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ORIGINAL RESEARCH

In Vivo Responses of Arcuate Tyrosine Hydroxylase (TH) Neurons to Hormonal Challenges Under Chronic High-Fat Diet

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ABSTRACT

Diet-induced obesity disrupts arcuate nucleus (ARC) hormonal signalling, including the development of ghrelin and leptin resistance, phenomena well-characterised in canonical AgRP/NPY neurons but largely unexplored in other orexigenic ARC populations. Tyrosine hydroxylase (TH)-expressing ARC neurons represent one such population with established roles in feeding control, yet whether chronic high-fat diet (HFD) exposure impairs their responsiveness to ghrelin and leptin in vivo remains unknown. Here, we investigated how HFD shapes ARC TH neuron Ca^{2+} dynamics across feeding-related states and during acute hormonal challenges using fibre photometry in freely moving TH-Cre mice.

A total of 11 male TH-Cre mice were maintained on standard diet (SD, n=5) or HFD (n=6) for three months. A Cre-dependent AAV.Syn.Flex.GCaMP6s virus was injected into the ARC, followed by optical fibre implantation. After at least two weeks of expression, in vivo fiber photometry recordings were performed. Fluorescence signals were calculated as ΔF/F and summarized as area under the curve per minute (AUC/min). Basal activity was assessed during baseline (P1), food exposure (P2), and digestion (P3) under fed and fasted conditions. For hormonal challenges, fed mice received intraperitoneal ghrelin (1 mg/kg) and fasted mice received leptin (5 mg/kg), with analyses conducted across P1–P4 epochs. Basal ARC TH activity was comparable between chow and HFD groups across P1–P3 in both metabolic states. Ghrelin significantly increased ARC TH activity during P2 in chow controls, whereas this response was attenuated in HFD mice ($p = 0.043$). Leptin produced modest and similar effects in both groups. Chronic HFD selectively blunts ghrelin-evoked activation of ARC TH neurons while largely preserving basal activity and leptin responsiveness.

Keywords: Arcuate nucleus. Tyrosine hydroxylase. High-fat diet. Ghrelin. Leptin. Obesity.

Kronik Yüksek Yağlı Diyetin Arkuat Tirozin Hidroksilaz (TH) Nöronlarında Ghrelinle Uyarılan Kalsiyum Yanıtları Üzerindeki İn Vivo Etkileri

ÖZET

Diyet kaynaklı obezitenin, arkuat çekirdek (ARC) hormonal sinyalleşmesini bozarak ghrelin ve leptin direncinin gelişimine yol açtığı yapılan çalışmalar ile ortaya konmuştur. Direnç gelişiminde başta AgRP/NPY nöronları olmak üzere kanonik ARC popülasyonlarının rolleri iyi karakterize edilmiş olsa da diğer oreksijenik ARC popülasyonlarında büyük ölçüde araştırılmamıştır. Tirozin hidroksilaz (TH) eksprese eden ARC nöronları, beslenme kontrolündeki yerleşik rolleriyle böyle bir popülasyonu temsil etmekle birlikte, kronik yüksek yağlı diyet (HFD) maruziyetinin bu nöronların ghrelin ve leptine in vivo yanıt verme kapasitesini olası etkisi henüz bilinmemektedir. Bu çalışma, serbest hareket eden TH-Cre farelerde fiber fotometri kullanılarak HFD'nin ARC TH nöronlarının Ca^{2+} dinamiklerini beslenmeyle ilişkili evreler boyunca ve akut hormonal uyarılar sırasında nasıl şekillendirdiği araştırmak üzere gerçekleştirilmiştir.

Toplamda 11 erkek TH-Cre fareler üç ay boyunca standart diyet (SD, n=5) veya HFD (n=6) ile beslenmiştir. ARC'ye Cre-bağımlı AAV.Syn.Flex.GCaMP6s virüsü enjekte edilmiş ve optik fiber implantasyonu gerçekleştirilmiştir. En az iki haftalık ekspresyon süresinin ardından in vivo fiber fotometri kayıtları alınmıştır. Floresan sinyaller ΔF/F olarak hesaplanmış ve dakika başına eğri altındaki alan (AUC/dk) şeklinde özetlenmiştir. Bazal aktivite, tokluk ve açlık koşullarında başlangıç (P1), besine maruziyet (P2) ve sindirim (P3) evrelerinde değerlendirilmiştir. Hormonal uyarılar için tok farelere intraperitoneal ghrelin (1 mg/kg), aç farelere ise leptin (5 mg/kg) uygulanmış ve analizler P1–P4 evrelerinde yapılmıştır. Bazal ARC TH aktivitesi her iki metabolik durumda da P1–P3 boyunca chow ve HFD grupları arasında benzer bulunmuştur. Ghrelin, chow grubunda P2 sırasında ARC TH aktivitesini anlamlı biçimde artırırken, bu yanıt HFD farelerde baskılanmıştır ($p = 0.043$). Leptin ise her iki grupta benzer ve sınırlı etkiler göstermiştir. Sonuç olarak, kronik HFD maruziyeti, ARC TH nöronlarının bazal aktivitesini ve leptin yanıtını büyük ölçüde korurken, ghrelin ile indüklenen aktivasyonu seçici olarak zayıflatmaktadır.

Anahtar Kelimeler: Arkuat çekirdek. Tirozin hidroksilaz. Yüksek yağlı diyet. Ghrelin. Leptin. Obezite.

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The arcuate nucleus (ARC) of the hypothalamus is a pivotal hub for energy homeostasis, integrating circulating adiposity signals and meal-related cues to orchestrate feeding, neuroendocrine outputs, and autonomic function. Classic ARC neuron populations, AgRP/NPY neurons that promote feeding and POMC neurons that suppress it, translate peripheral metabolic information into coordinated behavioural and physiological responses[1–5]. In obesity, chronic exposure to high-fat diet (HFD) disrupts ARC signalling and is closely linked to central leptin resistance, inflammatory remodelling, and impaired responsiveness to hormonal cues that normally regulate food intake[5–7]. Although the AgRP/POMC axis has been extensively characterized, increasing evidence supports an important contribution from additional ARC neuronal subtypes that interact with feeding circuits and may shape hedonic and homeostatic components of feeding[8,9].

Among these, tyrosine hydroxylase (TH)-expressing neurons in the ARC have emerged as a functionally relevant orexigenic population beyond their classical neuroendocrine role. ARC TH neurons largely overlap with A12 dopaminergic neurons but also display marked heterogeneity. They are not a uniform population, and can release classical neurotransmitters including GABA, communicate locally within the ARC microcircuit, and respond to multiple neuropeptides implicated in energy balance[10]. This heterogeneity suggests that ARC TH neurons occupy a unique interface between dopaminergic neuroendocrine signalling and hypothalamic feeding control. Functionally, optogenetic activation of ARC TH neurons increases food intake, while their silencing reduces body weight, firmly implicating these neurons in feeding behaviour rather than solely prolactin-related neuroendocrine pathways[10]. Mechanistically, ARC TH neurons can inhibit local POMC neurons, providing a route through which dopaminergic and GABAergic signalling directly interfaces with melanocortin pathways central to satiety control[10]. Together, these findings position ARC TH neurons as a candidate node linking peripheral metabolic state to both hypothalamic feeding circuits and broader motivation- and reward-related processes.

In obesity, chronic HFD exposure disrupts ARC hormonal signalling, most prominently through the development of leptin and ghrelin resistance in AgRP/NPY neurons: HFD diminishes ghrelin-induced ARC activation and electrophysiological excitability, with hyperleptinemia proposed as a key driver[5-7,11,12]. However, whether chronic HFD similarly impairs ghrelin and leptin responsiveness in ARC TH neurons, a distinct orexigenic population increasingly implicated in feeding control, remains unknown.

A key barrier to resolving these questions is the limited knowledge of real-time, in vivo Ca^{2+} dynamics of ARC TH neurons across feeding states and during acute hormonal challenges. While slice electrophysiology indicates that ghrelin can excite ARC TH neurons[10], comparable in vivo measurements under chronic diet manipulation are scarce. We hypothesised that chronic HFD would alter basal Ca^{2+} dynamics and impair ghrelin and leptin responsiveness in ARC TH neurons, thereby contributing to the hormonal resistance that develops in the context of diet-induced obesity. To test this, we employed fibre photometry with Cre-dependent GCaMP6s expression in TH-Cre mice to longitudinally record ARC TH neuron activity in freely moving animals across feeding- and fasting-related epochs as well as following acute ghrelin and leptin administration under SD versus HFD conditions.

Materials and Methods

Animals

A total of 11 male TH-Cre transgenic mice were used. TH-Cre mice were originally obtained from The Jackson Laboratory (USA; JAX #008601) and bred in-house. In this line, Cre recombinase expression is driven by the tyrosine hydroxylase (TH) promoter, thereby restricting Cre activity to TH-expressing neurons[10,13]. Because the viral vectors used in this study were Cre-dependent, transgene expression occurred selectively in TH neurons. Mice were housed under standard conditions (12 h light/dark cycle; 21 ± 1 °C) with ad libitum access to food and water unless otherwise specified. All experimental procedures were performed in accordance with the ARRIVE 2.0 guidelines and the European Directive 2010/63/EU on the protection of animals used for scientific purposes, and were approved by the Local Ethics Committee for Animal Experiments of the Yeditepe University Experimental Animal Research Center (YÜDETAM; approval number: 2023/01-02, date: 27/01/2023).

A priori sample size was calculated using G*Power v3.1.9.7. The expected effect size was estimated from previously published ARC neuronal activity studies in diet-induced obesity models, which consistently demonstrated large effect sizes (Cohen's d =1.8) for HFD-induced attenuation of ghrelin-evoked responses in arcuate neurons[11,12]. Using an unpaired two-tailed t-test with α=0.05, power (1 − β)=0.80, and Cohen's d=1.80, the calculation yielded a minimum required sample of n=6 per group (total n=12; actual power=0.815). Accordingly, 12 male TH-Cre mice were enrolled and allocated to chow (n=6) or HFD (n=6) using block randomisation stratified by baseline body weight. One chow animal was excluded from the final analysis due to displacement of the optical fibre during the recording session, yielding a final analysed cohort of n = 5 (chow) and n = 6 (HFD). No animals were excluded post-recording on the basis of signal magnitude or statistical outcome.

Male mice were exclusively used in this study for two reasons. First, oestrous cycle-related fluctuations in female mice introduce significant variability in ghrelin and leptin sensitivity, which would complicate the interpretation of hormone-evoked Ca^{2+} responses[22]. Second, male mice develop a more consistent and robust metabolic phenotype under chronic HFD exposure, including more pronounced weight gain, hyperleptinemia, and insulin resistance, making them a more reliable model for studying diet-induced hormonal dysregulation[23,24].

Diet Induced Obesity Model

After one week of acclimatisation, mice were randomly allocated to one of two dietary groups:

Chow group: standard rodent chow (~18% kcal from fat)

HF group: high-fat diet (60% kcal from fat)

Diets were maintained for 12 consecutive weeks. Body weight was measured (every two weeks) between 09:00–11:00 using a calibrated digital balance. At the end of the 12-week feeding period, mice were euthanised by decapitation under isoflurane anaesthesia. The following adipose depots were carefully dissected, blotted dry, and weighed on a precision balance:

scWAT; inguinal subcutaneous white adipose tissue (bilateral)

pgWAT: perigonadal white adipose tissue (epididymal in males; bilateral)

BAT: interscapular brown adipose tissue

Each depot mass was normalised to terminal body weight and expressed as a percentage (tissue mass / body weight × 100).

Stereotaxic Surgery
Recombinant Adeno-Associated Virus Microinjections

All stereotaxic procedures were conducted under isoflurane/O_2 anaesthesia. Following induction, the scalp was shaved and disinfected with 70% ethanol and povidone-iodine. A ~0.5 cm midline incision was made to expose the skull. Hydrogen peroxide (3% H_2O_2) was applied to facilitate exposure of the cranial surface, and a cranial window (≤1 mm diameter) was drilled at the injection site.

ARC was targeted using the following stereotaxic coordinates: anteroposterior (AP) −1.25 mm, mediolateral (ML)±0.30 mm, dorsoventral (DV) −6.00 mm (relative to bregma). Depending on experimental aims, Cre-dependent AAV-Syn-Flex-GCaMP6s vectors were used. For viral delivery, the injection needle was advanced to the target depth and left in place for 5 min prior to infusion. Each animal received 0.5 µL viral suspension delivered slowly over 10 min. To minimize backflow, the needle was kept in place for an additional 5 min before withdrawal. The incision was sutured and 0.25% bupivacaine was applied locally.

Postoperative analgesia was provided using meloxicam (2-4 mg/kg), and mice were observed for 5-10 min immediately after surgery. Postoperative recovery was monitored for 1 week, and no experimental procedures were initiated until wound healing was complete.

Fibre-Optic Ferrule Implantation

TH-Cre mice received a Cre-dependent GCaMP6s virus injection into the ARC (AAV.Syn.Flex. GCaMP6s.WPRE.SV40) using the coordinates described above (AP −1.25 mm, ML ±0.3 mm, DV −6.0 mm). Following viral delivery, fibre-optic implantation was performed during the same surgical session.

For Ca^{2+} recordings from ARC TH neurons, the fibre was positioned 150–200 µm above the injection site. A 200 µm core diameter, NA 0.37 multimode optical fibre (BFH37-200, Thorlabs) was used. After placement, alignment was optimized to achieve >80% light transmission. The ceramic ferrule was secured to the skull using C&B Metabond Quick Cement and dental acrylic[14]. Animals were then allowed to recover, and 2 weeks were allotted to permit viral expression before *in vivo* recordings.

In vivo Fibre Photometry

After postoperative recovery, Ca^{2+} signals were recorded from ARC TH neurons using a fibre photometry Mini Cube system equipped with two excitation ports and one emission port (Doric Lenses) (Fig.1a). The first excitation channel was 400–420 nm, the second excitation channel was 460–490 nm,

and the emission channel was 500–550 nm. Throughout all experiments, light power at the fibre tip was maintained at 10–15 µW. Non–Ca^{2+}-dependent components of the signal were filtered, and traces were processed as ΔF/F using the following formula (Cui et al., 2013): ΔF/F = (473 nm − 405 nm) / 405 nm.

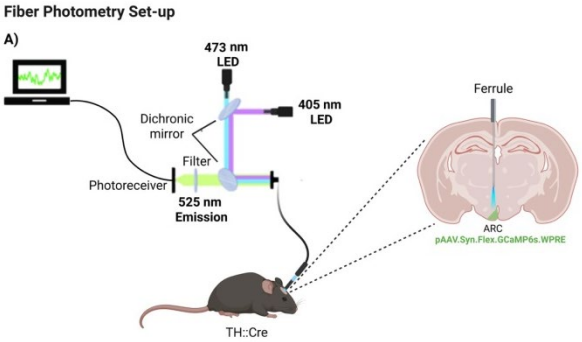

Fig. 1:
Methodological Framework for In Vivo ARC TH Activity Monitoring. (a) Schematic of Fiber Photometry Setup and Viral Strategy. Experimental design and in vivo fiber photometry protocols.

All *in vivo* recordings were analysed using Doric Studio software. Following recovery from stereotaxic surgery and viral expression, *in vivo* fibre photometry recordings were performed in TH-Cre mice expressing Cre-dependent GCaMP6s in ARC-TH neurons. Animals were studied under two diet conditions (control chow, SD vs high-fat diet, HFD) as indicated in the study groups (control diet: n = 5; HFD: n = 6). For all recording sessions, animals were connected to the patch cord and allowed to habituate briefly prior to acquisition. Photometry signals were acquired continuously across predefined time epochs (P1–P4) as outlined below (Fig. 2). The optical power at the fibre tip was kept constant (10–15 µW). Fluorescence signals were processed as ΔF/F using the isosbestic correction approach: ΔF/F = (473 nm − 405 nm) / 405 nm.

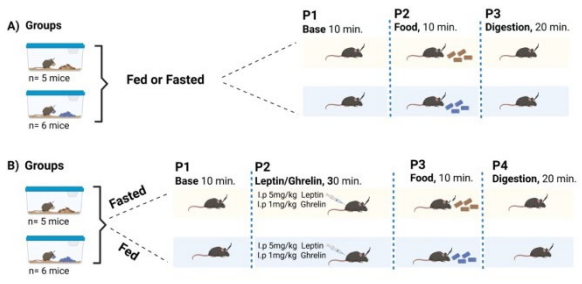

Fig 2:
Schematic representation of the study design. (a) Protocol 1 monitors fed and fasted mice across P1–P3 epochs (baseline, feeding, and digestion). (b) Protocol 2 evaluates leptin or ghrelin effects during P2 across P1–P4 epochs. All illustrations were created using BioRender.

Protocol 1: Feeding-state monitoring (Control protocol; Fig. 2a)

To characterize ARC TH neuronal activity across feeding states, mice were recorded under either fed (ad libitum access to food) or fasted (food removed for a defined fasting period) conditions. Each session consisted of three consecutive epochs:

P1 (Baseline; 10 min): animal in the recording arena without food exposure (baseline activity).

P2 (Food; 10 min): food was introduced and animals were allowed to eat freely (feeding epoch).

P3 (Digestion; 20 min): food was removed (or feeding was stopped) and animals were monitored during the postprandial/digestion period.

Activity measures (e.g., mean ΔF/F, peak responses, and/or AUC) were quantified within each epoch and compared between fed vs fasted states and between diet groups.

Protocol 2: Hormone challenge (Leptin/Ghrelin protocol; Fig. 2b)

To determine acute hormonal modulation of ARC TH activity, mice were studied in fed or fasted conditions using a four-epoch design with intraperitoneal (i.p.) hormone delivery during P2:

P1 (Baseline; 10 min): pre-injection baseline recording.

P2 (Hormone; 30 min): animals received an i.p. injection of either leptin (5 mg/kg)[15,16], or ghrelin (1 mg/kg)[17], doses widely used for acute hormonal challenge paradigms in mice, at the beginning of P2, followed by continuous recording for 30 min to capture the hormone-evoked response.

P3 (Food; 10 min): food was provided and animals were monitored during feeding.

P4 (Digestion; 20 min): animals were monitored post-feeding during digestion.

Leptin and ghrelin sessions were performed on separate days (with appropriate washout) and, where possible, the order of hormone administration was counterbalanced across animals. Hormone-evoked photometry responses were quantified by aligning ΔF/F traces to the injection time (t = 0) and extracting response metrics within predefined post-injection windows (e.g., peak ΔF/F and AUC during P2), followed by comparisons across diet (chow vs HFD) and metabolic state (fed vs fasted).

Statistical Analysis

All data are presented as mean ± SEM with individual data points shown, and error bars represent SEM. Repeated-measures fibre photometry data (AUC/min across epochs) and body weight were analysed using a linear mixed-effects model (LMM) with restricted maximum likelihood (REML) with Diet and Epoch as fixed effects, including their interaction, and matched within-subject measurements modelled as a repeated

factor, as implemented in GraphPad Prism v10.5.0 (GraphPad Software, San Diego, CA, USA). The Geisser–Greenhouse correction was applied to account for potential violations of sphericity. Post hoc pairwise comparisons were performed using Bonferroni's multiple comparisons test. Terminal adipose depot weights (scWAT, pgWAT, BAT), normalised to body weight and expressed as percentages, were compared between dietary groups using unpaired two-tailed Student's t-tests; Welch's correction was applied when variances differed significantly by F-test. Normality was confirmed using the Shapiro–Wilk test. Statistical significance was set at $p < 0.05$.

Results

Chronic High-Fat Diet Feeding Induced Obesity and Selective Expansion of Adipose Depots

To establish the diet-induced obesity model, TH-Cre mice were fed either standard chow or a high-fat diet for 12 weeks. HF-fed mice gained significantly more weight than chow controls from week 4 onwards ($p<0.001$ from week 4; Fig. 3a). Terminal analyses revealed marked increases in adipose depot mass normalised to body weight in HF compared with chow mice for scWAT (4.1-fold; $p=0.0008$), pgWAT (2.7-fold; $p<0.001$), and BAT (4.0-fold; $p=0.0053$) (Fig. 3b-d), confirming successful establishment of the diet-induced obesity phenotype prior to fibre photometry recordings.

Activity of Arcuate Nucleus Tyrosine Hydroxylase Neurons Across Feeding States and Dietary Conditions

To determine whether chronic HFD alters basal ARC TH neuronal calcium dynamics across behavioural epochs, we quantified fibre photometry signals as area under the curve per minute (AUC/min) during P1 (baseline), P2 (food/feeding epoch), and P3 (digestion/post-feeding) under fed and fasted conditions (Fig. 4).

Fed Condition: Chow versus High-Fat Diet

In fed chow animals (Fig. 4a), ARC TH activity showed a pattern in which P1 exhibited relatively higher AUC/min, followed by a lower mean AUC/min during P2, with P3 returning toward P1-like levels. Individual data points indicated inter-animal variability, but the overall trajectory suggested modest within-session modulation across epochs. In fed HFD animals (Fig. 4b), ARC TH activity appeared broadly comparable across P1–P3, with mean AUC/min values remaining within a similar range across the three epochs. As in the chow group, variability between animals was evident, but no clear shift toward either the feeding (P2) or digestion (P3) epoch dominated the group response.

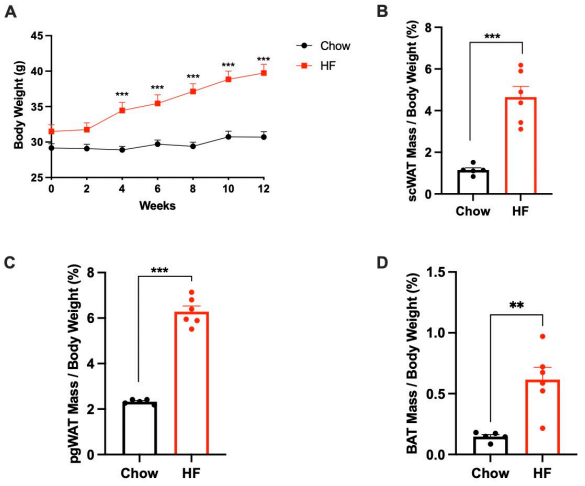

Fig 3:

*Chronic high-fat diet induces obesity and selective expansion of adipose depots in TH-Cre mice. Body weight (a) of mice maintained on standard chow (n = 5) or high-fat diet (n = 6) over 12 weeks of dietary intervention. Data were analysed using a linear mixed-effects model (LMM) with restricted maximum likelihood (REML) estimation, with Diet and Time as fixed effects (including their interaction) and matched within-subject measurements modelled as a repeated factor, followed by Bonferroni's multiple comparisons test. Terminal adipose depot mass (b-d) normalised to body weight (%) for (b) subcutaneous white adipose tissue (scWAT), (c) perigonadal white adipose tissue (pgWAT), and (d) interscapular brown adipose tissue (BAT). Data were compared between dietary groups using unpaired two-tailed Student's t-test. Data represent mean ± SEM with individual data points. **$p < 0.01$, ***$p < 0.001$.*

Direct diet comparisons under fed conditions (Fig. 4c) indicated that chow and HFD groups exhibited similar AUC/min levels at P1, P2, and P3, with overlapping dispersion of individual points and comparable mean ± SEM. Collectively, these data suggest that, in the fed state, chronic HFD exposure does not produce a large divergence in basal ARC TH activity across baseline–feeding–digestion epochs.

Fasted Condition: Chow versus High-Fat Diet

In fasted chow animals (Fig. 4d), ARC TH activity tended to show a relative increase during P2 compared with P1, followed by P3 values returning toward baseline. Notably, one or more animals showed higher AUC/min values during P2, contributing to an elevated mean and greater spread during this epoch. In fasted HFD animals (Fig. 4e), the group mean suggested a progressive increase from P1 to P3, with P3 showing the highest mean AUC/min. As in other groups, individual variability was present, including

higher AUC/min values in some animals during later epochs.

When chow and HFD were compared in the fasted condition (Fig. 4f), mean AUC/min values were similar and largely overlapping across P1–P3, though the pattern of within-group epoch modulation differed modestly (chow: more P2-weighted; HFD: more P3-weighted). Overall, these observations indicate that fasting does not reveal a strong diet-dependent separation in basal ARC TH activity magnitude across epochs, although it may subtly influence the temporal profile of activity during the session.

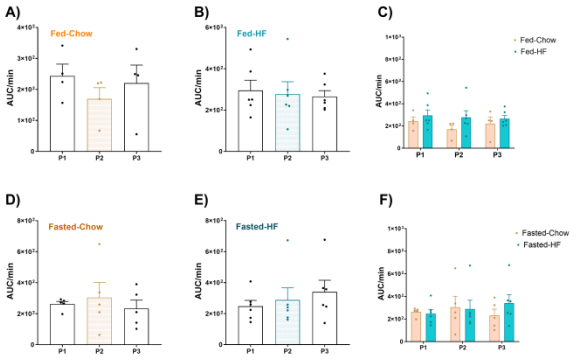

Fig 4:

ARC TH Neuron Activity (AUC/min) Across Feeding States Under Chow and HFD Conditions.

Fibre photometry recordings of ARC TH neuronal activity, quantified as area under the curve per minute (AUC/min) across three behavioural epochs: P1 (baseline, 10 min), P2 (food exposure, 10 min), and P3 (digestion, 20 min). (a-b) Intra-group epoch comparisons under the fed condition for (A) Fed-Chow (n = 5) and (B) Fed-HF (n = 6) mice. (c) Diet comparison (Chow vs. HF) across P1–P3 under the fed condition. (d-e) Intra-group epoch comparisons under the fasted condition for (d) Fasted-Chow (n = 5) and (e) Fasted-HF (n = 6) mice. (f) Diet comparison (Chow vs. HF) across P1–P3 under the fasted condition. All analyses were performed using a linear mixed-effects model (LMM) with restricted maximum likelihood (REML) estimation, with Diet and Epoch as fixed effects (including their interaction) and matched within-subject measurements modelled as a repeated factor, followed by Bonferroni's multiple comparisons test. Data represent mean ± SEM with individual data points; error bars represent SEM.

Hormonal Modulation of Arcuate Nucleus Tyrosine Hydroxylase Neuron Activity: Effects of Diet, Ghrelin and Leptin

To assess how peripheral metabolic hormones modulate ARC TH neuronal activity and whether these responses are altered by chronic HFD, we quantified fiber photometry activity as AUC/min across four recording epochs: P1 (baseline, 10 min), P2 (post-injection hormone window, 30 min), P3 (food/feeding, 10 min), and P4 (digestion, 20 min). Ghrelin responses were evaluated in the fed condition, while leptin responses were evaluated in the fasted condition (Fig. 5).

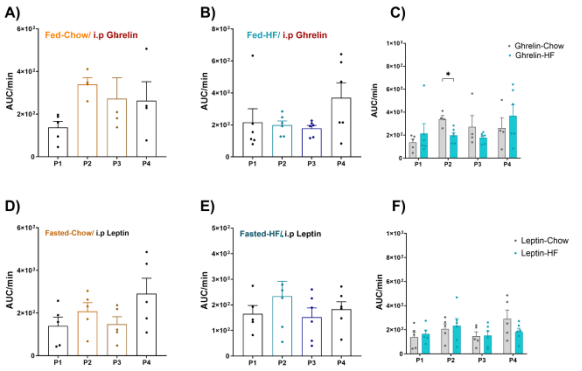

Fig 5:

Modulation of ARC TH Neuron Activity by Ghrelin and Leptin in Fed and Fasted Mice.

*Fiber photometry recordings of ARC TH neuronal activity (AUC/min) across four recording epochs (P1–P4). (a–b, d–e) Intra-group comparisons across P1–P4 for (a) Fed-Chow + Ghrelin, (b) Fed-HF + Ghrelin, (d) Fasted-Chow + Leptin, and (e) Fasted-HF + Leptin groups. (c, f) Diet comparisons (Chow vs. HF) for (c) Ghrelin and (f) Leptin effects. All analyses were performed using a linear mixed-effects model (LMM) with restricted maximum likelihood (REML) estimation, with Diet and Epoch as fixed effects (including their interaction) and matched within-subject measurements modelled as a repeated factor, followed by Bonferroni's multiple comparisons test. Data represent mean ± SEM with individual data points (*p < 0.05).*

Ghrelin in Fed Mice

In fed chow mice injected i.p. with ghrelin (Fig. 5a), ARC TH activity showed a robust increase during the hormone epoch (P2) relative to baseline (P1), indicating that ghrelin acutely enhances ARC TH calcium activity in the fed state. Following this peak, activity during P3 (food) and P4 (digestion) remained elevated compared with baseline in several animals, although the group mean suggested that the strongest effect was centred on P2. In contrast, in fed HFD mice (Fig. 5b), the ghrelin-associated increase during P2 was attenuated, with mean AUC/min values remaining closer to P1 and showing less pronounced elevation during the hormone epoch. Notably, some HFD animals displayed higher activity in P4, contributing to larger variability at later time points; however, the early ghrelin-locked increase (P2) was not as evident as in chow-fed mice. Direct diet comparisons for ghrelin (Fig. 5c) confirmed a significant difference between chow and HFD groups specifically during the

P2 hormone window, where ghrelin-evoked ARC TH activity was reduced in HFD relative to chow ($p = 0.0430$). In the remaining epochs (P1, P3, P4), AUC/min values showed overlap between diet groups, suggesting that the principal diet-sensitive component of the ghrelin response emerges during the immediate post-injection period.

Leptin in Fasted Mice

In fasted chow mice receiving i.p. leptin (Fig. 5d), ARC TH activity showed modest fluctuations across P1–P4, with no consistent suppression or enhancement tightly linked to the leptin window (P2) across animals. Variability increased in later epochs for some mice (notably P4), but the group mean did not indicate a strong leptin-locked shift. Similarly, in fasted HFD mice injected with leptin (Fig. 5e), AUC/min values exhibited inter-individual variability, with some mice showing higher signals during P2; however, at the group level, activity across epochs remained broadly comparable and did not present a clear, stereotyped leptin-evoked pattern. Consistent with these observations, diet comparisons for leptin (Fig. 5f) showed substantial overlap between chow and HFD groups across P1–P4, indicating no prominent diet-dependent divergence in leptin modulation of ARC TH activity under the fasted condition in this dataset.

Discussion and Conclusion

In the present study, we used Cre-dependent GCaMP6s fibre photometry in TH-Cre mice to track real-time Ca^{2+} dynamics of ARC TH neurons under chronic HFD (3 months) versus SD (chow diet), and across behavioural epochs (baseline-food-digestion) as well as following acute peripheral ghrelin or leptin injections (study design and photometry workflow as implemented in our protocol). The key finding is that HFD markedly attenuated the ghrelin-evoked increase in ARC TH neuron activity during the early post-injection period (P2), whereas baseline/epoch-related activity patterns and leptin-associated changes were comparatively modest and overlapped between diets.

A consistent finding in diet-induced obesity models is the development of central ghrelin resistance, where peripheral ghrelin becomes less effective at stimulating feeding and ARC activation. In seminal work, 12 weeks of HFD suppressed the ghrelin system and abolished ghrelin-induced feeding and ARC Fos responses in obese mice[11]. Subsequent mechanistic evidence indicates that diet-induced hyperleptinemia (rather than hypothalamic gliosis per se) can be a major driver of ghrelin resistance within ARC NPY/AgRP neurons[12]. In line with this literature, our data show that in fed mice, ghrelin robustly increased ARC TH neuron activity in chow, whereas the same ghrelin challenge elicited a substantially smaller (blunted) increase in HFD mice, with the clearest diet separation during the immediate post-injection epoch (P2). Importantly, this extends ghrelin resistance from a property of canonical orexigenic AgRP/NPY neurons to a non-canonical ARC subtype with distinct neurochemical identity and circuit connectivity, suggesting that diet-induced ghrelin resistance is a circuit-wide rather than cell-type-restricted phenomenon.

Several non-mutually-exclusive mechanisms may underlie blunted ghrelin signalling in ARC TH neurons after chronic HFD. First, GHSR-mediated signal transduction can be downregulated at the receptor or post-receptor level under chronic energy surplus, including reduced GHSR expression, impaired Gαq/IP3-dependent Ca^{2+} mobilisation, and altered AMPK activation[21]. Second, HFD induces a sustained pro-inflammatory state in the mediobasal hypothalamus, with microglial activation, cytokine release (IL-1β, TNF-α), and astrocytic gliosis that collectively disrupt synaptic transmission and reduce neuronal responsiveness to metabolic cues[6,7]. Third, diet-induced hyperleptinemia itself has been shown to drive ghrelin resistance in NPY/AgRP neurons by tonically engaging downstream signalling cascades that desensitise the ghrelin pathway[12], and the same circuit-level mechanism may extend to ARC TH neurons given their integration of multiple peptidergic inputs[10]. Finally, chronic HFD remodels excitatory/inhibitory synaptic balance in the ARC, including changes in presynaptic GABAergic inputs onto orexigenic populations, which could selectively dampen acute hormone-evoked recruitment without altering tonic population activity. The selective vulnerability of the P2 window in our data (without uniform shifts across P1–P4) is consistent with impaired acute transduction rather than a global change in baseline excitability.

Across baseline/feeding/digestion epochs (P1-P3), group means were broadly overlapping between diets in both fed and fasted conditions, suggesting that chronic HFD does not impose a large tonic change in ARC TH population Ca^{2+} activity under our recording conditions. Rather, diet-induced dysfunction emerged primarily when the system was acutely challenged with ghrelin, consistent with the view that rapid, state-dependent dynamics in ARC circuits can be masked when averaged across long epochs[5] and may require finer temporal or behavioural alignment (e.g., to feeding bouts or consumption microstructure) to be resolved.

The absence of a clear leptin-evoked Ca^{2+} response in either diet group, in contrast to the robust ghrelin response in chow controls, suggests that ARC TH neurons are not directly driven by acute peripheral

leptin but rather operate downstream of leptin-sensitive circuits. This functional asymmetry has important implications: diet-induced hyperleptinemia, a hallmark of obesity, has been mechanistically linked to ghrelin resistance in ARC orexigenic neurons via cell-autonomous signalling crosstalk[12,20]. Our finding that ARC TH neurons exhibit preserved (modest) leptin responsiveness but markedly attenuated ghrelin responsiveness under HFD is consistent with this framework; chronic leptin tone, rather than direct loss of ghrelin sensitivity, may be the proximate driver of the diet-dependent ghrelin response in this population. ARC TH neurons may thus serve as an integration node where chronic leptin elevation reconfigures acute hormonal responsiveness, providing a circuit-level substrate for the leptin–ghrelin imbalance that characterises diet-induced obesity.

Together, our findings position ARC TH neurons as a candidate node contributing to the broader phenomenon of impaired hormonal control of feeding circuits in diet-induced obesity. Diet-induced obesity in humans is associated with parallel hyperleptinemia, ghrelin dysregulation, and impaired hypothalamic responsiveness to peripheral satiety/hunger signals; a constellation that underlies the limited efficacy of monotarget pharmacotherapy and the propensity for weight regain after caloric restriction. By identifying ARC TH neurons as a previously unrecognised site of HFD-induced ghrelin resistance, our findings broaden the cellular substrate of obesity-associated hypothalamic dysfunction and suggest that therapeutic strategies targeting GHSR-related signalling, hypothalamic inflammation, or leptin–ghrelin crosstalk may need to consider non-canonical ARC populations beyond AgRP/NPY neurons. Combined modulation of multiple ARC subtypes, or upstream interventions that restore acute hormonal transduction across the population, may offer mechanistic advantages over single-cell-type approaches.

This study has several limitations that point to clear next steps. First, fibre photometry reports population-level Ca^{2+} dynamics and can't resolve whether HFD selectively shifts activity within heterogeneous ARC TH subpopulations[10]; combining photometry with cell-type intersectional strategies or single-cell approaches, together with finer temporal alignment to feeding bouts and injection-locked kinetics, would provide higher resolution. Second, the present work did not include direct molecular or hormonal readouts (circulating leptin/ghrelin, GHSR expression, markers of hypothalamic inflammation), which would strengthen mechanistic inference and enable direct comparison with established ghrelin-resistance frameworks[11,12]. Third, all recordings were performed exclusively in male mice, and extension to female cohorts will be essential to determine whether the diet-induced attenuation of ghrelin-evoked ARC TH activity generalises across sexes.

In summary, chronic HFD selectively attenuates ghrelin-evoked activation of ARC TH neurons while sparing baseline activity and leptin responsiveness, identifying this population as a novel, non-canonical contributor to diet-induced ghrelin resistance. These findings expand the cellular landscape of hypothalamic hormonal dysfunction in obesity and motivate cell-type-resolved therapeutic strategies targeting multiple ARC subtypes.

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References

1. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000;404(6778):661-671. doi:10.1038/35007534
2. Cowley MA, Smart JL, Rubinstein M, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*. 2001;411(6836):480-484. doi:10.1038/35078085
3. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. *Nature*. 2006;443(7109):289-295. doi:10.1038/nature05026
4. Chen Y, Lin YC, Kuo TW, Knight ZA. Sensory Detection of Food Rapidly Modulates Arcuate Feeding Circuits. *Cell*. 2015;160(5):829-841. doi:10.1016/j.cell.2015.01.033
5. Jais A, Brüning JC. Arcuate Nucleus-Dependent Regulation of Metabolism-Pathways to Obesity and Diabetes Mellitus. *Endocr Rev*. 2022;43(2):314-328. doi:10.1210/endrev/bnab025
6. De Souza CT, Araujo EP, Bordin S, et al. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology*. 2005;146(10):4192-4199. doi:10.1210/en.2004-1520
7. Thaler JP, Yi CX, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest*. 2012;122(1):153-162. doi:10.1172/JCI59660
8. Cone RD. Anatomy and regulation of the central melanocortin system. *Nat Neurosci*. 2005;8(5):571-578. doi:10.1038/nn1455
9. Betley JN, Xu S, Cao ZFH, et al. Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature*. 2015;521(7551):180-185. doi:10.1038/nature14416
10. Zhang X, Van den Pol AN. Dopamine/Tyrosine Hydroxylase Neurons of the Hypothalamic Arcuate Nucleus Release GABA, Communicate with Dopaminergic and Other Arcuate Neurons, and Respond to Dynorphin, Met-Enkephalin, and Oxytocin. *J Neurosci*. 2015;35(45):14966-14982. doi:10.1523/JNEUROSCI.0293-15.2015

11. Briggs DI, Enriori PJ, Lemus MB, Cowley MA, Andrews ZB. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. *Endocrinology*. 2010;151(10):4745-4755. doi:10.1210/en.2010-0556
12. Briggs DI, Lockie SH, Benzler J, et al. Evidence that diet-induced hyperleptinemia, but not hypothalamic gliosis, causes ghrelin resistance in NPY/AgRP neurons of male mice. *Endocrinology*. 2014;155(7):2411-2422. doi:10.1210/en.2013-1861
13. Aklan I, Sayar Atasoy N, Yavuz Y, et al. NTS Catecholamine Neurons Mediate Hypoglycemic Hunger via Medial Hypothalamic Feeding Pathways. *Cell Metab*. 2020;31(2):313-326.e5. doi:10.1016/j.cmet.2019.11.016
14. Davis GL, Minerva AR, Lario A, Simmler LD, Rodriguez CI, Gunaydin LA. Ketamine increases activity of a fronto-striatal projection that regulates compulsive behavior in SAPAP3 knockout mice. *Nat Commun*. 2021;12(1):6040. doi:10.1038/s41467-021-26247-2
15. Kabra DG, Pfuhlmann K, García-Cáceres C, et al. Hypothalamic leptin action is mediated by histone deacetylase 5. *Nature Communications 2016 7:1*. 2016;7(1):10782-. doi:10.1038/ncomms10782
16. Heinrich G, Russo L, Castaneda TR, et al. Leptin Resistance Contributes to Obesity in Mice with Null Mutation of Carcinoembryonic Antigen-related Cell Adhesion Molecule 1. *J Biol Chem*. 2016;291(21):11124. doi:10.1074/jbc.M116.716431
17. Reed F, Reichenbach A, Dempsey H, et al. Acute inhibition of hunger-sensing AgRP neurons promotes context-specific learning in mice. *Mol Metab*. 2023;77. doi:10.1016/j.molmet.2023.101803
18. Wang L, Saint-Pierre DH, Taché Y. Peripheral ghrelin selectively increases Fos expression in neuropeptide Y - synthesizing neurons in mouse hypothalamic arcuate nucleus. *Neurosci Lett*. 2002;325(1):47-51. doi:10.1016/S0304-3940(02)00241-0
19. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature*. 1998;395(6704):763-770. doi:10.1038/27376
20. Myers MG, Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: Distinguishing cause from effect. *Trends in Endocrinology and Metabolism*. 2010;21(11):643-651. doi:10.1016/j.tem.2010.08.002
21. Naznin F, Toshinai K, Waise TMZ, NamKoong C, Md Moin AS, Sakoda H, Nakazato M. Diet-induced obesity causes peripheral and central ghrelin resistance by promoting inflammation. J Endocrinol. 2015;226(1):81–92. doi: 10.1530/JOE-15-0139
22. Asarian L, Geary N. Sex differences in the physiology of eating. Am J Physiol Regul Integr Comp Physiol. 2013;305(11):R1215–R1267. doi:10.1152/ajpregu.00446.2012
23. Pettersson US, Waldén TB, Carlsson PO, Jansson L, Phillipson M. Female Mice are Protected against High-Fat Diet Induced Metabolic Syndrome and Increase the Regulatory T Cell Population in Adipose Tissue. PLoS ONE. 2012;7(9):e46057. doi:10.1371/journal.pone.0046057
24. Dorfman MD, Krull JE, Douglass JD, et al. Sex differences in microglial CX3CR1 signalling determine obesity susceptibility in mice. Nat Commun. 2017;8:14556. doi:10.1038/ncomms14556