tirzepatide.

Introduction

The worldwide epidemic of obesity, a risk and progression factor for ≥13 cancers including breast cancer[1], presents a global health threat, and effective strategies to mitigate this threat are needed. Estimates indicate that metabolic diseases, specifically diabetes and overweight/obesity, account for ~6% of all cancer diagnoses, and 30% of breast cancer diagnoses in women[2]. Epidemiological and preclinical studies suggest that significant intentional weight loss achieved through either lifestyle modifications or bariatric surgery is effective at reducing breast cancer incidence and progression[3-6]. Bariatric surgery is the current gold standard for long-term weight loss, with average total weight loss at 5-year follow-up of >25% and a 33% reduction in cancer risk 10 years post-surgery[7,8]. However, bariatric surgery is expensive, typically unavailable, and carries surgery-related risks[9,10]. Dietary weight loss may also offset the procancer effects of obesity but the extent of weight loss is typically less than that achieved through bariatric surgery and is challenging to sustain[11,12].

Weight-loss pharmaceuticals and supplements have historically been either ineffective (e.g., chitosan) or harmful (e.g., fenfluramine-phentermine)[13,14]. However, promising incretin-based therapies, including the dual glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptor agonist tirzepatide[15], have recently demonstrated impressive improvements in glycated hemoglobin levels and long-term, clinically-meaningful weight loss in individuals with type 2 diabetes[16,17]. While tirzepatide and retatrutide, a related GLP-1/GIP/glucagon receptor triple agonist, induce weight loss comparable to bariatric surgery[7,16,17], their anticancer benefits are unknown.

Using a mouse model of diet-induced obesity (DIO) and triple-negative breast cancer (TNBC), we tested whether long-term tirzepatide administration not only promotes sustained weight loss but also ameliorates obesity-associated metabolic dysregulation and suppresses mammary

cancer progression. Additionally, we compared the effects of tirzepatide and chronic calorie restriction (CCR), an effective weight loss and anticancer dietary regimen in preclinical models[4,5].

Results

We interrogated the weight loss, metabolic, and anticancer effects of long-term tirzepatide and CCR interventions in female DIO mice. Control and obese mice were generated via low-fat and high-fat diet feeding, respectively, then control mice (mean±SD body weight=23.9±1.8g) were maintained on low-fat diet while obese mice (mean±SD body weight=40.3±5.8g) were randomized to high-fat diet without tirzepatide (DIO) or with tirzepatide (TZP), or to CCR (30% of daily calories relative to controls; CCR) for 13 weeks (**Fig 1**). All mice received subcutaneous injections q.o.d. of vehicle or tirzepatide (escalating doses, 3–40 nmol/kg), and were orthotopically injected with E0771 cells after 27 weeks of diet treatments to induce mammary tumors.

Tirzepatide and CCR each promoted sustained weight loss (mean±SD terminal body weight change: -25.4±10.1% in tirzepatide-treated mice and -46.0±3.4% in CCR mice, versus 4.8±6.5% in DIO mice and 7.2±6.0% in controls) (**Fig 2A**). Tirzepatide reduced food consumption relative to DIO (**Fig 2B**). Tirzepatide-treated mice and CCR mice had less body weight and fat mass than DIO mice, with better conservation of lean mass in mice treated with tirzepatide versus CCR (**Fig 2C-F**).

Tirzepatide and CCR each mitigated DIO-related metabolic dysregulation, including improvement in glycemic control and normalization of several metabolic hormones (**Table 1**). Serum insulin-like growth factor 1 (IGF-1), insulin, and leptin levels were lower in tirzepatide-treated and CCR mice than DIO mice, with IGF-1 and insulin achieving levels not different from

controls. Circulating adiponectin, ghrelin, and glucagon concentrations were increased in CCR (but not tirzepatide-treated) mice, versus DIO mice.

Tirzepatide and CCR each suppressed mammary tumor growth relative to DIO, with CCR exerting superior antitumor activity (**Fig 2G**). Terminal body weight strongly correlated with tumor mass, with each 5g increase in body weight predicting a 0.70g increase in tumor weight (**Fig 2H**).

Discussion

To our knowledge, this is the first report of an anticancer benefit of extended (13 weeks) tirzepatide treatment in a preclinical model of obesity and breast cancer. Specifically, tirzepatide reduced caloric intake, produced sustained weight loss, decreased adiposity, ameliorated obesity-induced changes in glycemic control and circulating metabolic hormone levels (particularly IGF-1, insulin, and leptin), and suppressed tumor growth—albeit to lesser extents than 30% CCR—in a model of DIO and TNBC in female mice. These findings indicate that tirzepatide is a promising drug for mitigating the procancer effects of obesity.

The parallel but less robust effects of the tirzepatide regimen tested, as compared with CCR, along with the strong positive correlation between body weight and tumor mass, suggest additional approaches to increase weight loss may augment the benefits of tirzepatide. In the SURMOUNT-3 trial, tirzepatide taken following an intensive calorie restriction and exercise program produced the greatest weight loss in the SURMOUNT trial program to date[18], and although not yet studied, may also enhance anticancer effects. To prohibit rapid weight regain, we continued q.o.d. tirzepatide dosing after tumor cells were transplanted. However, clinicians may not advise weight loss drugs for patients with a cancer diagnosis due to concerns

surrounding malnutrition and cachexia[19]. Therefore, future studies assessing the possibility of using lifestyle approaches to maintain tirzepatide-induced weight loss and/or bolster lean mass after discontinuing the drug are warranted.

The murine E0771 orthotopic transplant model of TNBC enables evaluation of energy balance modulation of tumor progression but not tumorigenesis[4,5]. Thus, tirzepatide effects on tumor initiation remain to be explored as do its anticancer effects in obesity-related cancers besides TNBC.

These findings are timely and important because incretin therapies, which show promise as highly effective weight loss drugs, are increasingly being prescribed[20] and the tumor suppressive effects of tirzepatide described herein newly suggest incretin agonism as another propitious option for breaking the obesity-cancer link.

Methods

Animals

The University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee approved all animal studies and procedures. All diets were purchased from Research Diets, Inc (New Brunswick, NJ). Sixty 10-week-old C57BL/6NCrl female mice (Charles River, Wilmington, MA) were individually housed in a specific pathogen-free facility and randomized to receive high-fat diet ad libitum to induce DIO (60 kcal% fat; D12492; n=45) or sucrose-matched low-fat diet (10 kcal% fat; D12450J; n=15) for 18 weeks. Tirzepatide (LY3298176, Selleck Chemicals, Houston, TX) or dietary weight loss interventions began when body weight of obese mice averaged >40g. Power analysis based on prior weight-loss studies determined that 12 mice would reach 0.9 power (1-β) and α=0.05. We therefore included 3 additional mice/group to

ensure attrition would not render the study underpowered. Two mice were euthanized due to unresolved dermatitis; 1 mouse died of unknown causes.

Weight loss intervention

DIO mice were randomized to receive high-fat diet ad libitum with tirzepatide (TZP; n=13) or without tirzepatide (DIO; n=15), or to receive a daily serving of a diet (D15032801, Research Diets) formulated to achieve a 30% reduction in caloric intake with adequate micronutrient consumption relative to controls (CCR; n=15) (**Fig 1**). Control mice continued the low-fat diet (n=14). Every other day (q.o.d.) mice were weighed and injected subcutaneously with either 100 µl vehicle (5% DMSO, 95% 40 mM Tris HCl, pH 8.0) or 100 µl tirzepatide (week 1: 3 nmol/kg; weeks 2-3: 10 nmol/kg: weeks 4-7: 20 nmol/kg; weeks 8-11: 30 nmol/kg; weeks 12-13: 40 nmol/kg) in a random order between 16:00-18:00 to precede onset of the dark cycle.

At week 9 of the weight loss intervention, all mice were fasted 5-6h, blood was collected via tail nick, and blood glucose was measured via glucometer (Bayer, Pittsburgh, PA). Body composition was assessed using magnetic resonance imaging (EchoMRI, Houston, TX) in 10 randomly selected mice/group. E0771 tumor cells (CRL-3461, ATCC, Manassas, VA) were then orthotopically injected into the fourth mammary fat pad of every mouse ($3.5x10^4$/mouse).

Four weeks after tumor transplantation, mice were fasted 4-5h, euthanized, and tumors were excised. Serum hormones were quantified on a Luminex MAGPIX platform using the Bio-Plex Pro Mouse Diabetes 8-Plex and Adiponectin Assays (Bio-Rad, Hercules, CA) and the IGF-1 mouse Luminex Discovery Assay (R&D Systems, Minneapolis, MN). Personnel blinded to group identifiers weighed tumors and performed hormone assays.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software Inc., La Jolla, CA). $p \leq 0.05$ was considered significant. Unpaired t-tests, one-way ANOVAs with Tukey's post hoc test, and repeated measures two-way ANOVAs with Šídák's multiple comparisons test compared 2 groups, >2 groups, and ≥2 groups across time, respectively. Linear regression and Spearman correlation assessed the association between body weight and tumor mass. Serum hormone outliers identified by ROUT method (Q=5%) were removed, no other data points were removed.

Data Availability

The datasets analyzed for this study are available from the corresponding author on reasonable request.

Acknowledgements

This study was funded by the Breast Cancer Research Foundation (BCRF-22-073) and the UNC Triple Negative Breast Cancer Center. The funders played no role in study design, data collection, analysis and interpretation of data, or the writing of this manuscript. The UNC Preclinical Research Unit (P30CA16086) and Genomics and Energy Metabolic Core (P30DK056350) provided technical assistance.

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Figures and Tables

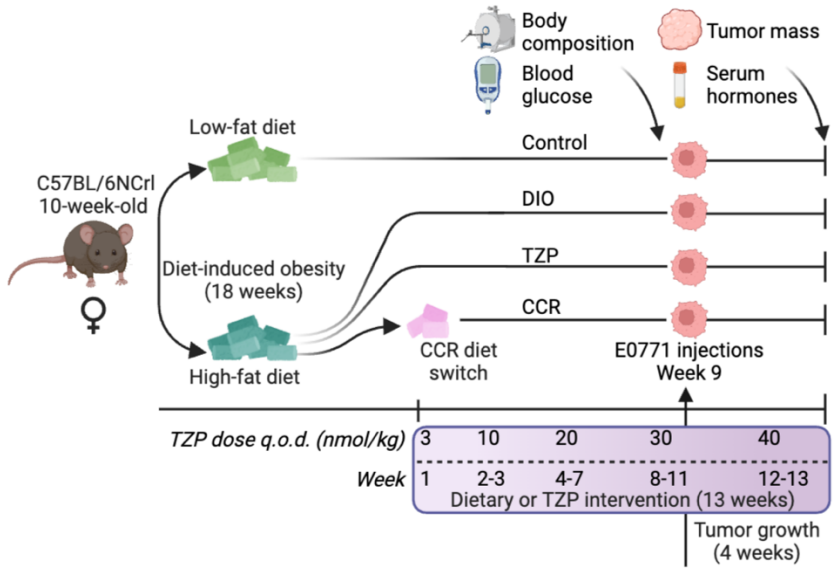

Figure 1. Study design. Schematic of study conducted to assess the potential of tirzepatide treatment to ameliorate tumor growth in a murine model of diet-induced obesity and triple-negative breast cancer. CCR, chronic calorie restriction; DIO, diet-induced obesity; TZP, tirzepatide. Created with BioRender.com.

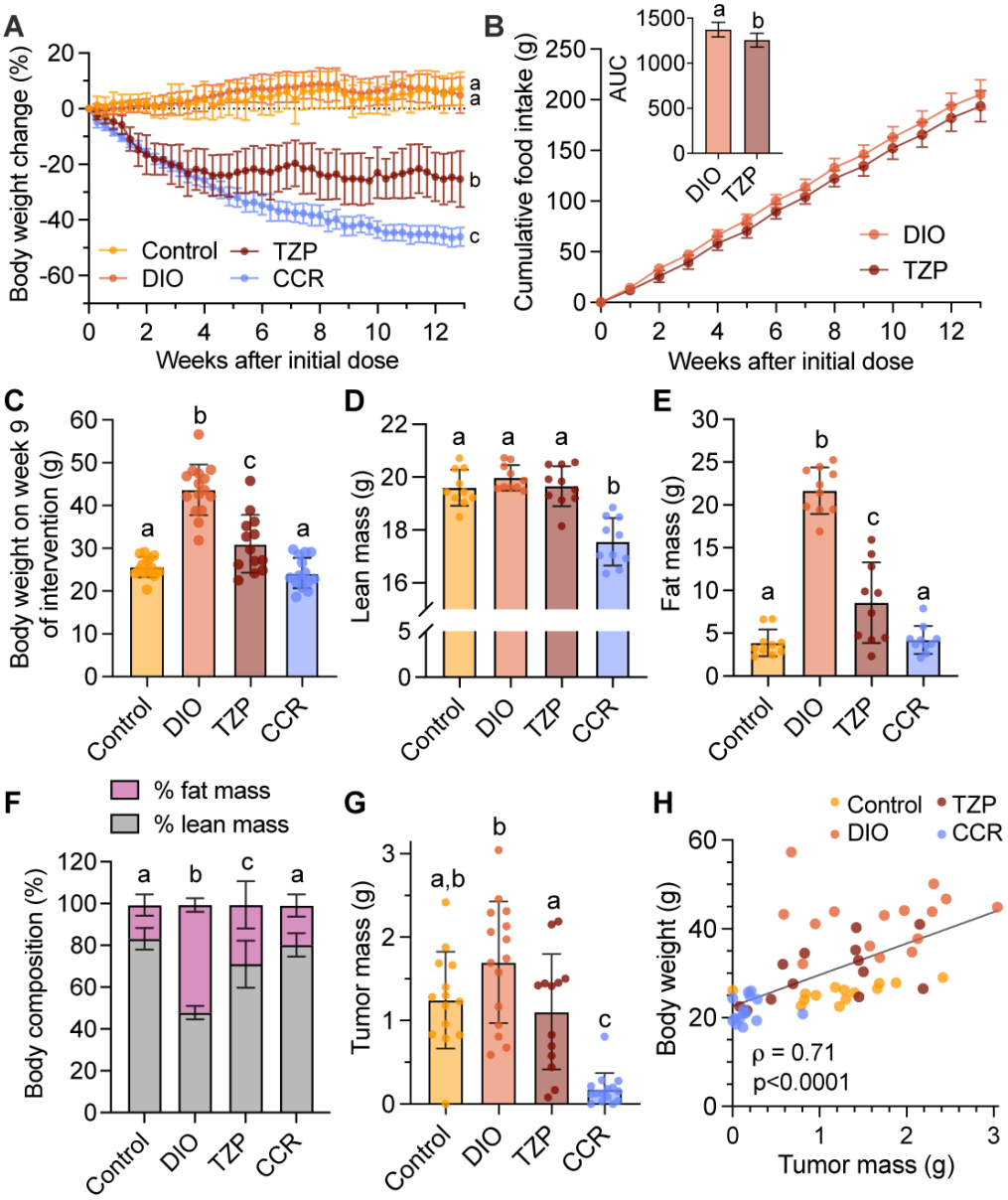

Figure 2. Chronic tirzepatide treatment reduces food intake, body weight, fat stores, and mammary tumor growth in obese female mice. (A) Body weight change and **(B)** cumulative food intake/mouse across 13-week intervention. **(C)** Body weight, **(D)** lean mass, **(E)** fat mass, and **(F)** percent fat and lean mass, at week 9 of the weight loss intervention. **(G)** Orthotopic E0771 tumor mass after 4 weeks of growth. **(H)** Correlation between terminal body weight and tumor mass.

Error bars indicate mean±SD. Repeated measures two-way ANOVA with Šídák's multiple comparisons test used for A. Unpaired t-test used for B. One-way ANOVA with Tukey's post hoc test used for C-G. Spearman correlation used for H. A-C,G: Control n=14, DIO n=15, TZP n=13, CCR n=15; D-F: n=10/group; H: n=57. Different letters denote p≤0.05 between groups.
AUC, area under the curve; CCR, chronic calorie restriction; DIO, diet-induced obesity; TZP, tirzepatide.

Table 1. Fasting concentrations of circulating glucose and selected metabolic hormones.

Analyte	Control	DIO	TZP	CCR
Adiponectin (μg/ml)	20.0 (7.5)†	23.1 (6.8)†	28.7 (13.4)†	95.0 (40.0)*#
Ghrelin (ng/ml)	15.5 (6.5)†	10.7 (3.0)†	16.2 (5.1)†	27.4 (12.1)*#
GIP (ng/ml)	1.9 (0.4)	1.8 (0.6)	2.0 (0.5)	2.0 (0.3)
GLP-1 (pg/ml)	124.2 (66.2)	108.6 (48.0)	100.8 (48.2)	130.2 (36.7)
Glucose (mg/dl)	127.6 (15.0)†	149.5 (18.5)*#†	124.5 (15.5)	107.6 (18.8)*
Glucagon (pg/ml)	847.6 (279.7)	702.0 (185.3)†	696.3 (94.0)†	972.2 (252.7)#
IGF-1 (ng/ml)	30.5 (5.3)	53.8 (18.2)*#†	40.0 (10.3)	36.5 (7.9)
Insulin (ng/ml)	2.6 (0.9)	4.5 (1.2)*#†	2.6 (0.5)	3.1 (1.6)
Leptin (ng/ml)	0.6 (0.2)#	11.9 (4.4)*#†	3.8 (2.5)*	1.6 (1.2)
PAI-1 (ng/ml)	2.6 (1.4)#†	1.7 (0.6)	1.1 (0.5)*	1.0 (0.4)*
Resistin (ng/ml)	89.8 (25.2)	65.5 (15.3)*†	69.0 (17.7)	90.0 (16.5)

Mean (SD). Blood glucose measured on week 9 of weight loss intervention. Serum hormones assayed following 13-week intervention. One-way ANOVA with Tukey's post hoc test. *p≤0.05 vs. Control; #p≤0.05 vs. TZP; †p≤0.05 vs. CCR.

CCR, chronic calorie restriction; DIO, diet-induced obesity; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; IGF-1, insulin-like growth factor 1; PAI-1, plasminogen activator inhibitor 1; TZP, tirzepatide.