

Effect of the p75 Neurotrophin Receptor on Glycemic Control of Obese Mice during Time-Restricted Feeding

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ABSTRACT

Circulating glucose regulates organismal energy homeostasis and is tightly controlled in response to feeding behavior. In overweight individuals, glucose homeostasis is often perturbed due to resistance to normal satiety signals, such as insulin and leptin, leading to type 2 diabetes and its attendant complications. An emerging dietary intervention, time-restricted feeding (TRF), aims to ameliorate the adverse metabolic consequences of obesity, however, its effectiveness on glucose control is uncertain. Here, we demonstrate that TRF is only transiently effective in reducing pre-meal serum glucose levels in obese mice lacking leptin. However, in *Ob/Ob* mice that also lack a gene known to suppress behavior associated with TRF, the p75 neurotrophin receptor (p75NTR), a sustained reduction of glucose levels is observed. These results suggest that the effectiveness of TRF on glycemic control can be enhanced with concurrent targeting of modulators of glucose homeostasis and TRF response.

INTRODUCTION

Metabolic health is increasingly appreciated as a fundamental requirement in preventing many chronic diseases (1). Obesity and type 2 diabetes come with symptoms that are highly systemic, and confer an increased risk for further complications such as cardiovascular disease and cancer (2). Compounding their detrimental effects is the lack of a broadly effective means to reverse obesity or type 2 diabetes (3).

Recently a novel diet regimen, time-restricted feeding (TRF), has gained attention as a plausible option for improving metabolic health by leveraging our knowledge of circadian biology to better time food intake to match organismal metabolic activity (4). Benefits of TRF in several clinical studies of patients at risk for obesity and type 2 diabetes have included increased insulin sensitivity and lowering of blood pressure, with several other trials ongoing in those with obesity and the metabolic syndrome (5–7). However, while TRF has shown some improvement of glucose homeostasis in mice on a high fat diet, the effect in humans is less clear, with only modest benefits observed (4–6,8). As glucose control is one of the most important drivers of detrimental health outcomes in those with obesity and diabetes, this suggests that combinatorial strategies may be necessary to achieve optimal health benefits from TRF (9).

Animal models of obesity have historically been used to study the control of glucose homeostasis in the context of increased weight and adiposity. One of the best characterized genetic models of obesity is the leptin-deficient *Ob/Ob* mouse, which develops massive obesity due to increased food intake and decreased energy expenditure, and subsequent hyperglycemia (10,11). Notably, the *Ob/Ob* background has been used previously to identify that the soluble ligand TNF α and its receptors (TNFR1, *Tnfrsf1*; TNFR2, *Tnfrsf2*) are necessary to promote hyperglycemia and insulin resistance in obesity (12,13). More recently we found a novel signaling interaction between TNF α /TNFR signaling with a paralogous TNFR superfamily

member, the p75 neurotrophin receptor (p75NTR, *Ngfr*) (14,15). Given these connections, we questioned whether there may be a similar role for p75NTR in obesity.

Similar to the TNF α receptors, p75NTR has been implicated in insulin sensitivity and glucose control in adipocytes, and has also been found to be necessary to promote weight gain on a high fat diet (16,17). Recently, we found that mice lacking p75NTR (*Ngfr*-KO) exhibit a phenotype of decreased food intake with increased weight loss on TRF, but while maintaining blood glucose levels similar to that of controls (18). However, due to their resistance to diet-induced obesity, it is unknown whether p75NTR may have a role in modulating glucose homeostasis during TRF in the setting of obesity.

To study this possibility, we generated a genetic model of *p75NTR* deletion on a background of leptin-deficient obesity (*ob/ob*). We used this model to test the hypothesis that targeting p75NTR function during TRF would lead to improvements in glycaemic control despite obesity.

Unexpectedly, we find that this dual loss of leptin and p75NTR leads to a variable weight phenotype, with increased mortality in adolescent and young adult mice due to an unknown cause. In mice that reach full adulthood, however, we find strikingly improved glucose homeostasis independent of body weight during TRF.

RESULTS

Loss of p75NTR in leptin-deficient *ob/ob* mice leads to obesity and hyperglycemia with increased mortality

To examine the physiologic effect of p75NTR loss in leptin-deficient mice, body weight and food intake were measured over the course of 15 weeks. Male *Ob-Ngfr*KO mice and WT controls had similar body weights until around week 7. From week 7 to week 15, approximately 40% of *Ob-Ngfr*KO mice began losing weight (**Fig 1A**). This weight loss occurred concurrently with decreased food intake, and all cases of weight loss in *Ob-Ngfr*KO mice progressed to the point of death or removal from the study by 15 weeks of age (**Fig 1B, C**). In those *Ob-Ngfr*KO mice that survived, we find they gained weight similarly to controls, with a similar ratio of feed efficiency despite a lower level of absolute food intake (**Fig 1D-F**). While the discordance between body weight and food intake may suggest a possible change in energy output, as has been previously demonstrated for *Ngfr*KO mice (17), it remains unknown whether *Ob-Ngfr*KO mice have decreased energy expenditure. We also demonstrate that baseline blood glucose is similar between genotypes (**Fig 1G**).

As p75NTR has been implicated in other metabolic functions regulated by leptin, including feeding and glucose homeostasis, we next assessed whether leptin responsiveness was intact (16,18). Following intraperitoneal vehicle administration, *Ob-Ngfr*KO mice ate significantly less than controls (**Fig 1G**), agreeing with the prior trend in food intake data (**Fig 1B, 1E**). Upon leptin administration (1ug, i.p.), however, both genotypes exhibited similar reductions in food intake (**Fig 1G**).

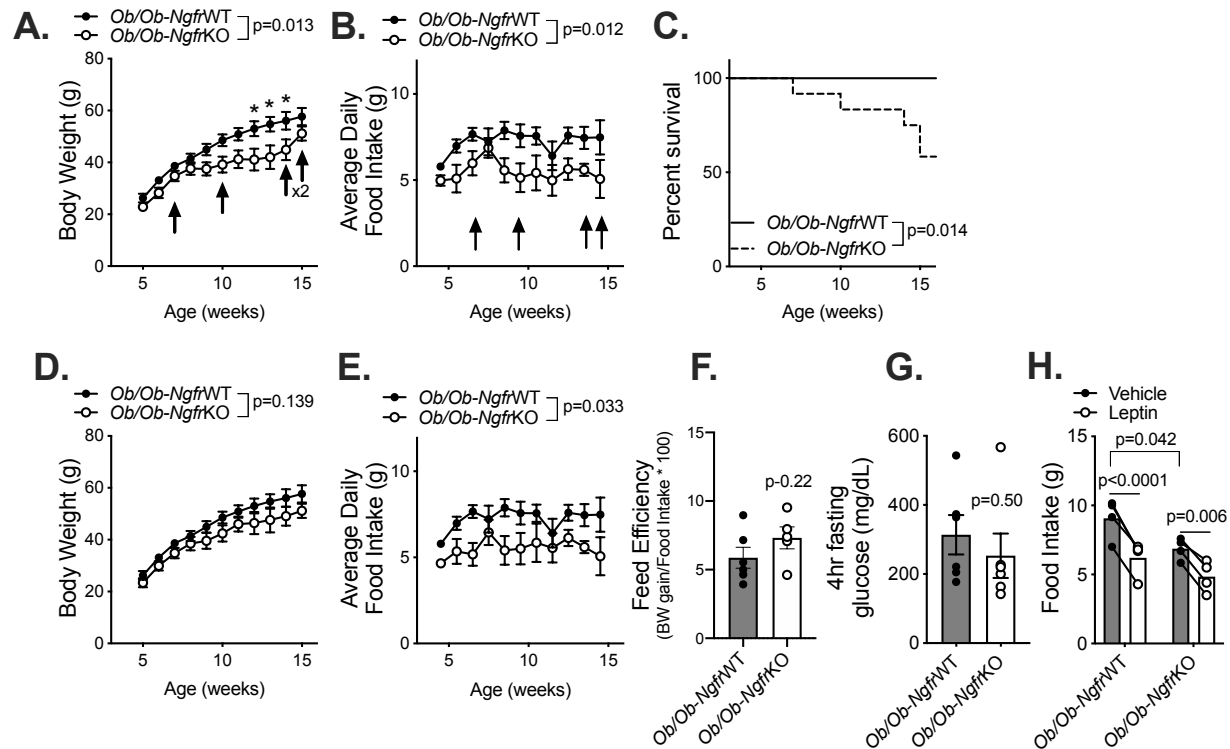


Figure 1- *Ob-NgfrKO* mice develop obesity and hyperglycemia, but with increased mortality. (A)

Male mouse body weight curves during adolescence into adulthood, $n=12/\text{group}$. Arrows indicate the death of an *Ob-NgfrKO* mouse. $F(1,22)=7.256$, $p=0.013$ overall between genotypes by mixed effects Anova. * $p<0.01$ at time points between genotypes by mixed effects Anova with Bonferroni's multiple comparisons post-hoc test. **(B)** Daily food intake averaged over the course of 7 days during development, $n=7$ *Ob-NgfrWT*, 8 *Ob-NgfrKO*. Arrows indicate the death of an *Ob-NgfrKO* mouse. $F(1,13)=8.446$, $p=0.012$ between genotypes by mixed effects Anova. **(C)** Survival curve during development, $n=12/\text{group}$. $p=0.014$ by Mantel-Cox test ($\chi^2=6.06$, $df=1$). **(D)** Male mouse body weight curves of surviving mice from adolescence into adulthood (replotted from A), $n=12$ WT, 7 KO. $F(1,17)=2.408$, $p=0.139$ between genotypes by two-way Anova. **(E)** Daily food intake of surviving mice averaged over the course of 7 days during development (replotted from B), $n=7$ *Ob-NgfrWT*, 5 *Ob-NgfrKO*. $F(1,10)=6.131$, $p=0.033$ between genotypes by two-way Anova. **(F)** Feed efficiency (10 week body weight gain (g) divided by 10 week food intake (g)) of survivors. $n=7$ *Ob-NgfrWT*, 5 *Ob-NgfrKO*. $F(1,10)=6.131$, $p=0.22$ between genotypes by Student's t-test. **(G)** Blood glucose following a 4 hour daytime fast in 12 week old mice, $n=6/\text{group}$. $p=0.50$ by Student's t-test. **(H)** Food intake over 24 hours following i.p. vehicle or leptin (1 ug) administration just before lights out, $n=4/\text{group}$. $F(1,6)=4.79$ between genotypes by two-way Anova with Bonferroni's multiple comparisons test.

Blood glucose levels are improved in *Ob-Ngfr*KO mice during time restricted feeding

To examine the effect of p75NTR on glucose homeostasis in the setting of obesity, *Ob-Ngfr*WT and surviving *Ob-Ngfr*KO mice were placed on a five day TRF (**Fig 2**). While *Ob-Ngfr*KO mice weighed around 9% less than controls, we observed similar trends in body weight and food intake over the course of restriction, with no statistically significant differences at any time point (**Fig 2A, B**). Both genotypes also experienced a similar reduction in blood glucose during the initial fast, however, blood glucose levels returned to normal in *Ob-Ngfr*WT mice by day 3 while they remained significantly lower in *Ob-Ngfr*KO mice (**Fig 2C**). While loss of p75NTR alone does not significantly alter fed or fasting insulin levels, *Ngfr*KO mice have been demonstrated to have improved insulin sensitivity (16,18). However, it is unknown whether *Ob-Ngfr*KO mice have similarly improved insulin sensitivity, nor whether this could explain the observed changes in glucose. Thus, it appears that *Ob-Ngfr*KO mice on TRF are able to adaptively correct their hyperglycemia in response to food restriction, while *Ob-Ngfr*WT mice are not.

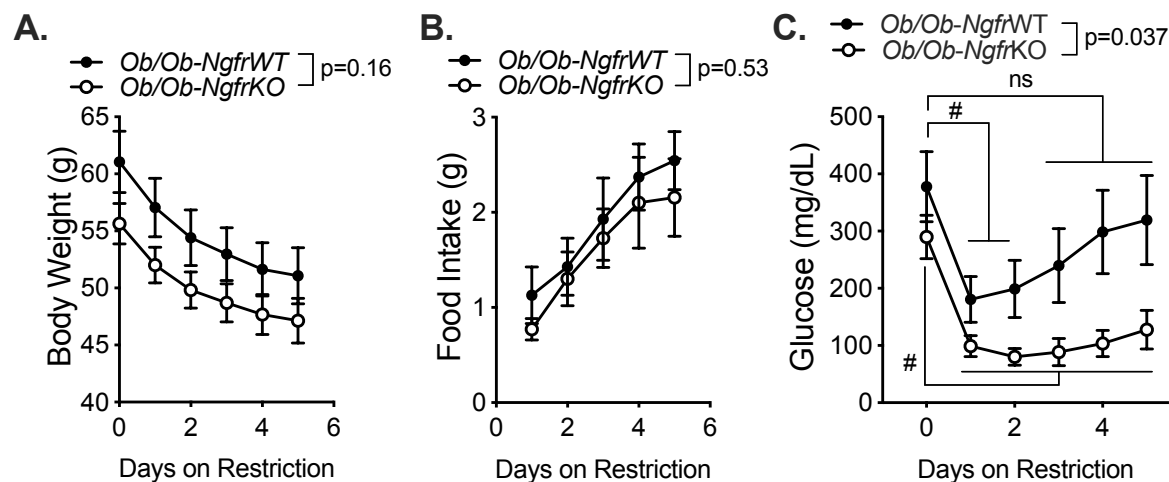


Figure 2- *Ob-NgfrKO* mice have significantly lower blood glucose during TRF compared to controls. 16-20 week old mice were placed onto TRF, whereby food was removed just before lights off on day 0, and was made available for only three hours per day on each of the next five days. Mice were allowed *ad libitum* access to food during the 3 hour meal time, and body weight and blood glucose measurements were made daily just after lights on. **(A)** Body weight of mice on TRF. $F(1,12)=2.29$, $p=0.16$ between genotypes by two-way Anova. **(B)** *Ad libitum* food intake during 3 hour mealtime. $F(1,12)=0.428$, $p=0.53$ between genotypes by two-way Anova. **(C)** Blood glucose measurements of mice on TRF. $F(1,12)=5.48$, $p=0.037$ between genotypes by two-way Anova. # $p<0.05$ compared to day 0 blood glucose by two-way Anova with Bonferroni's multiple comparisons.

DISCUSSION

Time restricted feeding is a metabolic intervention with increasing evidence for wide ranging benefits, though with mixed support for improvements in glycemic control. Pairing TRF with a targeted therapy against molecular players involved in glucose homeostasis and the response to TRF could be an effective strategy to further improve metabolic outcomes. Here, we demonstrate that obese mice with a loss of the p75NTR have similar baseline body weight and hyperglycemia as controls, but exhibit a larger and longer lasting reduction in blood glucose on TRF. A major limitation of this study, however, is the survival heterogeneity of mice with a double knockout of leptin and p75NTR. Elucidating the cause of their loss of body weight and appetite could provide a new window on our understanding of leptin and neurotrophin signaling in energy balance.

Previous work has delineated a role for p75NTR in reducing insulin sensitivity by decreasing Rab5 activity and increasing Rab31 activity, thereby reducing GLUT4 in the plasma membrane (16). In *Ngfr*KO mice fed a normal chow diet, this leads to an increase of glucose uptake in adipocytes and myocytes, decreasing overall serum glucose levels (16). It was unknown, however, whether these improvements would be maintained in the setting of increased weight, as *Ngfr*KO mice are resistant to diet-induced obesity. We suggest here that these improvements are indeed maintained during obesity, and that this provides preliminary evidence that targeting p75NTR function could improve the efficacy of TRF regimens with the aim of improving glycemic control. However, the approximate 9% baseline difference in body weight observed limits this interpretation, and warrants further investigation using strategies of weight matched cohorts. Further, given the unexpected lethality of some of our *Ob-Ngfr*KO mice, further study is warranted to establish the cause of death and possible adverse side effects in the acute setting, especially with the use of continuous glucose monitoring for possible drop-out effects.

Achieving improved glucose control is a clinically important endpoint for individuals with obesity and type 2 diabetes, and has been shown to significantly improve health outcomes. We believe coupling TRF with strategies to improve glucose homeostasis provides a promising path forward to improving metabolic health.

METHODS

Mice.

All experiments were carried out in compliance with the Association for Assessment of Laboratory Animal Care policies and approved by the University of Virginia Animal Care and Use Committee. Animals were housed on a 12-h light/dark cycle with food (Teklad Diet 8664) and water *ad libitum* unless otherwise indicated. *Ngfr*-KO mice were purchased from Jackson Labs (Bar Harbor, ME, USA) (#002213)(19), and were maintained on a B6;129s mixed background and genotyped with primers against intron II in *Ngfr* - Intron II (*Ngfr*-IntII, 5'-CGA TGC TCC TAT GGC TAC TA), Intron III (*Ngfr*-IntIII, 5'-CCT CGC ATT CGG CGT CAG CC), and the pGK-Neo cassette (pGK, 5'-GGG AAC TTC CTG ACT AGG GG). *Ob/Ob* mice were purchased from Jackson Labs (#000632) and genotyped by PCR amplifying (Forward primer: TGT CCA AGA TGG ACC AGA CTC, Reverse primer: ACT GGT CTG AGG CAG GGA GCA) a region around the *Ob* point mutation, followed by restriction digest using Dde1. All crosses were carried out using *Ob*/+ and *Ngfr*+/- or *Ngfr*-/- mice. All data shown is for male mice.

Body Weight and Food Intake.

Food intake was performed on individually housed male mice that were acclimated for 7 days, followed by food and body weight measurements weekly (for development curves), or daily during restricted feeding experiments.

Leptin Administration.

Vehicle (100 ul saline) or recombinant leptin (1 ug in 100 ul saline; Peprotech, Rocky Hill, NJ, USA) was injected intraperitoneally into 12-16 week old *ad libitum* fed mice just prior to lights out. Food intake was measured 24 hours later.

Time Restricted Feeding.

For scheduled feeding, mice were first acclimated to single housing for 7 days. For daytime scheduled feeding, mice were fasted at lights off (ZT12) on day 0. Mice were weighed, and glucose (one touch ultra 2, Bayer, Leverkusen, Germany) measures were taken 12 hours later at lights on (ZT0). Mice were then refed 4 hours later at ZT4, with food removed 3 hours later at ZT7. Mice were fed between ZT4-7 on each of the next four days.

Statistical Analysis.

Data are presented as mean $\pm$ SEM. Statistical analysis was carried out using Prism version 8.0 (Graphpad, San Diego, CA, USA). Student's t-test was used to compare single means between genotypes, and 2-way Anova was used to compare genotype by time interactions. Differences were considered significant if $p < 0.05$.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

1. Bauer UE, Briss PA, Goodman RA, Bowman BA. Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. *Lancet*. 2014 Jul 5;384(9937):45–52.
2. Khaodhilar L, McCowen KC, Blackburn GL. Obesity and its comorbid conditions. *Clin Cornerstone*. 1999;2(3):17–31.
3. Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. *N Engl J Med*. 2017 Jan 19;376(3):254–66.
4. Chaix A, Zarrinpar A, Miu P, Panda S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab*. 2014 Dec 2;20(6):991–1005.
5. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. *Cell Metab*. 2018 Jun 5;27(6):1212-1221.e3.
6. Hutchison AT, Regmi P, Manoogian ENC, Fleischer JG, Wittert GA, Panda S, et al. Time-Restricted Feeding Improves Glucose Tolerance in Men at Risk for Type 2 Diabetes: A Randomized Crossover Trial. *Obesity (Silver Spring)*. 2019 Apr 19;27(5):724–32.
7. Chaix A, Manoogian ENC, Melkani GC, Panda S. Time-Restricted Eating to Prevent and Manage Chronic Metabolic Diseases. *Annu Rev Nutr*. 2019 Aug 21;39:291–315.
8. Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab*. 2012 Jun 6;15(6):848–60.
9. Herman WH. Clinical evidence: glycaemic control in diabetes. *BMJ*. 1999 Jul 10;319(7202):104–6.
10. Ingalls AM, Dickie MM, Snell GD. Obese, a new mutation in the house mouse. *J Hered*. 1950 Dec;41(12):317–8.
11. Lindström P. The physiology of obese-hyperglycemic mice [ob/ob mice]. *ScientificWorldJournal*. 2007 May 29;7:666–85.
12. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature*. 1997 Oct 9;389(6651):610–4.
13. Uysal KT, Wiesbrock SM, Hotamisligil GS. Functional analysis of tumor necrosis factor (TNF) receptors in TNF-alpha-mediated insulin resistance in genetic obesity. *Endocrinology*. 1998 Dec;139(12):4832–8.
14. Kuhn KD, Edamura K, Bhatia N, Cheng I, Clark SA, Haynes CV, et al. Molecular dissection of TNFR-TNFα bidirectional signaling reveals both cooperative and antagonistic interactions with p75 neurotrophic factor receptor in axon patterning. *Mol Cell Neurosci*. 2020 Jan 28;103:103467.

15. Gamage KK, Cheng I, Park RE, Karim MS, Edamura K, Hughes C, et al. Death receptor 6 promotes wallerian degeneration in peripheral axons. *Curr Biol*. 2017 Mar 20;27(6):890–6.
16. Baeza-Raja B, Li P, Le Moan N, Sachs BD, Schachtrup C, Davalos D, et al. p75 neurotrophin receptor regulates glucose homeostasis and insulin sensitivity. *Proc Natl Acad Sci USA*. 2012 Apr 10;109(15):5838–43.
17. Baeza-Raja B, Sachs BD, Li P, Christian F, Vagena E, Davalos D, et al. p75 neurotrophin receptor regulates energy balance in obesity. *Cell Rep*. 2016 Jan 12;14(2):255–68.
18. Podyma B, Johnson D-A, Sipe L, Remcho TP, Battin K, Liu Y, et al. The p75 neurotrophin receptor in AgRP neurons is necessary for homeostatic feeding and food anticipation. *elife*. 2020 Jan 29;9.
19. Lee KF, Li E, Huber LJ, Landis SC, Sharpe AH, Chao MV, et al. Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. *Cell*. 1992 May 29;69(5):737–49.