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Neurometabolic Plasticity in Denervated Adipose Tissue: Remodeling of Lipogenic Carbonic Anhydrase Isoenzymes

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Abstract

Background

The sympathetic nervous system significantly influences the metabolic processes of white adipose tissue via norepinephrine signaling. Nevertheless, the adaptive mechanisms enabling adipose tissue to preserve metabolic stability following the loss of sympathetic innervation are not well understood.

Objective

The objective was to investigate alterations in CA isoenzyme expression within surgically isolated white adipose tissue subsequent to sympathetic denervation, given their crucial involvement in lipogenic pathways

Methods

Sixteen male Sprague Dawney rats were assigned to 1-month (Group 1) or 3-month (Group 2) groups (n = 8 each). Right retroperitoneal WAT was surgically denervated; the contralateral depot served as intra-animal control. Denervation was verified by tissue norepinephrine ELISA. Car2, Car5A, and Car5B mRNA expression was quantified by RT-qPCR using GAPDH as reference gene.

Results

Tissue norepinephrine was significantly reduced in denervated tissue in both groups ($p < 0.05$), confirming successful denervation. At 1 month, denervated adipose tissue was significantly heavier than controls ($p = 0.024$). Car2, Car5A, and Car5B mRNA were upregulated 2.09, 1.98, and 2.58 fold at 1 month, respectively. At 3 months, Car2 and Car5A returned to control levels, while Car5B remained modestly elevated (1.37-fold).

Conclusion

The presented data illustrate a specific temporal profile of carbonic anhydrase isoenzyme mRNA expression subsequent to sympathetic denervation. The profile demonstrates an initial, synchronized elevation of Car2, Car5A, and Car5B at the one-month interval, with Car2 and Car5A subsequently normalizing by three months, and Car5B continuing to exhibit a moderately enhanced expression. This dynamic gene expression profile indicates that denervated adipose tissue is not transcriptionally quiescent, but rather experiences a period of adaptation within its lipogenic gene network. We are tentatively designating this as neurometabolic plasticity, pending comprehensive mechanistic study. The functional metabolic adaptation stemming from this transcriptional plasticity necessitates validation via

protein-level assessment and direct quantification of lipogenic flux. Further investigations utilizing β-adrenergic agonist/antagonist interventions and metabolic disease models will be paramount in determining if CA isoenzyme remodeling is causally implicated in the metabolic sequelae of sympathetic denervation.

Introduction

White adipose tissue (WAT) is not simply a passive energy reservoir. Its metabolic activity is continuously shaped by neural signals, with the sympathetic nervous system (SNS) serving as a primary regulator. SNS efferent fibers innervate WAT depots and release norepinephrine (NE), which acts on beta-adrenergic receptors to promote lipolysis and suppress preadipocyte differentiation [1, 2]. In parallel, sensory afferent neurons convey information about depot lipid content back to the central nervous system, forming a bidirectional brain-adipose communication axis [3]. This neural dialogue allows the organism to dynamically adjust adipose tissue metabolism in response to changing energy demands. When this communication is disrupted adipose tissue does not simply shift to a new steady state (ref). Denervation consistently reduces lipolysis, increases adipose tissue mass and adipocyte size, and promotes adipocyte differentiation [1, 4, 5]. Yet these changes are not permanent: evidence suggests that over time, adipose tissue partially recovers metabolic homeostasis, likely through compensatory hormonal signaling that reaches the brain via the circulation even in the absence of intact neural pathways. This temporal dimension points to a form of metabolic plasticity at the tissue level, analogous in principle to the neuroplastic remodeling seen in denervated neural and muscular targets. The molecular basis of this plasticity, particularly at the level of intracellular lipogenic pathways, remains poorly understood.

Carbonic anhydrases (CAs; EC 4.2.1.1) are zinc-containing metalloenzymes that catalyze the reversible hydration of CO_2 to bicarbonate (HCO_3^-) [8]. In the context of lipogenesis, bicarbonate is not merely a by product — it is an obligate substrate. Cytosolic CA II supplies bicarbonate for acetyl-CoA carboxylase (ACC), the rate-limiting enzyme of fatty acid synthesis [6]. Mitochondrial CA VA and CA VB provide bicarbonate for pyruvate carboxylase, enabling mitochondrial acetyl-CoA export to the cytosol via the citrate shuttle [6, 7]. Through this mechanism, CA isoenzymes sit at a critical junction between cellular carbon metabolism and lipid biosynthesis.

Given that CA isoenzymes sit at a critical junction between neural metabolic regulation and lipid biosynthesis, we propose that this temporal metabolic adaptation may be underpinned by dynamic remodeling of lipogenic CA isoenzyme expression. We term this phenomenon “neurometabolic plasticity”: the capacity of adipose tissue to adaptively reprogram its metabolic machinery specifically its lipogenic enzymatic networks in response to altered sympathetic signaling. To test this phenomenon, we surgically denervated rat retroperitoneal WAT and examined whether Car2, Car5A, and Car5B expression patterns are dynamically altered during the early adaptive phase and potential recovery phase.

Materials and Methods

Animals and Experimental Design

The investigation was undertaken at the Surgical Application and Research Center of Karadeniz Technical University (KTU) Faculty of Medicine. All experimental procedures were reviewed and approved by the Local Ethics Committee for Animal Experiments of Karadeniz Technical University (protocol number 2018/41). All methods were performed in accordance with the relevant guidelines and regulations. Animals were housed and handled in the Surgical Application and Research Centre of the Karadeniz Technical University Faculty of Medicine, an institution licensed for the housing and use of laboratory animals. Sixteen male Sprague-Dawley rats, weighing 180–220 g and aged 4–6 weeks, were housed under controlled environmental conditions (22 ± 2°C, 12-hour light/dark cycle, with unrestricted access to standard chow and water). Animals were distributed into two groups randomly (n = 8 per group): The first group was sacrificed one month subsequent to denervation surgery, and the second group three months thereafter. In both groups, right retroperitoneal WAT was surgically denervated, while the left depot remained intact as an intra-animal control. Anaesthesia was induced by a single intraperitoneal injection of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg). Adequate depth of anaesthesia was confirmed before the first incision by the absence of the pedal withdrawal reflex. No postoperative analgesic was administered, in accordance with the protocol approved by the institutional ethics committee. At the end of the assigned survival period (1 or 3 months), animals were deeply anaesthetised using the same ketamine–xylazine regimen and euthanised by decapitation. At the time of sacrifice, Group 1 animals were aged 8–10 weeks and Group 2 animals were aged 17–19 weeks. Terminal body weights were not recorded in the study records and are therefore not reported; retroperitoneal adipose depot wet weights were recorded for every animal and are reported in the Results.

Surgical Denervation

Under general anesthesia, ketamine (80 mg/kg) and xylazine (10 mg/kg) were administered intraperitoneally, and a ventral midline incision was made to expose the right retroperitoneal adipose pad. Every observable nerve fiber that innervated the pad was identified and transected. The contralateral pad underwent surgical access; however, it was not subjected to manipulation. Upon sacrifice, both retroperitoneal adipose depots were surgically removed and their masses determined.

Tissue Norepinephrine Measurement

Tissue NE concentration was measured as a marker of denervation success. Homogenized tissue samples were assayed by ELISA (Rat NE ELISA kit, IBL, Fujioka, Japan; Lot no. END171). Results are expressed as ng/g wet tissue weight.

Total RNA Isolation

Total RNA was extracted from approximately 300 mg of adipose tissue using TRIpure Isolation Reagent (Roche, Basel, Switzerland) combined with GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA; catalog no. TN70), with on-column DNase I digestion (Qiagen, Hilden, Germany). RNA concentration and purity (A260/A280 ≈ 1.8) were determined with a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

cDNA Synthesis and RT-qPCR

cDNA was synthesized from 10 µL total RNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland; catalog no. 04896866001; 25°C/10 min, 55°C/30 min, 85°C/5 min). Concentrations were normalized to 100 ng/µL. RT-qPCR was performed on a LightCycler® 480 II (Roche, Basel, Switzerland) with SYBR Green I Master Mix (Roche). Primers were designed using UCSC Genome Browser, NCBI ORF Finder, and Primer3 software (Table 1). PCR protocol: 95°C/8 min pre-incubation; 55 cycles (95°C/10 s, 58°C/15 s, 72°C/20 s); melt curve analysis. Relative expression was calculated by the $2 - \Delta\Delta Ct$ method [9] with GAPDH as reference gene. Results are expressed as fold change relative to contralateral control tissue (set as 1.0). GAPDH was selected for consistency with the adipose denervation literature; reference gene stability at longer denervation intervals was not independently validated in this study [10].

Table 1
Primer sequences used for RT-qPCR

Gene	Direction	Sequence (5' → 3')
Car2	Forward	5'-CAGCCTCTGCTCATATGTTACG-3'
	Reverse	5'-TTTGTTCACGGTGTGCTCAG-3'
Car5A	Forward	5'-TAGTGTCTATGACCCGAAGCTG-3'
	Reverse	5'-AGAGCCCCATTCATCTGTTG-3'
Car5B	Forward	5'-GCAGTCACCCATCAACATCC-3'
	Reverse	5'-TAGTTATGTTCCAGGGGTCCTC-3'
GAPDH	Forward	5'-GCAAACAGAGTGAGCCCTTC-3'
	Reverse	5'-TGGCTGTTGAGGTGCTTG-3'

Statistical Analysis

Data are expressed as mean ± SD. Denervated vs. control tissue comparisons within each group were performed using paired t-tests or Wilcoxon signed-rank tests as appropriate. $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS.

Results

Verification of Denervation

Tissue NE was significantly lower in denervated vs. control adipose tissue in both groups (Group 1: 182.01 ± 3.55 vs. 195.74 ± 10.88 ng/g, p = 0.018; Group 2: 181.75 ± 1.81 vs. 203.20 ± 16.76 ng/g, p = 0.017; Fig. 1), confirming successful sympathetic denervation.

Adipose Tissue Weight

At 1 month, denervated tissue was significantly heavier than control (0.88 ± 0.32 g vs. 0.68 ± 0.27 g; p = 0.024; ~29% increase). At 3 months, the difference was no longer statistically significant (1.61 ± 0.36 g vs. 1.41 ± 0.43 g; p = 0.436; ~14% difference; Fig. 2).

Carbonic Anhydrase Gene Expression

At 1 month, *Car2* mRNA was 2.09-fold higher in denervated tissue relative to controls. At 3 months, no significant change was detected (fold change: 0.94). *Car5A* was 1.98-fold upregulated at 1 month; expression was comparable to controls at 3 months (fold change: 0.92). *Car5B* showed the largest upregulation at 1 month (2.58-fold) and remained modestly elevated at 3 months (1.37-fold). Results are summarized in Fig. 3.

Discussion

This study demonstrates that surgical sympathetic denervation of retroperitoneal white adipose tissue alters the expression of carbonic anhydrase isoenzymes involved in lipid synthesis. Beyond documenting coordinated changes in Car2, Car5A, and Car5B expression, our findings illuminate a broader principle: adipose tissue possesses a capacity to recalibrate its metabolic machinery in response to altered sympathetic input. We propose that this phenomenon—which we term neurometabolic plasticity—represents an adaptive rewiring of intracellular lipogenic networks, enabling metabolic homeostasis to persist even when neural control is compromised [1, 2].

Neurometabolic Plasticity as an Adaptive Response

The sympathetic nervous system exerts continuous metabolic governance over white adipose tissue through norepinephrine (NE)-mediated β-adrenergic signaling. This neural regulation is so fundamental to adipose physiology that disruption of the sympathetic-adipose dialogue fundamentally alters the tissue's metabolic identity [3, 4]. In the healthy state, NE suppresses adipogenesis, promotes lipolysis, and maintains the dynamic equilibrium between lipid storage and mobilization [5]. Oour findings support that when this neural input is severed, adipose tissue does not remain metabolically inert. Instead, a temporal pattern emerges: initial lipogenic upregulation (1 month) followed by partial metabolic normalization (3 months). We possibly interpret this temporal trajectory as the molecular signature of

neurometabolic plasticity—a form of metabolic remodeling that allows adipose tissue to adapt to the loss of sympathetic signaling through reconfiguration of its enzymatic infrastructure.

The coordinated upregulation of all three CA isoenzymes at 1 month, coinciding with maximal adipose tissue mass accumulation, points to a transcriptional program optimized for enhanced bicarbonate availability and lipogenic flux [6, 7]. At the biochemical level, this makes mechanistic sense: cytosolic CA II supplies bicarbonate for acetyl-CoA carboxylase, the rate-limiting step of fatty acid synthesis, while mitochondrial CA VA and CA VB support the citrate-pyruvate shuttle, enabling cytosolic acetyl-CoA regeneration [7, 8]. The sustained elevation of tissue mass at 3 months, despite normalization of Car2 and Car5A expression, suggests that the tissue has achieved a new metabolic equilibrium—one that may be supported by post-transcriptional mechanisms, altered protein stability, or compensatory hormonal signaling [9, 10].

The Puzzle of Catecholamine Responsiveness for Obesity: Plasticity or Resistance?

A significant, unresolved issue has arisen from our temporal information. Does reduced catecholamine responsiveness result from neurometabolic plasticity, or does it come before and cause it? At both time points, tissue NE levels were substantially reduced in denervated depots, validating effective sympathetic denervation and the cessation of neural catecholamine supply [11]. However, the tissue that had lost its nerve supply did not settle into a predetermined metabolic condition. Rather, it underwent significant changes in how its genes were expressed. This observation presents a conceptual difficulty: if sympathetic denervation decreases NE in tissues, one might expect that adaptive responses would be triggered by compensatory endocrine signals (adipokines, insulin, glucocorticoids) traveling to the brain through the bloodstream [12]. Yet, the fact that CA is upregulated early, at one month, and before the brain has completely readjusted systemic metabolism, indicates that the denervated adipocyte might have its own internal mechanisms for detecting changes in sympathetic activity. Recent evidence hints at a broader phenomenon termed catecholamine resistance in metabolic tissues: the loss of sensitivity to catecholamine signaling despite sustained or elevated circulating NE levels [13, 14]. We propose that both phenomena may represent manifestations of neurometabolic plasticity: in the first case, the tissue adapts to excess catecholamine by downregulating receptors or downstream signaling; in our case, the tissue adapts to catecholamine absence by reprogram intracellular lipogenic networks. Future studies employing selective β-adrenergic agonists or antagonists in denervated tissue could directly test whether CA upregulation is a primary adaptive response or a secondary consequence of altered catecholamine signaling capacity. In the intact organism, the sympathetic nervous system provides rapid, reversible control over lipolysis in response to energy demands, thermoregulatory challenges, and stress [5, 15]. This neural control preserves metabolic flexibility: the tissue can shift between lipid storage and mobilization on a timescale of minutes to hours. Denervation removes this flexibility, trapping the tissue in a constitutively lipogenic state, at least in the early phase [1, 4]. The observation that adipose tissue, when denervated, demonstrates sustained growth in cell size and mass, elevated adipogenesis, and diminished lipolysis highlights the critical nature of this metabolic imbalance [16]. From a somatic perspective, one might wonder. What are the physiological consequences of losing this

neural control? Our model of neurometabolic plasticity offers a partial solution. Although the denervated tissue can retain a certain level of metabolic stability due to CA upregulation and compensatory endocrine signaling, this stability comes at the expense of metabolic autonomy. The tissue's reliance on hormonal control, especially from insulin and adipokines, grows, diminishing its dynamic reaction to sympathetic signals [3, 12]. During metabolic stress, such as high-fat diets, insulin resistance, and obesity, this reduction in neural adaptability can result in considerable physiological consequences.

The dissociation between Car5B and the other isoenzymes at 3 months is particularly instructive. While Car2 and Car5A normalized, Car5B remained modestly elevated (1.37-fold). This suggests differentiated regulation of cytosolic versus mitochondrial lipogenic pathways, with CA VB potentially sustaining enhanced bicarbonate supply for pyruvate carboxylase-mediated acetyl-CoA export [8, 17]. The mechanistic basis for this isoform-specific trajectory remains unclear and warrants investigation with compartment-resolved protein and activity assays. Critically, we emphasize that mRNA abundance does not establish functional significance. Confirmation through Western blot, immunohistochemistry, and direct enzyme activity measurement will be necessary to link CA upregulation to enhanced lipogenic capacity. Initial findings from a related study within our team suggest that total CA enzymatic activity is, in fact, higher in denervated tissue, indirectly corroborating the suggested mechanism [18].

Several limitations warrant acknowledgment. This study was conducted in healthy, chow-fed rats; metabolic disease states (high-fat diet, insulin resistance, diabetes) may profoundly alter CA expression responses to denervation [19, 20]. The surgical approach denervated both efferent sympathetic and afferent sensory fibers, preventing attribution of effects to either component independently. As emphasized, protein-level validation and functional activity assays are essential before drawing mechanistic conclusions.

Future investigations should: (1) employ selective β-adrenergic agonists in denervated tissue to test whether CA expression responds to restored catecholamine signaling; (2) investigate whether catecholamine resistance and CA remodeling co-occur in obesity and metabolic disease; (3) employ compartment-specific genetic or pharmacological CA inhibition to establish causal relationships between CA-mediated bicarbonate supply and denervation-induced lipid accumulation; and (4) examine whether the temporal pattern of CA normalization reflects recovery of sensory feedback from adipose to brain, or rather systemic metabolic adaptation.

Conclusion

By proposing neurometabolic plasticity as a unifying framework, this study reframes denervation-induced metabolic changes not as pathological malfunction, but as an adaptive reconfiguration of intracellular lipogenic enzyme networks in response to loss of sympathetic control. The temporal dynamics of CA isoenzyme expression provide a molecular window into how adipose tissue recalibrates its metabolic identity when sympathetic neural governance is withdrawn. Yet this study also reveals a fundamental tension: the very plasticity that allows denervated adipose tissue to maintain metabolic

stability paradoxically costs the tissue its metabolic autonomy. Shifted from dynamic sympathetic regulation to dependence on hormonal cues, the tissue becomes metabolically inflexible—a condition that may exact significant physiological penalties in the context of metabolic stress or disease. This observation underscores a critical principle: sympathetic innervation of adipose tissue is not metabolic convenience, but rather fundamental to healthy tissue function and metabolic resilience. Future investigations employing selective β-adrenergic agonists, compartment-specific CA inhibition, and studies of catecholamine resistance in metabolic disease will be necessary to determine whether altered catecholamine sensitivity drives adaptive plasticity, or whether plasticity secondarily erodes catecholamine signaling capacity. Understanding these dynamics in healthy tissue may illuminate the dysregulation of sympathetic-metabolic crosstalk in obesity and metabolic disease, and could identify new therapeutic targets for restoring healthy adipose function and metabolic flexibility.

Declarations

Competing interests:

No, I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Ethics approval:

The KTU Local Ethics Committee for Animal Experiments provided approval for all experimental procedures undertaken, under protocol number 2018/41.

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Author Contribution

H.K., M.A., and A.A. conceived the study design. H.K. performed the surgical procedures and tissue collection. H.K. and M.A. conducted the molecular analyses. A.A. performed statistical analysis. All authors contributed to manuscript preparation and review.

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Data Availability

The datasets generated and/or analysed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.22134778

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Figures

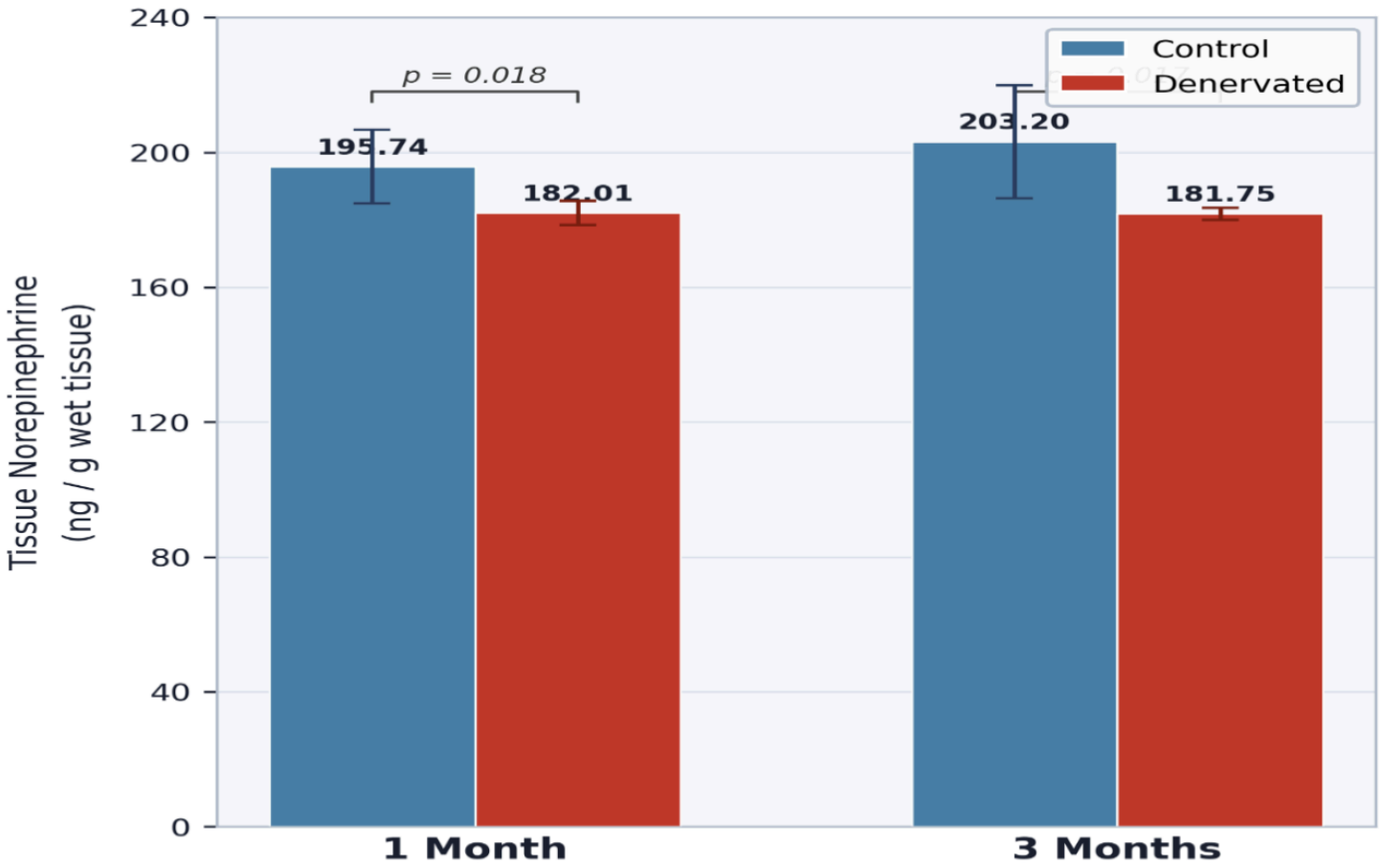


Figure 1

Tissue norepinephrine levels in control and denervated retroperitoneal adipose tissue at 1 and 3 months post-surgery. Data are mean ± SD (n = 8 per group).

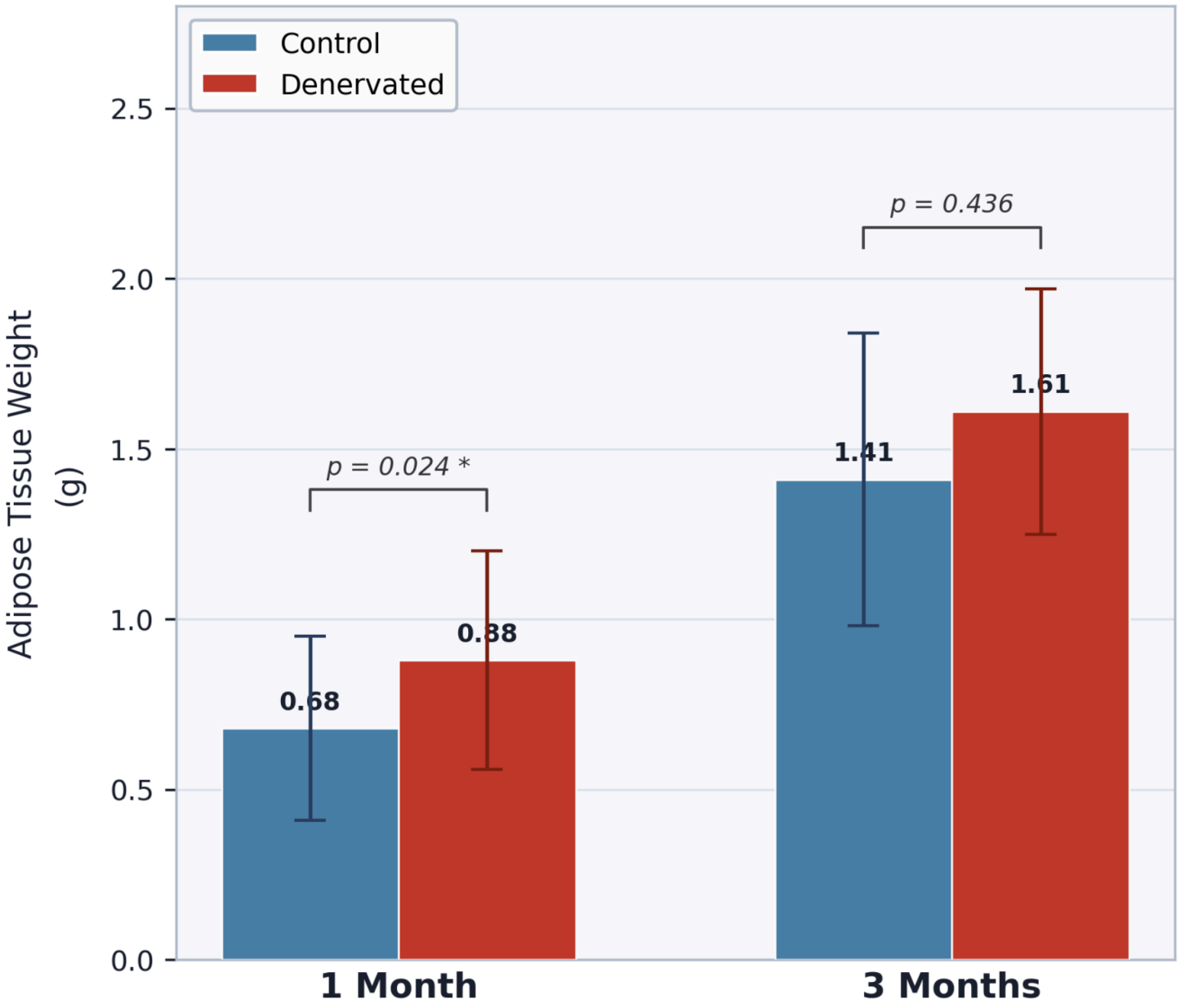


Figure 2

Retroperitoneal adipose tissue weight in control and denervated groups at 1 and 3 months post-surgery. Data are mean ± SD (n = 8 per group). * p < 0.05.

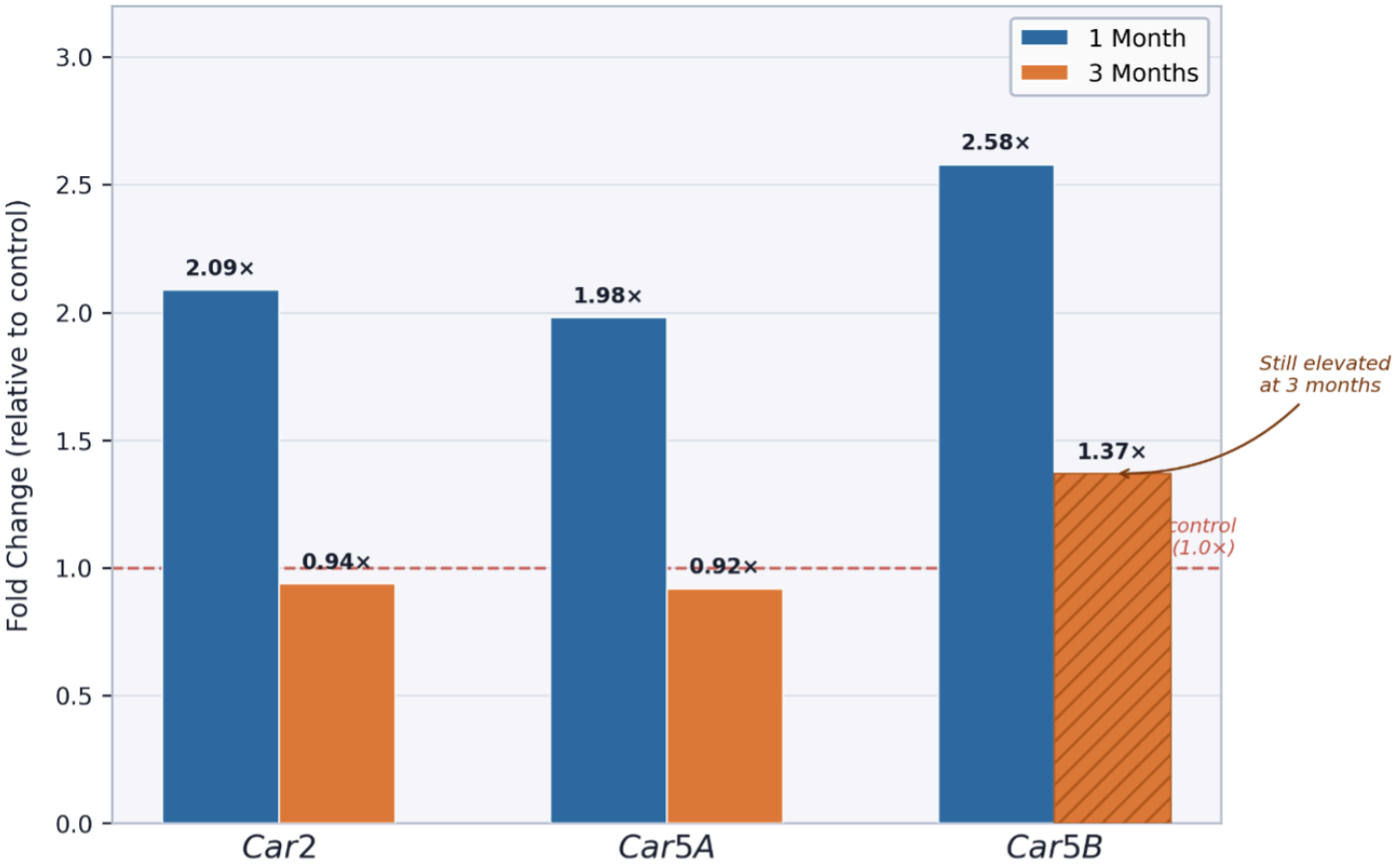


Figure 3

Fold change in Car2, Car5A, and Car5B mRNA expression in denervated vs. control retroperitoneal adipose tissue at 1 and 3 months post-sympathetic denervation ($2^{-\Delta\Delta}$Ct method; GAPDH reference gene). Dashed red line indicates control level (1.0×). Hatching on Car5B (3 months) denotes persistent modest elevation. Fold change expressed relative to contralateral control (set as 1.0). GAPDH used as reference gene.