THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF KINESIOLOGY

THE EFFECT OF STRAIN AND EXERCISE ON HYPOTHALAMIC GENE EXPRESSION IN C57BL/6J AND DBA/2J MICE

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ABSTRACT

Osteoporosis is a degenerative bone disease of major concern in the aging population of the United States. Within the past 10 years, researchers have begun to consider the neurological influences that may contribute to osteoporosis, and the important hormonal patterns that influence prevalence of osteoporosis. Exercise has been shown to have a positive impact on factors indicative of improved bone quality in studies that have examined gene expression in bone tissue, however little work has specifically addressed altered gene expression in brain tissue as a result of exercise.

The following study utilized 180 day old C57BL/6 (B6) and DBA/2J (D2) mice under two exercise treatment conditions: treadmill running and tower climbing and a sedentary non-exercised control. Ninety female mice were randomized into groups, with 15 mice from each strain in each exercise treatment and control group. RNA was extracted from the hypothalamus of each mouse and, after quality determination, from the six best samples, two samples were pooled onto three gene chips for each strain by treatment group three samples were pooled on to a gene chip from each treatment group in order to measure gene expression. Gene expression was compared across groups to determine the effects of strain and exercise. Although exercise treatment was not found to cause significant differences in gene expression, important suggestive differences included expression of the leptin receptor gene (Lepr) in B6 tower climbing and non-exercising controls. Approximately 450 genes were differentially expressed as a result of strain. The identification of genes that are differentially expressed in the hypothalamus of B6 and D2 mice may provide insight into central nervous system control of known differences in activity level and skeletal quality and lead to new pathways for research in the prevention and treatment of osteoporosis.

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Chapter 1

Introduction

Osteoporosis is a common disease of aging, especially in the United States. It is the most common degenerative bone disease and the leading cause of bone fractures [1, 2]. Approximately 10 million individuals older than 50 have osteoporosis while and additional 34 million have osteopenia, or low bone mass. Among Medicare enrollees above age 65, fractures are the most common musculoskeletal condition requiring hospitalization. According to the U.S. Surgeon General, it is expected that by 2020, one in two individuals older than 50 will be at risk of osteoporosis of the hip, with osteoporosis at any site in the skeleton being even more common [2]. As the U.S. population ages, bone health is likely to decrease.

Relatively recently, additional investigation has been conducted on the neuroskeletal basis of osteoporosis in order to elucidate the genes and pathways that control the biological and biological bases of bone health. The hypothalamus has been long-appreciated for its role in homeostasis, and in maintaining the skeleton, but recent research has focused on the gene and protein expression in differing environments. Although it is understood that osteoporosis is caused by a failure of bone homeostasis, not all factors that regulate bone quality have been described [1]. Currently intervention at the time of menopause has been suggested as a good preventative measure for osteoporosis. Understanding the specific neural pathways that regulate bone homeostasis and quality can yield information to support successful development of preventative measures and effective individualized treatment options for osteoporosis patients.

1.1 Study aims

The two-part hypothesis for this study describes the neuroendocrine pathways between brain and bone tissue that may increase bone quality:

(1) Within the hypothalamus, pathways involved in ostoeclast formation and activity will be down-regulated in exercise groups and the genes regulating these pathways will be differentially expressed in control and exercise animals regardless of strain.

(2) Within the hypothalamus, pathways involved in osteoblast differentiation will be up-regulated in the exercised groups, and the genes regulating these pathways will be differentially expressed in control and exercise animals regardless of strain.

The specific aim of this study is to determine changes in patterns of gene expression within hypothalamic tissue as a function of exercise and genetic strain, assuming that animals of the same strain are genetically identical.

1.2 Study limitations

(1) Some animals were more willing to participate in the exercise intervention program (particularly the voluntary tower climbing) than others, which would result in slight differences in the amount of physical activity experienced by each animal. Treadmill runners that experience electric shock as a result of reluctance to run may have experienced more stress than other treadmill runners.

(2) Although feed intake was measured, it was observed that some mice were shredding the pellets rather than ingesting them.

(3) Using only 3 biological replicates may have compromised the ability of the microarray to detect small changes in gene expression.

Chapter 2

Literature Review

2.1 Hippocampal Changes with Exercise

Although the specific biologic importance of changes in protein expression as a result of exercise is not completely understood, there are many proteins that increase expression in the hippocampus with exercise. Significantly ($p<0.05$) expressed proteins include heterogenous nuclear ribonucleoproteins K and H2, heat shock cognate 71 kDa and 60kDa and 75kDa heat shock proteins [3].

2.1.a. Exercise-induced neurogenesis

Brain-derived neurotrophic factor (BDNF) has been known to promote neurogenesis in many parts of the brain, including the hippocampus in mice [4]. Chronic moderate exercise has been shown to increase BDNF as well as tyrosine kinase (TrkB) in exercising B6 mice [5]. Exercise-induced increases in circulating insulin-like growth factor (IGF) -1 levels have been shown to stimulate neuronal expression of BDNF in many areas of the brain [6]. It has been suggested that exercise brings the glucocorticoid and mineralcorticoid receptor (MR) signaling into an optimal concentration, which creates conditions favorable to differentiation of neuronal lineages or new neuron survival [5]. Specifically, exercise has been shown to decrease levels of hippocampal MR [7].

2.2 Hypothalamic-Pituitary-Adrenal Axis

Some cases of osteoporosis have been directly attributed to corticosteroid pharmaceuticals. It has been suggested that exercise is critical to lowering plasma corticosterone levels, as in Figure **2-1**, modified from Droste et al. 2003. Although it has been observed that exercise may cause a transient increase in serum corticosterone levels, overall corticosterone levels are lower in exercise cohorts [5].

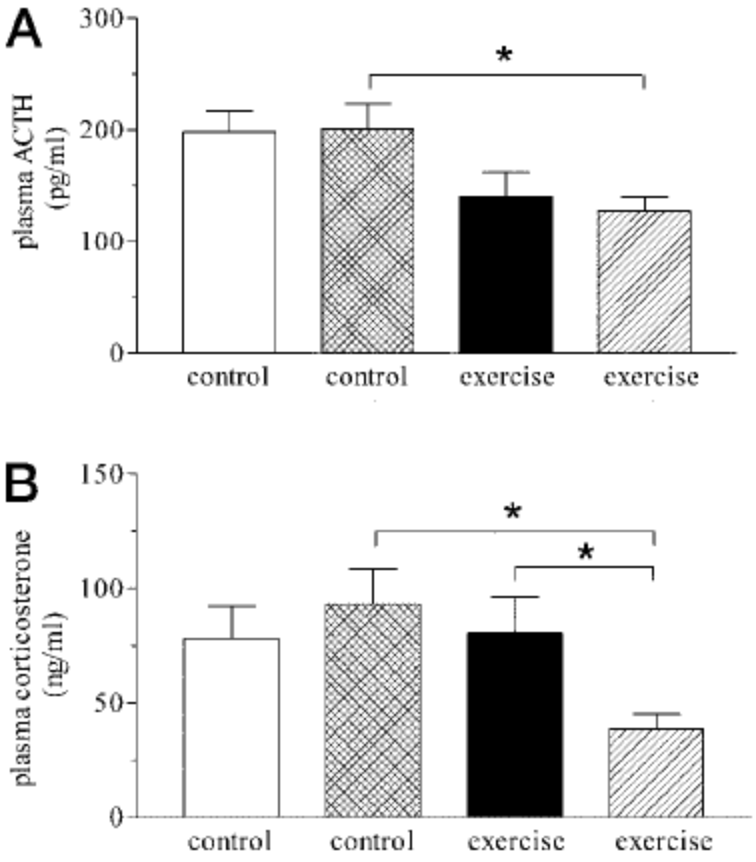

Figure **2-1:** The effect of exercise on plasma ACTH and corticosterone. (A) represents ACTH; (B) represents corticosterone. * indicates data expressed as mean ±SEM.

Exercise has been shown to significantly decrease corticotropin-releasing factor (CRF) in the paraventricular nucleus of the hypothalamus (PVN). Although levels of CRF are lower in

exercising mice, those mice are still capable of producing normal ACTH levels in response to potentially threatening situations (stressors) [7].

2.3 The Hypothalamic-Pituitary-Gonadal Axis

The current definition of the Hypothalamic-Pituitary-Gonadal (HPG) axis indicates that the hypothalamus produces Gonadotropin-releasing hormone (GnRH) which then acts on the anterior pituitary (AP) to stimulate the production of gonadotropins Follicle-stimulating hormone (FSH) and Leuteinizing hormone (LH). These gonadotropins in turn stimulate ovarian secretion of estrogens which inhibit bone resorption [8]. Figure **2-3** below explores a mechanism suggested by Sun et al. in which FSH stimulates bone resorption and leads to post-menopausal (hypogonadal) osteoporosis [9].

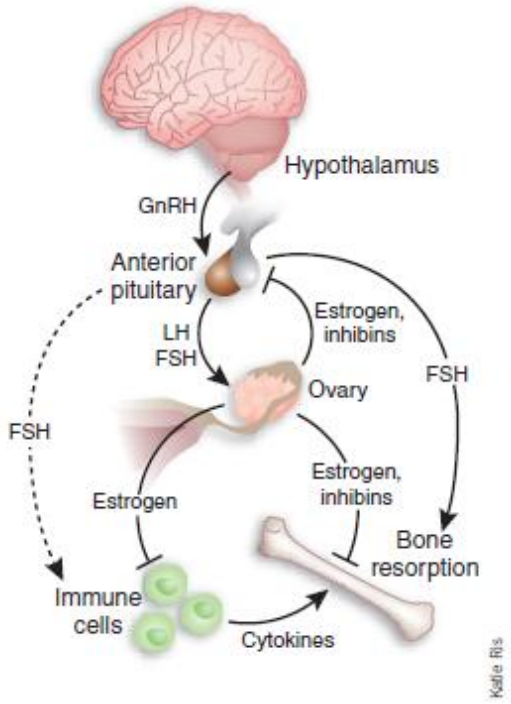

Figure **2-2**: A revised look at the HPG Axis [8]

Although men and women can suffer from osteoporosis, the disease is more common in women. Approximately 35 percent of post-menopausal women have osteoporosis of the hip, spine or distal forearm [2].

2.3.a. Amenorrhea

Amenorrhea is defined as the absence of menstrual periods. Amenorrhea is common in female athletes and the root of the observed reproductive abnormalities that result from hypothalamic dysfunction. Hypergonadotropic amenorrhea may occur in women of reproductive age, and is otherwise known as premature ovarian failure. Amenorrheic women of reproductive age have lower bone density than eumenorrheic women matched for age [Devleta 2004].

Elevated levels of FSH have been observed in hypergonadotropic women with lower bone density [Devleta 2004]. Similar to normal menopause, high levels of circulating FSH were caused by a loss of FSH receptor signaling; however it has been suggested that there is a direct effect of FSH on bone metabolism [Delveta 2004].

2.3.b. Galanin-like peptide

In male rats, galanin-like peptide (GALP) has been shown to increase the secretion of LH in male rats, specifically by stimulating the release of LH releasing hormone in the median preoptic area (MPA) of the hypothalamus [10].

2.4 Leptin mediates bone through receptor cascades

Leptin is a 16kDa cytokine-like hormone that is primarily produced by white adipose cells. Studies have indicated that leptin inhibits osteoblastic bone formation. Neither the *Leptin* gene nor the *ObRb* (*Lepr*) receptor gene have been detected in bone, so it has been determined that the expression of *Lepr* is highly specific to the hypothalamus [11]. Ob/ob and db/db mice are zygotes that are heterozygous leptin deficient and leptin receptor deficient mice respectively.

Although ob/ob and db/db mice show hypercortisolism and hypogonadism, they both display a similar high bone mass phenotype. Leptin is the only gene known to affect the aforementioned conditions, and increased serum leptin levels have been shown to decrease bone mass [11][12].

Leptin's action on the neuroendocrine activity has been shown to be mediated by the sympathetic nervous system (SNS), cocaine- and amphetamine- regulated transcript (CART), neuromedin U (NMU) and neuropeptide Y (NPY). Figure **2-3**, modified from Driessler and Baldock, 2010 shows a model of leptin signaling through the hypothalamus as mediated by the SNS and the leptin/serotonin cascade. The ventromedial hypothalamus (VMH) directs bone formation, while the neurons of the arcuate nucleus (ARC) control energy use and activity of adipose tissue. The mechanistic action of leptin on the ObRb receptors of the VMH and ARC nuclei has not been completely elucidated [Driessler 2010].

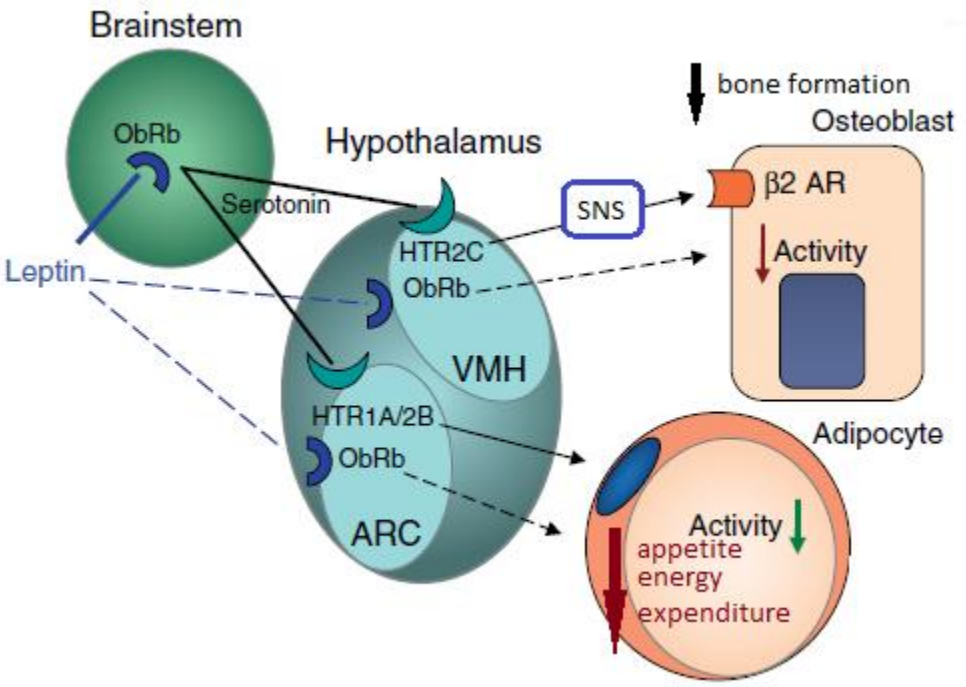

Figure **2-3:** Model of leptin signaling in bone and fat formation

2.4.a. Sympathetic Tone

Dopamine β-hydroxylase (DBH) is an enzyme that is required for the production of epinephrine and norepinephrine, important ligands for communication in the SNS. Mice deficient in DBH show a high bone mass phenotype; even on intracerebroventicular (ICV) infusion (central treatment) of leptin, DBH-deficient mice still maintained high bone mass. In order to maintain signaling in the SNS, mice are also required to have functioning β_2 – adrenergic receptors [13].

2.4.b. CART

It has been shown that the expression of CART in ARC neurons is dependent on circulating leptin levels or leptin signaling [14]. In contrast to the SNS signaling action of leptin, the CART action inhibits osteoclast differentiation, specifically through a decrease in the osteoblast-derived differentiation factor RANK-L [15].

2.4.c. Neuromedin U

It has been suggested that NMU does not alter bone resorption, but instead affects osteoblast proliferation. Deficiencies in NMU result in a high bone mass phenotype [16]. Like experiments for the effect of leptin on sympathetic tone, it was shown that ICV infusion of NMU resulted in decreases in bone mass in ob/ob mice [11][16]. Leptin ICV infusion into $NMU^{-/-}$ mice showed an increase in both bone volume and osteoblast number, suggesting that NMU acts downstream of leptin [16].

2.4.d. Neuropeptide Y

Although NPY and leptin appear to antagonize each other in the control of body weight, they do not appear to be antagonists in the regulation of bone formation [11]. It was reasoned that if NPY participated in leptin-dependent control of bone formation, a high bone mass phenotype would be observed upon ICV infusion of NPY in wild type mice. These mice however showed

bone loss [11]. It has additionally been shown that osteoblastic function is regulated by a NPY Y2 receptor circuit, even in the presence of normal levels of leptin [17].

2.5 Strain Differences between B6 and D2 Mice

Peak bone density is considered an important predictor of osteoporotic fractures. Human studies have shown that as much as 70% of bone mineral density is heritable [18]. Inbred mice can be used to study genetic effects because an inbred mouse strain provides multiple nearly-identical genetic replicates.

The mouse strains that were chosen for this study, C57BL/6J (B6) and DBA/2J (D2), are known to have different skeletal strength morphologies. Beamer et al. observed that B6 mice had significantly longer femurs than D2 mice, however D2 mice had more dense cortices and a higher bone mineral content than did B6 mice [19]. Voluntary exercise (duration, average speed and average running distance) has been shown to be significantly greater in B6 mice compared to that of D2 mice [20]][21]. In "home cage" activity and anxiety tests, D2 mice consistently displayed low activity, while B6 mice displayed intermediate, and in some cases high, activity [22].

A comparison study between hind limb muscle weights of B6 and D2 mice revealed that B6 mice had muscle weights 11-34% greater than D2 mice [25].

It is important to note that exercise and exercise performance is complex and expression of a certain exercise trait requires interaction between cardiac, lung and muscular function. Less-quantifiable variables, such as motivation and desire, can also affect exercise performance. It may be important to note that nociception studies suggest that B6 mice are distinctly more sensitive to pain than 10 other common strains used in laboratory experiments, while D2 mice are the most representative strain for evaluating pain sensitivity [23].

Gene expression in many individual organs is known to differ across different mouse strains. After exercise D2 mice have been shown to express more atrial natriuretic factor (ANF)

and β-myosin heavy chain (MHC) mRNA in cardiac tissue than 6 other inbred strains, including B6 [21]. Samples of liver mRNA and protein from B6 and D2 mice on high-fat/cholesterol diets has shown that distribution of cholesterol may vary in different mouse strains [24].

Brain characteristics also differ across mouse strains. B6 mice are considered seizure resistant while D2 mice are considered susceptible. Astrocytes have come under more scrutiny in recent years for their involvement inepilepsy. Midbrain astrocytes from D2 mice have reduced capacity for potassium and glutamate buffering, because of defects in the Kir channel, which is mediated by potassium inwardly-rectifying channel, subfamily J, member 10 (kcnj10) [26].

When considering the hypothalamus as a center for metabolic regulation differences in meal-eating behavior have been observed across mouse strains. D2 mice have been shown to have more motivation to sustain higher daily food intake and meal size than B6 mice [27].

Chapter 3

Materials and Methods

The following section was slightly modified for brain tissue from the Materials and Methods section of Chapter 4 in the Ph. D. dissertation by Holly M. Preston [34].

3.1 Animals and Experimental Design

Ninety adult (180 day old) female mice equally divided between C57BL/6 (B6) and DBA/2J (D2) inbred mouse strains were exposed to treadmill running, tower climbing or served as non-exercised controls (15 in each group) (Figure 1). The five week exercise intervention was conducted with five cohorts staggered over a span of nine months with 3 mice from each strain by treatment group in each cohort.

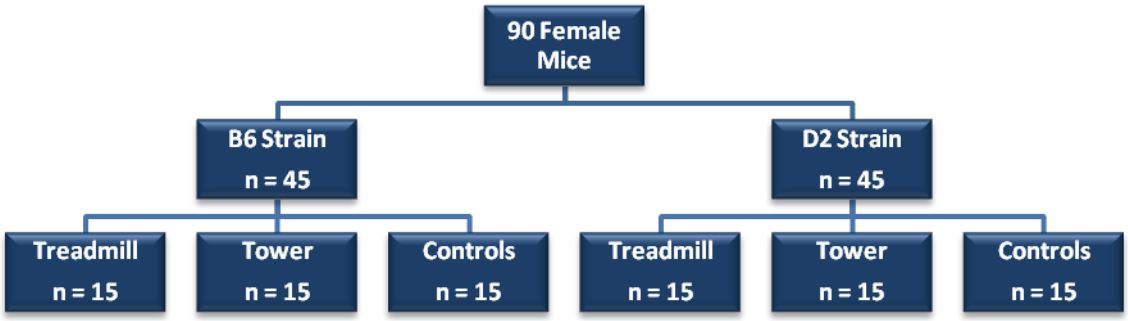

Figure **3-1**: Schematic of experimental design.

Mice in the treadmill group were run on a rodent treadmill 5 days per week for 5 weeks. The speed, incline and duration were gradually increased until a target speed of 15 m/min at a 25 degree incline for 30 minutes was attained. The mice were run for one week at the maximum incline, speed and duration (Table 1). See Appendix A for

instructions to treadmill operators on conditions for refusal to exercise. The tower climbers were housed in a standard mouse cage attached to a 120 cm tall mesh wire tower with a diameter of 17 cm, with water bottles placed at the top of the tower (See Appendix B). Tower climbers remained in the towers 24 hours per day 7 days/week for a 5 week period. To train the mice to climb during the first week, the water bottles were put at the bottom of the tower and gradually raised to the top. The mice then climbed to the top of the towers to drink during the remaining weeks of the intervention.

Table **3-2**: Protocol of settings used during treadmill running intervention

Week	Day	Incline (degrees)	Speed (m/min)	Duration (min)
1	1	5	10	10
1	2	5	10	20
1	3	5	10	20
1	4	5	10	30
1	5	10	10	20
2	8	10	10	30
2	9	10	12	30
2	10	15	12	20
2	11	15	12	30
2	12	15	13	30
3	15	20	13	20
3	16	20	13	30
3	17	20	14	30
3	18	25	14	20
3	19	25	14	30
4	22	25	14	30
4	23	25	15	30
4	24	25	15	30
4	25	25	15	30
4	26	25	15	30
5	29	25	15	30
5	30	25	15	30
5	31	25	15	30
5	32	25	15	30
5	33	25	15	30

All of the mice were housed in individual cages in the same room with monitored temperature and humidity, and with food and water available ad libitum. The lights were set on a reverse 12 hour light/dark cycle and treadmill mice were exercised during the dark cycle when mice are known to be more active [28]. All procedures complied with and were approved by the Pennsylvania State University Institutional Care and Use Committee (IACUC#: 22463).

3.2 Tissue Harvesting

Approximately two hours after the last exercise exposure, either after treadmill running or removal from the cages attached to towers, mice were euthanized by cervical dislocation. After euthanasia the head was separated from the body of the mouse. The brain was recovered from the skull by cutting along the midline of the dorsal skull. Immediately after recovery, brains were inserted into a microtube, which was in turn immersed in liquid nitrogen. Brain tissue was stored at -80 °C until preparation for hypothalamic RNA extraction.

3.3 RNA Extraction

3.3.a. Preparation

At a minimum of 16-24 hours prior to extraction, individual brains were removed from the microtube where they had been stored at -80 °C and moved into a second tube containing Ambion RNAlater-ICE (cat# AM7030) at a ratio of 10 volumes relative to the total brain mass (assumed to be 400 mg).

3.3.b. RNA Extraction

Total RNA from the hypothalamus of all mice (n=90) was extracted using Qiagen RNeasy Fibrous Tissue Mini Kit (cat # 74704). There were two deviations to the protocol provided with the kit. In order to avoid overflow of the Qiazol lysis reagent in the micro tube, the amount of reagent was reduced from 1000µL to 800µL. Homogenization was accomplished using a rotor-stator homogenizer. The sample was then transferred to a new tube leaving any remaining foam to be discarded as is specified by the Department of Environmental Health and Safety. Deviation from the Qiagen kit protocol additionally occurred during aqueous and organic separation of the homogenate and chloroform solution where the sample was centrifuged for 20 min at room temperature rather than 4ºC.

3.3.c. RNA quality analysis

The concentration and quality of the total RNA samples were assessed by the Penn State Genomics Core Facility using a Thermo Scientific Nanodrop and Agilent Bioanalyzer. The Nanodrop was used to obtain concentrations and 260/280 ratios. Ratios between 1.8 and 2.1 were considered as an indication of good quality, while ratios under 1.8were considered unusable because such a value indicates sample degradation or contamination. The Bioanalyzer calculated RIN (RNA Integrity Number) values which were used to evaluate the quality of the RNA as well. The RIN scale ranges from 1 to 10 with a score of 10 indicating intact or high quality RNA where a score of 1 indicates sample degradation. Samples with RIN values below 7 were not used. The Bioanalyzer also provided a pseudo-gel image and an electropherogram. These additional tools were employed to evaluate the quality of the RNA.

3.4 RNA Pooling and Labeling

RNA samples from two biological replicates were pooled for each microarray, reducing the sample variance. For each mouse strain by exercise group being evaluated (6 groups total; B6 and D2 strains split into 3 treatment groups: non-exercised controls, treadmill runners and tower climbers), the RNA samples were ranked according to quality (15 samples per group).

The six best RNA samples in terms of quality were chosen from the original 15 samples in each group. These six samples were then pooled in pairs of two for a total of three pooled samples per group. The resulting 18 samples were then labeled by the Penn State Genomics Core Facility staff using Ambion MessageAmp II-Biotin Enhanced Kit (cat # AM1791) and protocol. From each pooled sample, 300 ng of RNA was labeled (kit range = 50-5000ng). The amplified and labeled RNA samples were then quantified and evaluated at the Penn State Genomics Core Facility once again using the Agilent Technologies Nanodrop and Bioanalyzer. The Nanodrop results provided the concentration of amplified RNA and the Bioanalyzer results were used as a visual indication that the samples amplified properly (using the pseudo-gel and electropherogram images). Amplified RNA (15μg) was then fragmented using the buffer and protocol provided with the Ambion kit. The samples were evaluated once again using the Bioanalyzer which provided visual assurance that the samples were completely fragmented using the pseudo-gel and electropherogram results.

3.5 Microarray Processing and Data Analysis

Gene expression was examined using the Affymetrix GeneChip Mouse Gene 1.0 ST Array and 18 experiments (chips) were conducted for the brain tissue. Three chips

containing similar samples from different mice were used as biological replicates for each strain (B6 or D2) and treatment (control, treadmill running or tower climbing) group. Labeled and fragmented samples were processed (hybridized and scanned) at the Penn State Genomics Core Facility using the Affymetrix platform.

The resulting microarray data was analyzed using statistical software, R, with Bioconductor and Linear Models for Microarray Data (LIMMA) packages. The data were normalized using Robust Multichip Averaging (RMA) and differential expression was deemed significant at $p \leq 0.05$ after appropriate adjustment for multiple comparisons using False Discovery Rate (FDR). Differential expression was compared relative to mouse strain and exercise treatment within brain tissue.

Chapter 4

Results

4.1 Differential Expression as a Function of Genetic Strain

Comparisons between genetic mouse strains (B6 vs. D2) resulted in the differential expression of over 450 probe sets ("genes"), when evaluated for significance against multiple comparisons. An adjusted p-value ≤ 0.05 and a minimum fold change of 2 were required for gene expression to be considered significant.

In total 285 genes were expressed at a significantly higher level in the hypothalamus of B6 mice compared to D2 mice. Forty-five of these genes had a 5-fold or greater change (Table **4-1**), 127 with a 3- or 4-fold change (Table **4-2**), and 113 genes with a 2-fold change (Table **4-3**).

In total, 200 genes were expressed at greater levels in the hypothalamus of D2 mice than in B6 mice. Of those, 38 genes had a 5-fold or greater change (Table **4-4**), 81 genes had a 3- or 4-fold change (Table **4-5**), and 81 genes had a 2-fold change (Table **4-6**).

Table **4-1**: Genes expressed at higher levels in the hypothalamus of B6 mice with a 5-fold change or greater

Gene	Title	Adj Pval	Fold Change
Acp1	acid phosphatase 1, soluble	0.000	11
Agxt2l1	alanine-glyoxylate aminotransferase 2-like 1	0.000	7
Actr6	ARP6 actin-related protein 6 homolog (yeast)	0.000	10
Creg1	cellular repressor of E1A-stimulated genes 1	0.000	6
Ccl28	chemokine (C-C motif) ligand 28	0.000	5
Chrna6	cholinergic receptor, nicotinic, alpha polypeptide 6	0.001	16
Coq2	coenzyme Q2 homolog, prenyltransferase (yeast)	0.000	5
Cyb5r3	cytochrome b5 reductase 3	0.000	5
Dnm3os	dynamin 3, opposite strand	0.002	7
Dnahc7b	dynein, axonemal, heavy chain 7B	0.000	8
Dnahc7b	dynein, axonemal, heavy chain 7B	0.000	6
Eapp	E2F-associated phosphoprotein	0.000	12
Fam55d	family with sequence similarity 55, member D	0.000	7
Fcrls	Fc receptor-like S, scavenger receptor	0.000	17
Fggy	FGGY carbohydrate kinase domain containing	0.000	24
Fggy	FGGY carbohydrate kinase domain containing	0.000	33
Fggy	FGGY carbohydrate kinase domain containing	0.000	38
Fggy	FGGY carbohydrate kinase domain containing	0.000	45
Hspa8 /// LOC624853	heat shock protein 8 /// hypothetical LOC624853	0.000	7
H2-Q2 /// H2-Q1	histocompatibility 2, Q region locus 2 /// histocompatibility 2, Q region locus 1	0.000	11
Hdac1	histone deacetylase 1	0.000	8
Hdac1	histone deacetylase 1	0.000	6
Klk6	kallikrein related-peptidase 6	0.002	8
Mrpl35	mitochondrial ribosomal protein L35	0.000	6
Mkl2	MKL/myocardin-like 2	0.000	14
Myoc	myocilin	0.000	28
Myo7a	myosin VIIA	0.000	9
Ninj2	ninjurin 2	0.000	5
Ociad2	OCIA domain containing 2	0.001	5
Oscar	osteoclast associated receptor	0.000	7
Pttg1	pituitary tumor-transforming gene 1	0.000	56
Plekhh2	pleckstrin homology domain containing, family H (with MyTH4 domain) member 2	0.000	16
Pdxdc1	pyridoxal-dependent decarboxylase domain containing 1	0.000	6
Rnasel	ribonuclease L (2', 5'-oligoisoadenylate synthetase-dependent)	0.000	5
Rpl3	ribosomal protein L3	0.000	1165
Rpl31	ribosomal protein L31	0.000	5
Rps2	ribosomal protein S2	0.000	7
Slc39a2	solute carrier family 39 (zinc transporter), member 2	0.000	5
Slc6a3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	0.007	28
Sat2	spermidine/spermine N1-acetyl transferase 2	0.000	12
Trim12a	tripartite motif-containing 12A	0.000	23
Trim30d	tripartite motif-containing 30D	0.000	7
Tnfaip6	tumor necrosis factor alpha induced protein 6	0.000	10
Ublcp1	ubiquitin-like domain containing CTD phosphatase 1	0.000	1760
Zfp125	zinc finger protein 125	0.000	1216

Table **4-2a**: Genes expressed at higher levels in B6 mice with a 3- or 4-fold change Part I

Gene	Title	Adj Pval	Fold Change
Bdh1	3-hydroxybutyrate dehydrogenase, type 1	0.001	3
Phgdh	3-phosphoglycerate dehydrogenase	0.001	4
Abhd10	abhydrolase domain containing 10	0.000	3
Abhd14a	abhydrolase domain containing 14A	0.001	3
Acot11	acyl-CoA thioesterase 11	0.000	4
Adipor2	adiponectin receptor 2	0.016	3
Aldh7a1 /// Phax	aldehyde dehydrogenase family 7, member A1 /// phosphorylated adaptor for RNA export	0.000	3
Aasdh	aminoadipate-semialdehyde dehydrogenase	0.002	3
Apobec3	apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3	0.005	3
Armcx1	armadillo repeat containing, X-linked 1	0.000	4
Dars2	aspartyl-tRNA synthetase 2 (mitochondrial)	0.001	3
Arid5b	AT rich interactive domain 5B (MRF1-like)	0.000	3
Bub1b	budding uninhibited by benzimidazoles 1 homolog, beta (S. cerevisiae)	0.005	3
Cml3 /// Cml5	camello-like 3 /// camello-like 5	0.001	3
Cd33	CD33 antigen	0.000	4
Cd38	CD38 antigen	0.002	3
Cetn4	centrin 4	0.001	4
Chia	chitinase, acidic	0.000	3
Cbx7	chromobox homolog 7	0.000	3
Cldn10	claudin 10	0.001	3
F2rl2	coagulation factor II (thrombin) receptor-like 2	0.001	3
F3	coagulation factor III	0.000	3
Commd7	COMM domain containing 7	0.000	4
C1qc	complement component 1, q subcomponent, C chain	0.002	4
Cntnap3	contactin associated protein-like 3	0.003	4
Cyb5r4	cytochrome b5 reductase 4	0.002	3
Cyb5r4	cytochrome b5 reductase 4	0.001	3
Cyp2r1	cytochrome P450, family 2, subfamily r, polypeptide 1	0.000	4
Dlg4	discs, large homolog 4 (Drosophila)	0.000	3
Darc	Duffy blood group, chemokine receptor	0.000	3
Dynll1	dynein light chain LC8-type 1	0.001	3
Dnahc7a /// Dnahc7b	dynein, axonemal, heavy chain 7A /// dynein, axonemal, heavy chain 7B	0.002	3
Dbndd2	dysbindin (dystrobrevin binding protein 1) domain containing 2	0.000	3
Entpd2	ectonucleoside triphosphate diphosphohydrolase 2	0.000	3
Entpd3	ectonucleoside triphosphate diphosphohydrolase 3	0.000	4
Entpd4	ectonucleoside triphosphate diphosphohydrolase 4	0.000	4
Efhd1	EF hand domain containing 1	0.005	3
Efnb3	ephrin B3	0.000	3
Eps8l1	EPS8-like 1	0.001	3
Fancl	Fanconi anemia, complementation group L	0.000	3
Fancl /// Vrk2	Fanconi anemia, complementation group L /// vaccinia related kinase 2	0.001	3
Fcgr3	Fc receptor, IgG, low affinity III	0.005	3
Fggy	FGGY carbohydrate kinase domain containing	0.003	4
Fut10	fucosyltransferase 10	0.000	3
Glul	glutamate-ammonia ligase (glutamine synthetase)	0.000	3
Gapdhs	glyceraldehyde-3-phosphate dehydrogenase, spermatogenic	0.009	3

Table **4-2b**: Genes expressed at higher levels in B6 mice with a 3- or 4-fold change Part II

Gene	Title	Adj Pval	Fold Change
Gpnmb	glycoprotein (transmembrane) nmb	0.004	3
Gadd45gip1 /// Rad23a	growth arrest and DNA-damage-inducible, gamma interacting protein 1 /// RAD23a homolog (S. cerevisiae)	0.000	3
H2-Ke2	H2-K region expressed gene 2	0.000	3
Hspb6	heat shock protein, alpha-crystallin-related, B6	0.000	4
Hfm1	HFM1, ATP-dependent DNA helicase homolog (S. cerevisiae)	0.000	4
Hfm1	HFM1, ATP-dependent DNA helicase homolog (S. cerevisiae)	0.001	3
H2-K2	histocompatibility 2, K region locus 2	0.004	3
Hpgd	hydroxyprostaglandin dehydrogenase 15 (NAD)	0.000	3
Il33	interleukin 33	0.002	4
Ica1	islet cell autoantigen 1	0.000	3
Ivd	isovaleryl coenzyme A dehydrogenase	0.000	4
Kpna2	karyopherin (importin) alpha 2	0.004	3
Klhl1	kelch-like 1 (Drosophila)	0.018	3
Klra2	killer cell lectin-like receptor, subfamily A, member 2	0.000	3
Kif1c	kinesin family member 1C	0.001	4
Lama2	laminin, alpha 2	0.015	3
Lcmt2	leucine carboxyl methyltransferase 2	0.000	3
Lrrc2	leucine rich repeat containing 2	0.004	3
Mro	maestro	0.001	3
Man2b1	mannosidase 2, alpha B1	0.001	3
Miip	migration and invasion inhibitory protein	0.001	3
Mcm6	minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)	0.000	4
Mal	myelin and lymphocyte protein, T-cell differentiation protein	0.001	3
Mobp	myelin-associated oligodendrocytic basic protein	0.006	3
Naaa	N-acylethanolamine acid amidase	0.000	4
Asah1	N-acylsphingosine amidohydrolase 1	0.002	3
Ntsr2	neurotensin receptor 2	0.000	3
Nfu1	NFU1 iron-sulfur cluster scaffold homolog (S. cerevisiae)	0.000	3
Nsun7	NOL1/NOP2/Sun domain family, member 7	0.002	4
Odf4	outer dense fiber of sperm tails 4	0.028	3
Palmd	palmdelphin	0.000	3
Ppdpf	pancreatic progenitor cell differentiation and proliferation factor homolog (zebrafish)RIKEN cDNA 2700038C09 gene	0.001	3
Pank2	pantothenate kinase 2	0.009	3
Pon2	paraoxonase 2	0.001	4
Park2	Parkinson disease (autosomal recessive, juvenile) 2, parkin	0.000	3
Prdx2	peroxiredoxin 2	0.001	3
Polr1a	polymerase (RNA) I polypeptide A	0.001	3
Paqr8	progestin and adipoQ receptor family member VIII	0.000	3
Prrg4	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)	0.040	3
Psmb6	proteasome (prosome, macropain) subunit, beta type 6	0.001	3
Rassf8	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8	0.001	3
Rassf2	Ras association (RalGDS/AF-6) domain family member 2	0.000	3
Rec8	REC8 homolog (yeast)	0.001	4
Rsl1	regulator of sex limited protein 1	0.000	3
Rbp1	retinol binding protein 1, cellular	0.007	3
Rprl2	ribonuclease P RNA-like 2	0.002	3

Table **4-2c**: Genes expressed at higher levels in B6 mice with a 3- or 4-fold change Part III

Gene	Title	Adj Pval	Fold Change
Rnase6	ribonuclease, RNase A family, 6	0.000	4
Rpl26	ribosomal protein L26	0.000	3
Rps2	ribosomal protein S2	0.001	3
Rps4y2	ribosomal protein S4, Y-linked 2	0.000	3
Rplp0	ribosomal protein, large, P0	0.000	4
Rplp0	ribosomal protein, large, P0	0.000	4
S100a11	S100 calcium binding protein A11 (calgizzarin)	0.026	3
Sec24d	Sec24 related gene family, member D (S. cerevisiae)	0.000	4
Skint3	selection and upkeep of intraepithelial T cells 3	0.002	3
Skint4	selection and upkeep of intraepithelial T cells 4	0.002	3
Spink10	serine peptidase inhibitor, Kazal type 10	0.004	3
Sft2d2	SFT2 domain containing 2	0.003	3
Srp54c /// Srp54a /// Srp54b	signal recognition particle 54C /// signal recognition particle 54A /// signal recognition particle 54B	0.007	3
Snord14e	small nucleolar RNA, C/D box 14E	0.003	3
Srd5a1	steroid 5 alpha-reductase 1	0.000	4
Sult2b1	sulfotransferase family, cytosolic, 2B, member 1	0.001	3
Sbsn	suprabasin	0.001	3
Tatdn3	TatD DNase domain containing 3	0.001	3
Tcea1	transcription elongation factor A (SII) 1	0.002	3
Tada1	transcriptional adaptor 1	0.001	3
Tmem159	transmembrane protein 159	0.001	3
Tmem195	transmembrane protein 195	0.032	3
Tmem56	transmembrane protein 56	0.013	3
Tnni1	troponin I, skeletal, slow 1	0.001	3
Tnnt2	troponin T2, cardiac	0.001	4
Ttll3 /// Arpc4	tubulin tyrosine ligase-like family, member 3 /// actin related protein 2/3 complex, subunit 4	0.000	3
Usp53	ubiquitin specific peptidase 53	0.001	3
Ulk4	unc-51-like kinase 4 (C. elegans)	0.000	3
Usmg5	upregulated during skeletal muscle growth 5	0.001	4
Vit	vitrin	0.000	3
Wdr49	WD repeat domain 49	0.001	3
Zbtb16	zinc finger and BTB domain containing 16	0.010	3
Zfp277	zinc finger protein 277	0.001	3
Zfp708	zinc finger protein 708	0.002	3
Zfp738	zinc finger protein 738	0.005	4

Table **4-3a**: Genes expressed at higher levels in B6 mice with a 2-Fold Change Part I.

Gene	Title	Adj Pval	Fold Change
Oxsm	3-oxoacyl-ACP synthase, mitochondrial	0.014	2
Haus8	4HAUS augmin-like complex, subunit 8	0.039	2
Arl3	ADP-ribosylation factor-like 3	0.002	2
Aff1	AF4/FMR2 family, member 1	0.013	2
Arrdc2	arrestin domain containing 2	0.032	2
Arnt2	aryl hydrocarbon receptor nuclear translocator 2	0.002	2
B3gat2 /// Smap1	beta-1,3-glucuronyltransferase 2 (glucuronosyltransferase S) /// stromal membrane-associated protein 1	0.007	2
Bmpr1b	bone morphogenetic protein receptor, type 1B	0.019	2
Cmbl	carboxymethylenebutenolidase-like (Pseudomonas)	0.029	2
Comt1	catechol-O-methyltransferase 1	0.002	2
C1qb	complement component 1, q subcomponent, beta polypeptide	0.031	2
Csrp1	cysteine and glycine-rich protein 1	0.002	2
Cda	cytidine deaminase	0.007	2
Ddx4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	0.019	2
Dap3	death associated protein 3	0.012	2
Defb1	defensin beta 1	0.005	2
Dhdds	dehydrodolichyl diphosphate synthase	0.013	2
Dhrs11	dehydrogenase/reductase (SDR family) member 11	0.011	2
D14Ertd449e	DNA segment, Chr 14, ERATO Doi 449, expressed	0.009	2
Enpp4	ectonucleotide pyrophosphatase/phosphodiesterase 4	0.019	2
Epas1	endothelial PAS domain protein 1	0.003	2
Fam189a2	family with sequence similarity 189, member A2	0.011	2
Fan1	FANCD2/FANCI-associated nuclease 1	0.003	2
Fgf7	fibroblast growth factor 7	0.025	2
Fkbp10 /// Nt5c3l	FK506 binding protein 10 /// 5'-nucleotidase, cytosolic III-like	0.003	2
Fmn2	formin 2	0.023	2
Gpr77	G protein-coupled receptor 77	0.023	2
Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2	0.034	2
Gabrr2	gamma-aminobutyric acid (GABA) C receptor, subunit rho 2	0.035	2
Gcfc1	GC-rich sequence DNA-binding factor 1	0.020	2
Glul	glutamate-ammonia ligase (glutamine synthetase)	0.002	2
Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	0.003	2
Gdf1 /// Lass1	growth differentiation factor 1 /// LAG1 homolog, ceramide synthase 1	0.008	2
Gucy2c	guanylate cyclase 2c	0.032	2
Haus2	HAUS augmin-like complex, subunit 2	0.041	2
Hspa12b	heat shock protein 12B	0.041	2
Hspa2	heat shock protein 2	0.013	2
Idua	iduronidase, alpha-L-	0.004	2
Immp2l	IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae)	0.007	2
Itpripl2	inositol 1,4,5-triphosphate receptor interacting protein-like 2	0.021	2
Igtp /// Irgm2	interferon gamma induced GTPase /// immunity-related GTPase family M member 2	0.007	2

Table **4-3b**: Genes expressed at higher levels in B6 mice with a 2-Fold Change Part II

Gene	Title	Adj Pval	Fold Change
Josd2	Josephin domain containing 2	0.001	2
Katnal2	katanin p60 subunit A-like 2	0.026	2
Lgi2	leucine-rich repeat LGI family, member 2	0.029	2
Lsm4	LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae)	0.001	2
Med8	mediator of RNA polymerase II transcription, subunit 8 homolog (yeast)	0.015	2
Myg1	melanocyte proliferating gene 1	0.002	2
Morc2b	microrchidia 2B	0.003	2
Mtap7	microtubule-associated protein 7	0.008	2
Mis12	MIS12 homolog (yeast)	0.013	2
Mrpl11	mitochondrial ribosomal protein L11	0.007	2
Mrpl41	mitochondrial ribosomal protein L41	0.002	2
Nat8	N-acetyltransferase 8 (GCN5-related, putative)	0.044	2
Ndufa12	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12	0.032	2
Nav1	neuron navigator 1	0.001	2
Ncstn ///	nicastrin /// coatomer protein complex subunit alpha	0.017	2
Nr2c1	nuclear receptor subfamily 2, group C, member 1	0.019	2
Ociad1	OCIA domain containing 1	0.001	2
Odc1	ornithine decarboxylase, structural 1	0.029	2
Pmp22	peripheral myelin protein 22	0.042	2
Pign	phosphatidylinositol glycan anchor biosynthesis, class N	0.009	2
Plscr4	phospholipid scramblase 4	0.000	2
Plac9	placenta specific 9	0.006	2
Pllp	plasma membrane proteolipid	0.024	2
Pvr	poliovirus receptor	0.036	2
Pkd2l1	polycystic kidney disease 2-like 1	0.032	2
Pgap2	post-GPI attachment to proteins 2	0.015	2
Pstpip2	proline-serine-threonine phosphatase-interacting protein 2	0.032	2
Pot1b	protection of telomeres 1B	0.029	2
Pink1	PTEN induced putative kinase 1	0.003	2
Rabl2	RAB, member of RAS oncogene family-like 2	0.010	2
Rab5a	RAB5A, member RAS oncogene family	0.011	2
Rasgef1a	RasGEF domain family, member 1A	0.007	2
Rcbtb2	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2	0.010	2
Rpe65	retinal pigment epithelium 65	0.022	2
Arhgef19	Rho guanine nucleotide exchange factor (GEF) 19	0.013	2
Rnase4 /	ribonuclease, RNase A family 4 /// angiogenin, ribonuclease, RNase A family, 5	0.000	2
Rnps1	ribonucleic acid binding protein S1	0.005	2
Rnps1	ribonucleic acid binding protein S1	0.001	2
Rps15a	ribosomal protein S15A	0.012	2

Table **4-3c**: Genes expressed at higher levels in B6 mice with a 2-Fold Change Part III

Gene	Title	Adj Pval	Fold Change
Rplp0	ribosomal protein, large, P0	0.005	2
Rplp0	ribosomal protein, large, P0	0.002	2
Rfwd2	ring finger and WD repeat domain 2	0.007	2
Rnf122	ring finger protein 122	0.003	2
Rnf169	ring finger protein 169	0.017	2
S100a11	S100 calcium binding protein A11 (calgizzarin)	0.025	2
Sspn	sarcospan	0.004	2
Selplg	selectin, platelet (p-selectin) ligand	0.002	2
Scpep1	serine carboxypeptidase 1	0.035	2
Spint1	serine protease inhibitor, Kunitz type 1	0.040	2
Snord115	Small nucleolar RNA, C/D Box 115 cluster	0.020	2
Scnm1	sodium channel modifier 1	0.003	2
Slc25a13	solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 13	0.002	2
Slc38a11	solute carrier family 38, member 11	0.004	2
Slc5a5	solute carrier family 5 (sodium iodide symporter), member 5	0.037	2
Slc9a9	solute carrier family 9 (sodium/hydrogen exchanger), member 9	0.011	2
S1pr1	sphingosine-1-phosphate receptor 1	0.003	2
Stard9	START domain containing 9	0.001	2
Soat1	sterol O-acyltransferase 1	0.007	2
Them4	thioesterase superfamily member 4	0.032	2
Tmc4	transmembrane channel-like gene family 4	0.001	2
Trim3	tripartite motif-containing 3	0.009	2
Tubg2	tubulin, gamma 2	0.000	2
Tssc1	tumor suppressing subtransferable candidate 1	0.002	2
Ttyh2	tweety homolog 2 (Drosophila)	0.007	2
Uba2	ubiquitin-like modifier activating enzyme 2	0.001	2
Unc13c	unc-13 homolog C (C. elegans)	0.039	2
Use1	unconventional SNARE in the ER 1 homolog (S. cerevisiae)	0.002	2
Ucp3	uncoupling protein 3 (mitochondrial, proton carrier)	0.032	2
Wdr74	WD repeat domain 74	0.019	2
Zfp458	zinc finger protein 458	0.017	2
Zfp874a	zinc finger protein 874a	0.000	2
Zfyve21	zinc finger, FYVE domain containing 21	0.024	2

Table **4-4**: Genes expressed at a higher level in D2 mice with a 5-fold or greater change

Gene	Title	Adj. Pval	Fold Change
Adi1	acireductone dioxygenase 1	0.000	6
Adat2	adenosine deaminase, tRNA-specific 2, TAD2 homolog (S. cerevisiae)	0.000	5
Alad	aminolevulinate, delta-, dehydratase	0.000	11
Bex4	brain expressed gene 4	0.001	24
Catsperg1 /// Catsperg2	cation channel, sperm-associated, gamma 1 /// cation channel, sperm-associated, gamma 2	0.000	6
Cnot8	CCR4-NOT transcription complex, subunit 8	0.002	5
Cd59b	CD59b antigen	0.000	5
AI413582	expressed sequence AI413582	0.000	7
Fndc1 /// LOC100039091	fibronectin type III domain containing 1 /// fibronectin type III domain-containing protein 1-like	0.000	5
Gabra2	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2	0.000	9
Glo1	glyoxalase 1	0.000	7
Glo1	glyoxalase 1	0.000	6
Gzmk	granzyme K	0.000	8
Hdhd3	haloacid dehalogenase-like hydrolase domain containing 3	0.000	7
Mael	maelstrom homolog (Drosophila)	0.000	5
Me1	malic enzyme 1, NADP(+)-dependent, cytosolic	0.002	5
Mlycd	malonyl-CoA decarboxylase	0.000	8
Mela	melanoma antigen	0.000	38359
Npl	N-acetylneuraminate pyruvate lyase	0.000	6
Ocel1	occludin/ELL domain containing 1	0.000	5
Olfr1507	olfactory receptor 1507	0.000	7
Pla2g4e	phospholipase A2, group IVE	0.000	20
Prss41	protease, serine, 41	0.000	8
Ppp1r12b	protein phosphatase 1, regulatory (inhibitor) subunit 12B	0.000	5
Pcdhb3	protocadherin beta 3	0.000	11
Rpa3	replication protein A3	0.000	6
Rarres1	retinoic acid receptor responder (tazarotene induced) 1	0.000	9
Rfk	riboflavin kinase	0.000	11
Serpina1a /// Serpina1b	serine (or cysteine) peptidase inhibitor, clade A, member 1A /// serine (or cysteine) preptidase inhibitor, clade A, member 1B	0.000	47
Serpina1c	serine (or cysteine) peptidase inhibitor, clade A, member 1C	0.001	6
Serpina3n	serine (or cysteine) peptidase inhibitor, clade A, member 3N	0.000	7
Sfi1 /// C330046E03	Sfi1 homolog, spindle assembly associated (yeast) /// hypothetical protein C330046E03	0.000	11
Snrpe	small nuclear ribonucleoprotein E	0.000	5
Snord53	small nucleolar RNA, C/D box 53	0.000	176
Slc25a31	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 31	0.001	8
Stab2	stabilin 2	0.000	247
Wdfy1	WD repeat and FYVE domain containing 1	0.000	7
Zfand1	zinc finger, AN1-type domain 1	0.000	240

Table **4-5a**: Genes expressed at higher levels in D2 mice with a 3- or 4-fold change Part I

Gene	Title	Adj Pval	Fold Change
Mtrr	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	0.001	3
Adal	adenosine deaminase-like	0.001	4
Abcb8	ATP-binding cassette, sub-family B (MDR/TAP), member 8	0.000	4
Car2	carbonic anhydrase 2	0.001	4
Ctse	cathepsin E	0.000	4
Cd59a /// Cd59b	CD59a antigen /// CD59b antigen	0.000	4
Ccr6	chemokine (C-C motif) receptor 6	0.019	3
Clic1	chloride intracellular channel 1	0.001	3
Coq9	coenzyme Q9 homolog (yeast)	0.000	4
Cox18	COX18 cytochrome c oxidase assembly homolog (S. cerevisiae)	0.000	3
Cuedc1	CUE domain containing 1	0.004	3
Ccna2	cyclin A2	0.000	4
Dpp7	dipeptidylpeptidase 7	0.002	3
Dnahc8	dynein, axonemal, heavy chain 8	0.000	3
Eid3	EP300 interacting inhibitor of differentiation 3	0.005	3
Fam45a	family with sequence similarity 45, member A	0.000	4
Fam78b	family with sequence similarity 78, member B	0.000	3
Fcho1	FCH domain only 1	0.001	3
Fmo2	flavin containing monooxygenase 2	0.009	3
Fpr3 /// Fpr2	formyl peptide receptor 3 /// formyl peptide receptor 2	0.010	3
Fv1	Friend virus susceptibility 1	0.000	4
Qser1	glutamine and serine rich 1	0.022	3
Gsr	glutathione reductase	0.000	4
Gnrh1	gonadotropin releasing hormone 1	0.002	3
Il17d	interleukin 17D	0.000	3
Isoc2b	isochorismatase domain containing 2b	0.008	3
Lrrc61	leucine rich repeat containing 61	0.000	3
Mtvr2 /// Sssca1	mammary tumor virus receptor 2 /// Sjogren's syndrome/scleroderma autoantigen 1 homolog (human)	0.000	3
Mir377	microRNA 377	0.014	3
Mir410 /// Mirg	microRNA 410 /// miRNA containing gene	0.002	3
Mir1839	microRNA mir-1839	0.024	3
Mrpl20	mitochondrial ribosomal protein L20	0.000	4
Mudeng	MU-2/AP1M2 domain containing, death-inducing	0.027	3
Nmbr	neuromedin B receptor	0.009	3
Pigz	phosphatidylinositol glycan anchor biosynthesis, class Z	0.000	3
Pla2g5	phospholipase A2, group V	0.001	3
Pion	pigeon homolog (Drosophila)	0.000	3
Pih1d1 /// Aldh16a1	PIH1 domain containing 1 /// aldehyde dehydrogenase 16 family, member A1	0.000	3
Kcnj10	potassium inwardly-rectifying channel, subfamily J, member 10	0.000	3
P4ha3	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide III	0.001	4
Pmch /// 4930547N16Rik	pro-melanin-concentrating hormone /// RIKEN cDNA 4930547N16 gene	0.013	3
Pcdhb10	protocadherin beta 10	0.016	3
Pcdhb11	protocadherin beta 11	0.035	3
Pcdhb2	protocadherin beta 2	0.004	3

Table **4-5b**: Genes expressed at higher levels in D2 mice with a 3- or 4-fold change Part II

Gene	Title	Adj Pval	Fold Change
Pcdhb4	protocadherin beta 4	0.000	3
Pcdhb7	protocadherin beta 7	0.005	3
Pcdhb8	protocadherin beta 8	0.000	4
Arhgap4	Rho GTPase activating protein 4	0.000	4
Rpl29	ribosomal protein L29	0.004	4
Rpl29	ribosomal protein L29	0.039	4
Rps18	ribosomal protein S18	0.042	3
Rps9	ribosomal protein S9	0.001	3
Rufy4	RUN and FYVE domain containing 4	0.001	3
Rwdd3	RWD domain containing 3	0.000	3
Sgcg /// Sacs	sarcoglycan, gamma (dystrophin-associated glycoprotein) /// sacsin	0.001	3
Slfn8	schlafen 8	0.000	4
Scg5	secretogranin V	0.000	4
Szt2	seizure threshold 2	0.000	4
Szt2	seizure threshold 2	0.000	3
Sh3bp1	SH3-domain binding protein 1	0.000	3
Srp14	signal recognition particle 14	0.001	3
Snrnp25	small nuclear ribonucleoprotein 25 (U11/U12)	0.002	3
Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2	0.000	4
Senp3	SUMO/sentrin specific peptidase 3	0.000	3
Sycp1	synaptonemal complex protein 1	0.001	3
Tns1	tensin 1	0.000	3
Txndc9	thioredoxin domain containing 9	0.002	3
Txndc9	thioredoxin domain containing 9	0.005	3
Thoc7	THO complex 7 homolog (Drosophila)	0.001	3
Tor3a	torsin family 3, member A	0.001	3
Tmem132b	transmembrane protein 132B	0.000	4
Tmem203	transmembrane protein 203	0.004	3
Tmem87b	transmembrane protein 87B	0.019	3
Tmod4	tropomodulin 4	0.000	4
Ugcg	UDP-glucose ceramide glucosyltransferase	0.001	3
Vmn1r90	vomeronasal 1 receptor 90	0.001	3
Zfp58	zinc finger protein 58	0.003	4
Zfp658	zinc finger protein 658	0.000	3
Zfp69	zinc finger protein 69	0.000	3
Zfp71-rs1	zinc finger protein 71, related sequence	0.011	3
Zfp760	zinc finger protein 760	0.016	3

Table **4-6a:** Genes expressed at higher levels in D2 mice with a 2-fold change Part I

Gene	Title	Adj Pval.	Fold Change
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	0.032	2
Alkbh2	alkB, alkylation repair homolog 2 (E. coli)	0.012	2
Anapc13	anaphase promoting complex subunit 13	0.010	2
Anxa4	annexin A4	0.009	2
Ano5	anoctamin 5	0.038	2
Aifm1	apoptosis-inducing factor, mitochondrion-associated 1	0.044	2
Acap3	ArfGAP with coiled-coil, ankyrin repeat and PH domains 3	0.002	2
Arsa	arylsulfatase A	0.023	2
Atp6ap1l	ATPase, H+ transporting, lysosomal accessory protein 1-like	0.022	2
Abca3	ATP-binding cassette, sub-family A (ABC1), member 3	0.005	2
Btbd9	BTB (POZ) domain containing 9	0.011	2
Car11	carbonic anhydrase 11	0.032	2
Ccl17	chemokine (C-C motif) ligand 17	0.012	2
Ccl3	chemokine (C-C motif) ligand 3	0.023	2
Chi3l1	chitinase 3-like 1	0.007	2
Ccdc72	coiled-coil domain containing 72	0.007	2
Cstl1	cystatin-like 1	0.006	2
D17H6S56E-5	DNA segment, Chr 17, human D6S56E 5	0.011	2
Dnajc24	DnaJ (Hsp40) homolog, subfamily C, member 24	0.004	2
Dcdc5	doublecortin domain containing 5	0.032	2
Emp1	epithelial membrane protein 1	0.006	2
Exoc5	exocyst complex component 5	0.016	2
Fam20b	family with sequence similarity 20, member B	0.007	2
Fam71e1	family with sequence similarity 71, member E1	0.028	2
Gpr19	G protein-coupled receptor 19	0.003	2
Gmip	Gem-interacting protein	0.000	2
Gga3	golgi associated, gamma adaptin ear containing, ARF binding protein 3	0.001	2
Guk1	guanylate kinase 1	0.004	2
H2-Ke6	H2-K region expressed gene 6	0.001	2
Hes7	hairy and enhancer of split 7 (Drosophila)	0.039	2
Hddc3	HD domain containing 3	0.003	2
H2-Q8	histocompatibility 2, Q region locus 8	0.010	2
Hist1h2bb	histone cluster 1, H2bb	0.001	2
Ifi202b	interferon activated gene 202B	0.016	2
Irf2bp1	interferon regulatory factor 2 binding protein 1	0.015	2
Kptn	kaptin	0.005	2
Lrrc51	leucine rich repeat containing 51	0.001	2
Lcorl	ligand dependent nuclear receptor corepressor-like	0.033	2
Lyplal1	lysophospholipase-like 1	0.002	2
Mamdc2	MAM domain containing 2	0.003	2
Mia2	melanoma inhibitory activity 2	0.001	2
Micalcl	MICAL C-terminal like	0.006	2
Mir30c-2	microRNA 30c-2	0.030	2
Mir539	microRNA 539	0.022	2

Table **4-6b:** Genes expressed at higher levels in D2 mice with a 2-fold change Part II

Gene	Title	Adj Pval.	Fold Change
Mrps14	mitochondrial ribosomal protein S14	0.027	2
Myo19	myosin XIX	0.001	2
Ntn4	netrin 4	0.005	2
Nudc	nuclear distribution gene C homolog (Aspergillus)	0.000	2
Nup35	nucleoporin 35	0.005	2
Pacrg	PARK2 co-regulated	0.012	2
Parvg	parvin, gamma	0.006	2
Pdpn	podoplanin	0.004	2
Pdia3	protein disulfide isomerase associated 3	0.040	2
Ppp4r2	protein phosphatase 4, regulatory subunit 2	0.005	2
Pcdhb1	protocadherin beta 1	0.003	2
Pusl1	pseudouridylate synthase-like 1	0.014	2
Pyroxd2	pyridine nucleotide-disulphide oxidoreductase domain 2	0.003	2
Pdxp	pyridoxal (pyridoxine, vitamin B6) phosphatase	0.005	2
Rdh13	retinol dehydrogenase 13 (all-trans and 9-cis)	0.002	2
Rpl29	ribosomal protein L29	0.044	2
Rps20	ribosomal protein S20	0.004	2
Sfrp4	secreted frizzled-related protein 4	0.040	2
Szt2	seizure threshold 2	0.001	2
Sepn1	selenoprotein N, 1	0.019	2
Srp9	signal recognition particle 9	0.001	2
Sssca1 ///	Sjogren's syndrome/scleroderma autoantigen 1 homolog (human) /// mammary tumor virus receptor 2	0.002	2
Stard4	StAR-related lipid transfer (START) domain containing 4	0.002	2
Sae1	SUMO1 activating enzyme subunit 1	0.003	2
Stxbp4	syntaxin binding protein 4	0.044	2
Tctex1d2	Tctex1 domain containing 2	0.012	2
Tns1	tensin 1	0.003	2
Tns1	tensin 1	0.000	2
Tmprss7	transmembrane serine protease 7	0.004	2
Uba5	ubiquitin-like modifier activating enzyme 5	0.021	2
Ugdh	UDP-glucose dehydrogenase	0.005	2
Vgf	VGF nerve growth factor inducible	0.007	2
Zfp457	zinc finger protein 457	0.011	2
Zfp612	zinc finger protein 612	0.011	2
Zfp74	zinc finger protein 74	0.041	2
Zfand1	zinc finger, AN1-type domain 1	0.014	2
Zmynd17	zinc finger, MYND domain containing 17	0.003	2

4.2 Differential Expression as a Function of Exercise Treatment

One of the primary aims of this study was to elucidate significant genes that are differentially expressed as a result of exercise treatment. Although many genes were differentially expressed as a result of exercise, no significant differences were identified after adjustment for multiple comparisons. In the following tables, the adjusted p-value is shown with the p-value and the fold change to indicate the magnitude of differential expression.

As mentioned earlier leptin is an important hormone for controlling bone metabolism and energy homeostasis. The Lepr leptin receptor was expressed at lower levels in the B6 tower climbing mice than in the sedentary controls. Cyclic AMP response-element binding protein (CREB), a transcription factor in the hypothalamus, was down-regulated in B6 tower climbers as compared to the B6 controls. Melanin-concentrating hormone, alternatively known as Pmch, was additionally expressed at higher levels in the B6 tower climbing mice as compared to B6 controls. For genes involved in the leptin pathway, see Table **4-7.**

Table **4-7** Differential expression of genes involved in leptin signaling

Results	Gene	Title	Pval	Adj Pval	Fold Change
B6TO<C	Lepr	leptin receptor	0.02	0.579	3
B6TO>C	Pmch /// 4930547N16Rik	pro-melanin-concentrating hormone /// RIKEN cDNA 4930547N16 gene	0.02	0.579	3
B6TO<C	creb3l2	cAMP responsive element binding protein 3-like 2	0.01	0.579	2

Arginine vasopressin (AVP) and NMU are important in stimulation of the HPA axis.With both strains combined, AVP, showed increased expression in tower climbing mice compared to controls. Expression of NMU receptor 1 was increased only in B6 tower climbers compared to B6 controls. For genes that interact with the HPA axis see Table **4-8**.

Table **4-8** Differential expression of genes that interact with the HPA axis

Results	Gene	Title	Pval	Adj Pval	Fold Change
B6TO>C	Nmur1	neuromedin U receptor 1	0.01	0.579	2
TO>C	avp	arginine vasopressin	0.01	0.999	4

Dopamine-beta hydroxylase (DBH) is important in adrenergic signaling and was expressed at higher levels in B6 tower climbers compared to B6 controls. For differential expression of genes involved in neurotransmitter signaling see Table **4-9**.

Table **4-9** Differential expression of genes involved in regulation of neurotransmitters

Results	Gene	Title	Pval	Adj Pval	Fold Change
B6TO>C	dbh	dopamine beta-hydroxylase	0.024	0.579	2

Chapter 5

Discussion

The realization that genes on almost every chromosome may control skeletal phenotypes has led the scientific community to believe that osteoporosis risk is determined by a large number of genes with a relatively small magnitude of effect. Early osteoporosis reaserchers believed that only a few genes strongly determined susceptibility [29].

An animal's performance in voluntary exercise does not necessarily predict performance in a forced-exercise situation [21]. Hippocampal proteins that are regulated during voluntary exercise may differ from those of involuntary exercise [3]. In this circumstance, treadmill exercising was definitely involuntary, while tower climbing mice could modulate the number of trips they climbed each day.

5.1 Analysis of strain effect

A quantitative trait locus (QTL) has been determined for τ, a measure of intrinsic circadian rhythm, however not all genes that influence τ have been confirmed. Suggested candidate genes include kcnj10 on chromosome 1, which in the above results was expressed at a 3-fold change higher in D2 mice than in B6 mice [30].

Similar to τ, a QTL on chromosome 12 for free-running circadian rhythm has been investigated. Zinc finger protein 277 was shown to be differentially in whole-brain assays of B6 and D2 mice [31]. The only known function of zfp277 is for its role in cellular senescence; mouse embryonic fibroblasts from homozygous knockouts for zfp277 showed premature senescence [32].

One of many QTLs that has been evaluated for bone mineral density (BMD) includes another candidate gene on Chromosome 1, Duffy blood group, chemokine receptor (Darc). The

expression of Darc in most tissues has been determined to be a result of expression in endothelial cells of post-capillary venules [33]. It has been concluded that Darc is a negative regulator of osteoclasts, because bone resorption was reduced in the absence of Darc [29]. The above data from this study shows a 3-fold increased expression of Darc in B6 mice as compared to D2 mice, which may help explain why D2 mice have higher bone mineral density.

Candidate genes for a QTL on chromosome 4 have previously been identified in this lab. Aminolevulinate, delta-, dehydratase (Alad) was previously shown to be differentially expressed at higher levels in muscle of D2 mice as compared to B6 mice, which correlated with a QTL for femur total area and yield stress [34][35]. Again in this study, Alad was expressed at higher levels in D2 mice.

Serine (or cysteine) peptidase inhibitor, clade A, member 1A (serpina1a) was expressed at higher levels in D2 mice than B6 mice. Although it has not been previously shown to have an important role in the brain, its production in osteoblasts supports development of hematopoietic stem cells [36]. It is possible that some extracellular serpina1 may be produced in the brain and has its impact on long bones, which would support higher bone mineral density that is observed in D2 mice. Serpina1a is a candidate gene for a QTL on chromosome 12 for femur head diameter, and has been previously shown increased expression in D2 mice compared to B6 mice [34][35].

Genes involved in metabolism and intake were differentially expressed between B6 and D2 mice. Malonyl CoA decarboxylase (Mlycd) had 8-fold greater expression in D2 than in B6 mice. Because Mlycd is important in stimulating a desire to eat, it is possible that Mlycd contributes to the observed differences in food motivation between D2 and B6 mice [37]. Glyoxalase 1 (Glo1) is another gene that has been implicated in differences in intake and was expressed at higher levels in D2 mice than in B6 mice. It has been suggested that increased Glo1 expression correlates with increased carbohydrate intake [38].

Basal gene expression of the PVN, known as a stress control region, found by Tsolakidou et al. supports many of the genes differentially expressed in the above data see Table **5-1** [39]. A possible reason for large differences in fold change may be due to the gene extraction from the whole hypothalamus (author's study) vs. only the PVN in the study by Tsolakidou et al. However, it has been observed that in general genes that are differentially expressed as a result of strain in one region of the brain are either consistently or not expressed in other regions of the brain [40].

Table **5-1** Basal gene expression for the PVN in Tsolakidou et al. supports data from author's study

Result	Gene	Title	Fold Change reported by Tsolakidou	Fold Change in author's study
B6>D2	Glul	Glutamate-ammonia ligase (glutamine synthase)	3	3
B6>D2	Glul	Glutamate-ammonia ligase (glutamine synthase)	2	2
B6>D2	Gatm	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	3	2
B6>D2	H2-Q1	Histocompatibility 2, Q region locus 1	3	11
B6>D2	Pttg1	Pituitary tumor-transforming 1	3	56
D2>B6	4922501C03Rik	RIKEN cDNA 4922501C03 gene	2	2
D2>B6	Gsr	Glutathione reductase 1	2	4

5.2 Analysis of treatment effect

In B6 tower climbers, the leptin receptor was down-regulated. This same effect was observed after 12 weeks of exercise in rats [41]. Leptin is known to be important in many aspects of metabolism, and leptin-deficient ob/ob mice have skeletal abnormalities including decreased bone length and mass. Leptin deficiency has been shown to specifically cause decreased cortical bone mass [42]. These skeletal abnormalities, and other abnormalities involving selective accumulation of bone in certain regions, are the same as those that occur during starvation [43]. The same transcription factor that is responsible for regulation of NPY in the hypothalamus, CREB, may be responsible for leptin regulation as well, so it is important to note that CREB3 was down regulated in the same cohort as the leptin receptor [44].

Melanin-concentrating hormone is known to be critical in the prevention of osteoporosis. Knockout mice lacking the receptor for Pmch show a low bone mass phenotype [45]. The differential expression of Pmch as a result of exercise in this circumstance provides further evidence of the benefits of exercise in on skeletal health.

Arginine vasopressin interacts with CRH to increase ACTH, and corticosteroid production from the adrenals in exercising animals [7][46]. The synergistic actions with CRF indicate that AVP is pro-inflammatory. Specifically, AVP is expressed in the hypothalamus by the parvocellular paraventricular nucleus (pPVN) and by the magnocelluar supraoptin nucleus (SON) [47]. Exercising above the lactate threshold is considered a stressor and changes blood osmolarity. Although lower exercise intensity does increase release of AVP, as was observed in this experiment, treadmill running above the lactate threshold showed a significantly higher production of AVP by neurons in the PVN and SON [47].

Upon exposure of neurons of the PVN to NMU, rats show increased energy expenditure associated with activity. NMU was shown to increase physical activity and energy expenditure in a dose-dependent manner, regardless of rat strain [48]. Neuromedin U stimulates downstream effects through the release of CRF and is important in increasing locomotor activity [49].

Dopamine beta-hydroxylase is responsible for converting dopamine into norepinephrine. Adrenergic messengers are important in neuroendocrine and autonomic nervous system signaling, especially with regard to cardiac function [50]. Exercise training in Wistar-Kyoto (WKY) rats resulted in a greater density of DBH in the PVN [51].

It is possible that more suggestive genes were observed in B6 tower climbers than D2 tower climbers due to differences in natural activity levels. In a study measuring “home cage” activity, B6 mice had significantly greater vertical activity than D2 mice [22].

5.3 Recommendations for future investigation

Comparison of this data to mRNA expression in long bones should elucidate clear neuroskeletal (and neuroendocrine) pathways for the influence of exercise on skeletal remodeling. Comparison of the expression profile of the hypothalamus to those of adipose and uterine tissue may be able to further support the hormonal implications of hypothalamic control.

Future study might include increased distance for treadmill running, or a comparison of treadmill exercise in this protocol to voluntary wheel exercise. A setup of this sort could reveal compounding effects of treadmill stress that change the expression profile in the hypothalamus. Additionally, it might be useful to monitor intake and eating behavior of laboratory animals to normalize any of these (i.e. leptin, NPY) metabolic pathways that are involved in skeletal modeling.

It would be interesting to compare DBH levels in the hypothalamus with the distribution and composition of white adipose tissue in exercised mice. Additional opportunities exist for comparing exercise-induced hypothalamic gene expression as it changes with age, one protein of interest is zfp277, because of its role in cellular senescence and in the QTL for free-running circadian activity.

Overall, focusing studies on the PVN or the VMH may prove to better elucidate genes that control bone formation downstream.

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Appendix A

Treadmill Operator Protocol

Protocol used to monitor the mice during running on the treadmill and actual setting used throughout duration of treatment (speed, incline, duration).

Technician/Treadmill Operator:

1) Fill out log-in sheet with animal **ID, group, treadmill inclination, exercise intensity, and duration**.

2) Verify the following treadmill settings: 0.76 mA (equivalent **to 4 on the intensity dial**) and a repetition rate of 2 pulses per second (equivalent to **5 on the repetition rate dial**).

Acclimation:

3) Prior to each exercise session each mouse should be placed on the treadmill in its respective lane with the belt unmoving and shock grids off but with the belt motor on. The mice should be left **undisturbed for 5 minutes**.

Warm-up:

4) Turn on the shock grids and start the belt. The animals should be warmed up at the beginning of each session. The belt speed **should start at 10 m/min** and slowly be **ramped up** with an acceleration of **1 m/min^2**.

Exercise Training Regimen:

5) The timer should be started as soon as the belt speed reaches 10 m/min.

Treadmill Monitoring:

The red lamp next to the Repetition Rate knob indicates each time there is a shock pulse present at the shocker grid.6) The technician will observe the animals during the entire exercise period.

Record the following:

a) Using a stop watch: record the **time of each shock**.

b) **Time between each consecutive shock**.

c**) Number of** times each animal is willing to receive 2 seconds or more of shocking rather than return to the treadmill (this is equivalent to **4 consecutive shocks**). d) If the animal remains on the bar for a period longer than 5 seconds (this is equivalent to **10 consecutive shocks**).

7) Once the animal has received 1 interval of 2 seconds of shock (equivalent to 1 interval of 4 consecutive shocks) the technician should gently nudge the mouse on its rump to encourage the mouse to run.

8) If at any time the animal becomes exhausted during the exercise session the shock grid must be deactivated for that lane. If the electric stimulus is removed the technician will note the time and whether the animal resumes running during the remainder of the session.

9) If the electric stimulus is removed, the technician should continue to nudge the mouse with their hand to encourage the mouse to run. The technician should stop nudging the animal after 5 attempts to encourage the animal to run.

The stimulus consists of 200 millisecond 0.74 mA pulses that will be delivered at a rate of 2 pulses per second. Two (200 millisecond) pulses are equivalent to 0.4 seconds of shock in a 1 second period. This is equivalent to 0.8 seconds of shock in a 2 second period and 2 seconds of shock in a 5 second period.

The criteria for discontinuing shock exposure are as follows:

A) If the animal spends **more than 5 consecutive seconds** on the shock grid without attempting to reengage the treadmill.

(10 consecutive shocks)

(equivalent to 2 seconds of shock in a 5 second consecutive period)

B) The third time a mouse is willing to sustain **2 seconds or more of shocking** rather than return to the treadmill.

(3 X 4 consecutive shocks = 12 shocks)

(equivalent to 3 periods of 0.8 seconds of shock in a 2 second consecutive period)

(equivalent to 2.4 seconds of shock)

C) When the animal sustains **a total of 20 shocks within any 5 minute period** regardless of the spacing of the shocks during the 30 minute exercise period. (equivalent to 4 seconds of shock in a 5 minute period)

D) When the animal sustains **a total of 50 shocks regardless of the spacing** of the shocks during the 30 minute exercise period.

(equivalent to 10 seconds of shock)

10) Animals that repeatedly refuse to run after five attempts will be removed from the exercise intervention group. Records on each animal's performance will be maintained and the number of shocks each animal receives during each session will be recorded by the observer.

Appendix B

Tower Design

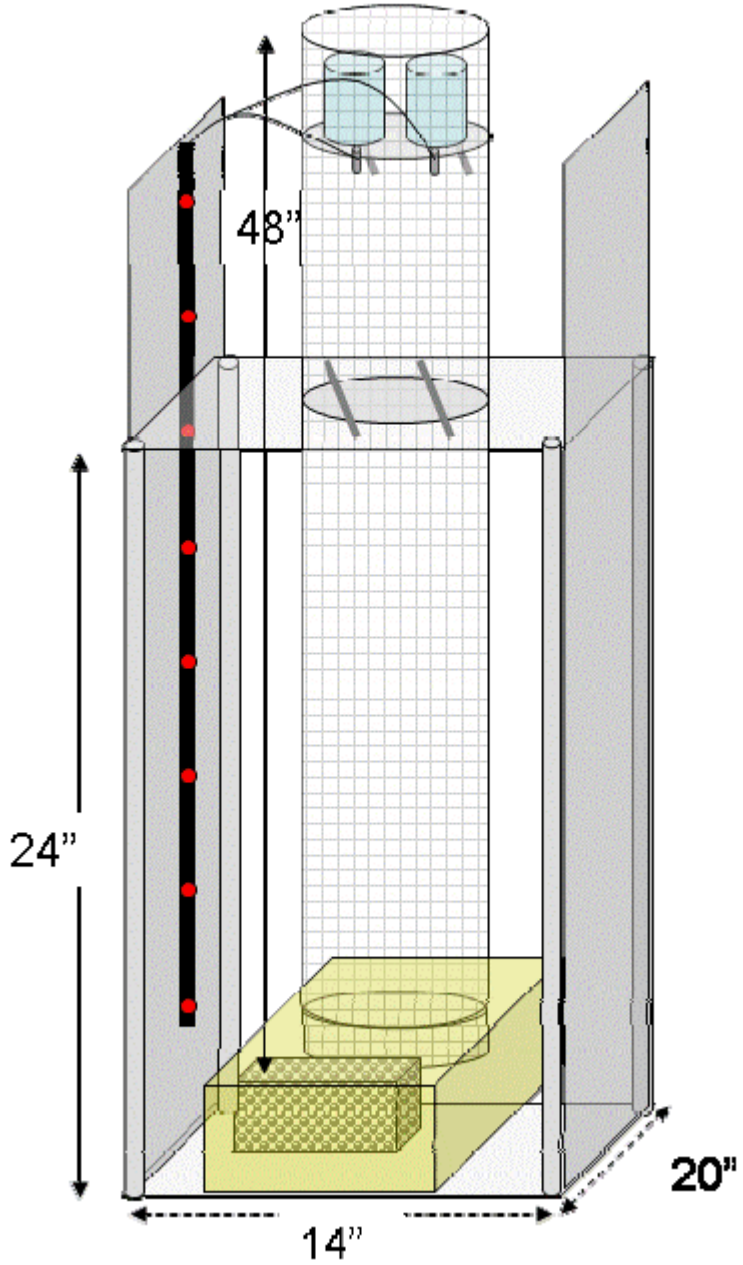

Figure **B-1**: Design of Tower for Exercise Intervention.

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Career Goals: Pursue a DVM with intentions to work in dairy production medicine or mixed-animal practice

Education

The Pennsylvania State University, University Park, PA
B.S. Veterinary and Biomedical Sciences with Honors in Kinesiology
Minors in Spanish and Biology
Schreyer Honors Scholar

Thesis Title: The effect of strain and exercise on hypothalamic gene expression in C57BL/6 and DBA/2J mice

Thesis Supervisor: Dr. Dena Lang, Research Faculty, Kinesiology, Penn State University

Honors/Awards

- Oswald Scholarship 2010-2012
- Russel Memorial Scholarship: 2009-2010
- Dean's List: Fall 2008, Fall 2009, Fall 2010, Spring 2010, Fall 2011
- Pennsylvania SpaceGrant Minorities in Undergraduate Research and Engineering Research Assistantship: awarded December 2008
- National AP Scholar: awarded September 2008
- Academic Excellence Scholarship: 2008-2012
- John N Adam Jr Scholarship for Excellence in Agriculture: 2008-2009

Work Experience

William H. Miner Agricultural Research Institute — Chazy, NY — May 2011 – August 2011

Farm Management Intern

- Rotated through farm operations including crop production, feeding, herd health, calf management and milking management
- Assisted with data collection for research trials in nutrition and animal behavior
- Participated in educational outreach

Penn State University Dairy Barns — University Park, PA — September 2010-present

Student Employee

- Monitor cattle for signs of disease
- Complete regular feeding, milking and cleaning procedure

Applebrook Veterinary Clinic Oxford, PA May 2010-present

Large Animal Shadow

- Perform physical examinations on patients
- Restrain bovine, equine, caprine and ovine patients
- Assist in medical and surgical treatment of routine and emergency cases

Penn State Biomechanics Laboratory University Park, PA January 2010 - present

Honors Thesis Research Assistant under Dr. Dena Lang

- Investigating the effects of genetics and exercise on hypothalamic gene expression in relation to osteoporosis.

Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania Philadelphia, PA May 2009 – August 2010

Emergency Service Volunteer

- Maintained stock of regular medical and food supplies
- Assisted clinicians with pharmacy, and pathology requests

The Animal Clinic Exton, PA May 2009 – August 2009

Veterinary Assistant

- Restrained canine and feline patients
- Completed office maintenance
- Assisted in scheduling of appointments and discharge of patients

Penn State Biomechanics Laboratory University Park, PA January 2009 – December 2009

Undergraduate Research Assistant under Dr. Dena Lang

- Investigating the effects of genetics and exercise on bone quality in relation to osteoporosis

Community and Leadership Activities

Member, Penn State Dairy Science Club	March 2011 – present
Member, Penn State Pre-Vet Club	March 2010 – December 2010
Mentor, Schreyer Honors Orientation	Fall 2010
Ministry Volunteer, Penn State Catholic Campus Ministry	September 2008 – present
Recruitment Volunteer, Schreyer Honors College Student Council	September 2008 – April 2011
Secretary, Service Chair, THON co-coordinator, Fundraising Chair, Newman Catholic Student Association	August 2008 - Present
Team Leader, Fresh START Day of Service	August 2008 – August 2011
Competitive Team Member, Penn State Club Cross Country	August 2008 – May 2011
Community Service Chair, Collegiate Horsemen Association at Penn State	August 2008 – May 2010
Member, Biomedical Sciences Club	August 2008 – December 2009

Language Proficiency: Working knowledge of Spanish