THE IMPACT OF MEDIUM-CHAIN TRIGLYCERIDES ON ENERGY INTAKE, ADIPOSITY, AND HIPPOCAMPAL BRAIN-DERIVED NEUROTROPIC FACTOR IN *AD LIBITUM* AND PAIR-FED RAT MODELS OF HIGH-FAT-DIET-INDUCED OBESITY

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Dedicated to my loving fiancé for her continued support and encouragement, to my father for always setting the example, to my mother for her unparalleled selflessness, to my sisters for their kindness, to all those that I get to call friend, and to God for granting me the courage and determination

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ABSTRACT

Dietary intervention remains a popular, albeit challenging, approach for combating obesity. In recent years, dietary interventions that increase consumption of medium-chain triglycerides (MCT) instead of long-chain triglycerides (LCT) have gained attention. Pre-clinical research has demonstrated that rats fed a high-fat diet (HFD) induce adiposity, but a dietary shift from LCT to MCT suppresses this effect. To date, the extent to which this effect operates via suppressed hyperphagia is not fully understood. In the present study, we sought to determine how consuming a HFD composed of different fat types affects energy intake, adiposity, and hippocampal brain-derived neurotropic factor (BDNF) levels. Rats were assigned to one of four diet groups – rat chow (CHOW), LCT-enriched HFD (LCT-HFD), MCT-enriched HFD (MCT-HFD), or coconut oil-enriched HFD (COCO-HFD), which composes a mixture of LCT and MCT. In Experiment 1, all animals were given *ad libitum* access to their assigned diet, whereas in Experiments 2 and 3, HFD-subjects were pair-fed to CHOW to prohibit hyperphagia. In Experiments 1 and 2, subjects were aged 20-24 weeks, whereas in Experiment 3, subjects were aged 10-11 weeks. Across experiments, we found that the effect of MCT consumption on suppressing HFD-induced adiposity is causally related to suppressed HFD-induced hyperphagia. Additionally, we failed to detect an effect of HFD consumption on hippocampal BDNF. Therefore, our findings did not support or oppose the hypothesis that MCT consumption attenuates HFD-induced BDNF deficiency. Future studies should focus on determining the causal relationship between MCT consumption, energy expenditure, and HFD-induced adiposity.

INTRODUCTION

The Modern Obesity Problem

Obesity is a complex disease that is characterized by excessive amounts of adipose tissue. Moreover, obesity poses a major public health concern. The World Health Organization (WHO) estimates that 13% of adults worldwide were obese as of 2016, with 4 million people dying per year from obesity-associated complications. Rates of obesity have also increased rapidly over the last half-century. WHO estimates that the prevalence of obesity tripled in adults from 1975 to 2016, and increased from 4% to 18% in children and adolescents during the same timeframe. The etiology of this dramatic increase in obesity rates is complicated, but the strongest evidence suggests that modern obesity is driven by increased energy availability in the environment, specifically in the form of refined carbohydrate and dietary fat, thereby increasing energy intake (Swinburn et al., 2011), and resulting in widespread positive energy balance (Hall et al., 2012). However, the relationship between the macronutrient composition of our modern diets and energy intake requires further investigation. Although sucrose consumption in the form of sugar-sweetened beverages is positively associated with body weight gain (van Dam & Seidell, 2007), solid sucrose consumption has no effect on energy intake or adipose tissue mass, while variable dietary fat consumption is highly related to both (Hu et al., 2018). Therefore, research investigating the mechanisms that motivate fat-induced energy intake is crucial not only to elucidating the cause of the current obesity epidemic, but also to providing novel solutions.

High-Fat Diets

High-fat diets (HFD) have been used extensively to study obesity in rodent models because they reliably increase energy intake and induce body fat accumulation (Hariri & Thibault, 2010). Interestingly, however, a dietary shift from long-chain triglycerides (LCT) to medium-chain triglycerides (MCT) suppresses HFD-induced obesity in rats (Ferreira et al., 2014), and reduces total body weight, waist circumference, hip circumference, total body fat, subcutaneous fat, and visceral fat in humans (Mumme & Stonehouse, 2015). MCT consumption leads to a negative energy balance through a combination of reduced energy intake (Ferreira et al., 2014; Maher & Clegg, 2020) and increased energy expenditure (Papamandjaris et al., 1998; Costa et al., 2012).

However, previous reports have not determined the extent to which these factors mediate the relationship between MCT consumption and body fat accumulation. Therefore, here we focus on assessing energy intake as a potential mediator of the relationship between MCT consumption and HFD-induced obesity *in vivo* using both a statistical mediation model and an experimental manipulation.

Metabolic Distinctions of MCT

The effects of MCT consumption on energy intake and energy expenditure are likely driven by metabolic advantages. Due to their shorter chain-length, medium-chain fatty acids (MCFA) bypass chylomicron transport and the carnitine acyltransferase system necessary for long-chain fatty acids (LCFA) to travel to the liver and enter mitochondria (Bach & Babayan, 1982; Schönfeld & Wojtczak, 2016). As a result, MCT consumption increases thermogenesis and fatty acid oxidation in brown adipose tissue (Zhang et al., 2015) and the liver (Rial et al., 2020). Interestingly, increased hepatic fatty acid oxidation after MCT consumption not only increases energy expenditure, but is also necessary for decreased energy intake (Ooyama et al., 2009), and thus may provide a better explanation than satiety hormones (Maher & Clegg, 2019). Nevertheless, these improvements in cellular energy metabolism also extend to the brain. Hughes et al. (2014) demonstrated that C10, a MCFA, increases citrate synthase activity in human neuroblasts, resulting in a downstream increase in mitochondrial complex I activity, and that this effect is dependent on peroxisome proliferator-activated receptor-γ (PPAR-γ) activation. These results suggest that MCT consumption improves cellular energy metabolism in the brain via PPAR-γ agonism.

Central Hypothesis

Human obesity contributes to the development of cognitive impairment, particularly in executive functioning, throughout the lifespan (Smith et al., 2011). The mechanisms responsible for this effect are still under investigation, but one hypothesis is that diet and obesity interact to produce low-grade peripheral and central inflammation that promotes neuronal death (Leigh & Morris, 2020). This was supported by Park et al. (2010), who found that mice fed a HFD for 7 weeks exhibited less BrdU-positive cells in the dentate gyrus than control mice. These effects were concomitant with increased hippocampal malondialdehyde (MDA) levels, a marker of oxidative

stress, and decreased brain-derived neurotrophic factor (BDNF) levels. Moreover, impaired neurogenesis was ameliorated with preexposure to exogenous BDNF. Similarly, in humans, Kaur et al. (2016) established an inverse relationship between the waist-to-hip circumference ratio, an indirect but reliable measure of body fat, and executive functioning that is mediated by lower circulating brain-derived neurotrophic factor (BDNF) levels. Therefore, interventions that restore BDNF levels may be able to combat the effects of diet and obesity on executive functioning.

Pharmacological activation of PPAR-γ in the brain has been shown to elevate hippocampal BDNF and alleviate diabetes-induced cognitive dysfunction (Kariharan et al., 2015). Therefore, MCFA may similarly permeate the blood-brain barrier (Oldendorf, 1973), agonize PPAR-γ (Liberato et al., 2012), elevate hippocampal BDNF, and attenuate diet-induced cognitive impairment. However, a connection between MCT consumption and BDNF has yet to be established. Here, we aimed to explore the effects of MCT consumption on hippocampal BDNF in *ad libitum* and calorie-controlled environments.

Study Preview

In the present study, we sought to address two research questions: first, does energy intake mediate the relationship between MCT consumption and HFD-induced obesity? And second, how does MCT consumption affect BDNF in the hippocampus? These questions were addressed with male and female Long Evans rats in three separate experiments. Across experiments, all subjects were assigned to one of four diet groups: standard rat chow (CHOW), a HFD rich in LCT (HFD-LCT), a HFD rich in MCT (HFD-MCT), or a HFD rich in coconut oil (HFD-COCO), which is composed of a mixture of LCT and MCT. In Experiment 1, all subjects were given *ad libitum* access to their assigned diet, but in Experiment 2 and Experiment 3, subjects assigned to the three HFD groups were pair-fed to the CHOW group to prevent hyperphagia. We hypothesized that MCT consumption would suppress HFD-induced obesity in Experiment 1, and that this effect would be statistically mediated by energy intake. However, in Experiment 2 and Experiment 3, we hypothesized that variation in HFD-induced obesity between diet groups will be diminished, further indicating mediation by energy intake. Additionally, we hypothesized that MCT consumption would attenuate HFD-induced hippocampal BDNF deficiency in Experiment 1, but that variation in hippocampal BDNF between groups would be significantly diminished in Experiment 2 and Experiment 3.

EXPERIMENT 1

Method

Subjects

Adult male (*n* = 16) and female (n = 16) Long Evans rats aged 20-22 weeks were individually housed in suspended wire cages in humidity (55-65%) and temperature (20.5 ± 1 °C) controlled rooms that were maintained with a 12:12 hour light/dark cycle. Males and females were housed in separate rooms with light/dark cycles staggered by 1 hour to maintain consistency of testing times. Animals had *ad libitum* access to chow diet (Teklad 2018, Envigo, Indianapolis, IN) unless specified otherwise in experimental conditions. All procedures were approved by the Purdue Animal Care and Use Committee (PACUC).

Dietary Intervention

Subjects were assigned to either a control group fed standard rat chow (CHOW) or one of three HFD groups: HFD-LCT subjects were fed a HFD mixture composed of 70% chow and 30% vegetable shortening; HFD-MCT subjects were fed a HFD mixture composed of 70% chow, 22.5% capric acid (C10), and 7.5% caprylic acid (C8); and HFD-COCO subjects were fed a HFD mixture composed of 70% chow and 30% coconut oil. The macronutrient compositions and energy densities of these diets are displayed in Table 1. All rats were allowed *ad libitum* access to their assigned diet for three weeks before euthanasia.

Table 1. Diet Compositions

Diet Type	% LCT	% MCT	% Carbohydrate	% Protein	Energy Density (kcal/g)
CHOW	18	0	58	24	3.10
HFD-LCT	63	0	27	10	4.93
HFD-COCO	8	55	27	10	4.78
HFD-MCT	28	35	27	10	4.69

Energy Intake

Food intake was recorded daily using a portable digital scale (Scout Pro Sp2001, Ohaus Corperation, Parsippany, NJ) as change in chow hopper weight for CHOW subjects, and as change in food cup weight for HFD subjects, after accounting for spillage. Each subject's daily food intake was multiplied by the energy density of their respective diet to calculate daily energy intake, then summed over the course of the three-week dietary intervention to calculate total energy intake.

Body Weight and Body Fat

Body weight was recorded three times per week using the same digital scale mentioned above. To assess total body fat mass, rats were placed in a restraining tube and inserted into an EchoMRI-900 (EchoMRI LLC, Houston, TX) for approximately 90s. Total body fat mass was measured at baseline, then weekly for three weeks of dietary intervention. Adiposity was then calculated by subtracting each subject's initial body fat mass from their final body fat mass after the three-week dietary intervention.

Perfusion

Rats were deeply anesthetized with a lethal dose of a euthanasia solution (Euthasol®, 0.77 ml/kg) and perfused with ice-cold 1X phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4). The brain was removed and hippocampi were dissected. Hippocampi were then frozen with dry ice and stored at -80^{o}C until ready for western blot analysis.

Western Blot (WB)

Hippocampi were thawed on ice and then homogenized in ice-cold 1XPBS by passing the sample through progressively smaller diameter needles (21G, 25G, 27G, and 30G, respectively). Protein concentration of each homogenate was determined by Coomassie Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Samples were diluted with Laemmli buffer (0.25 M Tris, pH 6.8, 6% sodium dodecyl sulfate (SDS), 40% sucrose, 0.04% Bromophenol Blue, 200 mM Dithiothreitol), loaded onto duplicate 12% Tris-glycine gels, separated via SDS-PAGE, and transferred to polyvinylidene fluoride membranes (Cat# 88518, GE Healthcare, Chicago, IL).

Duplicate membranes were blocked with 5% nonfat milk diluted in 1XPBS+ 0.1% Triton at room temperature (RT) for one hour on a rocking platform, then incubated with two series of antibodies: series 1) anti-BDNF (1:200; sc-546; Santa Cruz Biotechnology, Dallas, TX, USA) at 4^{o}C overnight and HRP-linked secondary anti-rabbit (1:3K; ab205718; Abcam) at RT for one hour; series 2) primary antibody anti-beta-actin (1:5K; #3700S; Cell Signaling) at RT for one hour and HRP-linked anti-mouse (1:3K; ab205719; Abcam) at RT for one hour. After each antibody incubation, three washes in 1XPBS with 0.1% Tween were performed (series 1: 10 minutes/wash, series 2: 5 minutes/wash). Between each series of antibodies, membranes were stripped from primary antibodies using stripping buffer (25 mM glycine, pH 2.0, 10% SDS) for two hours at RT then washed in 1XPBS with 0.1% Tween twice for 10 minutes/wash. Immunoreactive bands were visualized using an enhanced chemiluminescence (ECL) substrate for detection of HRP (Thermo Scientific, 32106) and captured on Blue Autoradiography Film (BX57, MIDSCI, St. Louis, MO). When bound to BDNF, duplicate membranes were exposed to film for 1 hour before development, and when bound to beta-actin, duplicate membranes were exposed for four epochs that ranged from one second to three minutes before development. Developed films were scanned and images were analyzed using FIJI image processing software (Schindelin et al., 2012) to obtain raw optical density (OD) values. Each sample's raw OD for BDNF and beta-actin were averaged, respectively, across duplicate gels and exposure times to obtain a grand mean OD for each protein. The grand mean OD of BDNF was then sum-normalized and divided by the sum-normalized grand mean OD for beta-actin to obtain the relative OD (ROD) of BDNF.

Statistical Analysis

All statistical analyses were conducted with RStudio Version 1.4.1103 (©2009-2021 RStudio, PBC). Descriptive statistics and scatter plot matrices were obtained using the "psych" R package Version 2.1.9 (Reville, 2021). Regression and ANOVA models were obtained using the "stats" R package Version 4.0.3 (R Core Team, 2020). Bar graphs were obtained using the "tidyverse" R package Version 1.3.1 (Wickham et al., 2019). Tables of regression and ANOVA models were obtained using the "apaTables" R package Version 2.0.8 (Stanley et al., 2021). Causal mediation analyses were performed using the "mediation" R package version 4.5.0 (Tingley et al., 2014). Effect sizes were obtained using the "lsr" R package Version 0.5.2 (Navarro, 2015). Power

analyses were conducted with GPower Version 3.1.9.7 (Faul et al., 2007; Faul et al., 2009). All significance tests set $\alpha = 0.05$.

Results

Assumptions

Descriptive statistics of these data are displayed in Table 2. Normality and homoskedasticity of adiposity, energy intake, and BDNF were tested using Shapiro-Wilks Test and Bartlett's Test for Homogeneity of Variances. Energy intake was normally distributed (W = 0.98, p = .850) and homoscedastic (K^2 = 7.76, df = 7, p = .715) across the sexes and across diets; adiposity was normally distributed (W = 0.95, p = .159) and homoscedastic (K^2 = 7.76, df = 7, p = .354) across the sexes and across diets; and BDNF was normally distributed (W = 0.94, p = .092) and homoscedastic across the sexes and across diets (K^2 = 10.45, df = 7, p = .165). As illustrated in Figure 1, energy intake and adiposity were approximately linearly related and independent from one another. Therefore, the subsequent analyses are appropriate for these data.

Sex, Diet, Energy Intake, and Adiposity

To evaluate the relationship between sex, diet, energy intake, and adiposity, two hierarchical regressions were performed, followed by a bootstrapping procedure for causal mediation analysis.

The first hierarchical regression predicted energy intake from sex and diet. These results are displayed in Table 3. Sex independently predicted energy intake, $F(1, 29) = 4.38$, $p < .001$, R^2 = 0.49, such that males consumed more energy than females (t = 5.29, $p < .001$). Diet type predicted energy intake over and above sex, $F(3, 26) = 14.94$, $p < .001$, $\Delta R^2 = 0.32$, such that HFD-LCT (t = 5.041, $p < .001$), but not HFD-COCO (t = 1.89, p = .070) or HFD-MCT (t = -1.23, p = .230) consumed more energy than CHOW. Furthermore, HFD-COCO (t = -2.98, p = .006) and HFD-MCT (t = -6.27, $p < .001$) consumed less energy than HFD-LCT, and HFD-MCT consumed less energy than HFD-COCO (t = -3.07, p = .005).

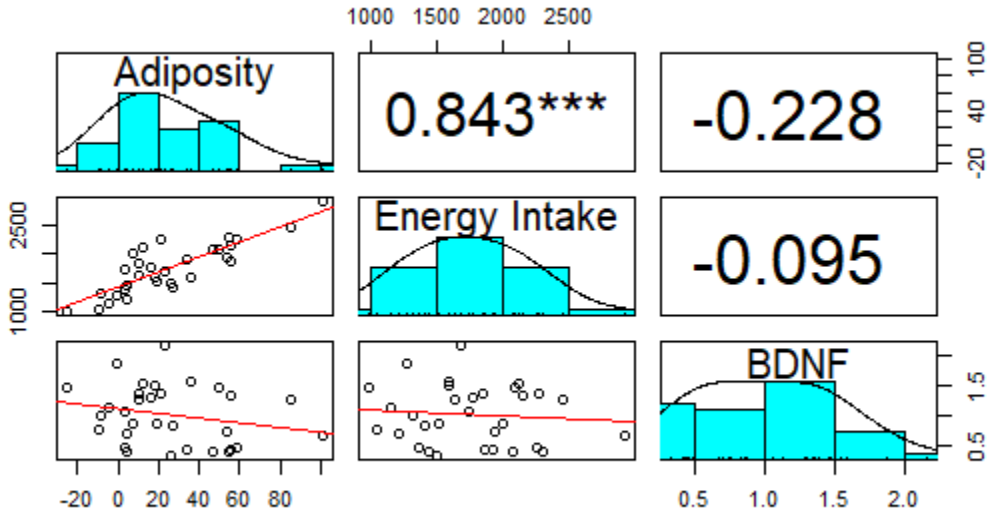
Table 2. Descriptive Statistics

	CHOW		HFD-LCT		HFD-COCO		HFD-MCT	
	Male	Female	Male	Female	Male	Female	Male	Female
Initial body weight (g)	549.32	304.32	544.85	305.35	524.27	311.28	540.10	306.42
	±50.66	±20.48	±41.70	±22.98	±14.15	±29.73	±27.26	±18.25
Final body weight (g)	566.10	304.40	629.10	351.68	573.13	342.65	551.60	300.80
	±59.37	±19.04	±62.25	±34.42	±31.90	±49.21	±23.72	±12.20
Change in body weight (g)	16.77	0.07	84.25	46.33	48.87	31.38	11.50	-5.62
	±9.54	±16.41	±31.29	±18.66	±17.76	±19.67	±8.28	±11.96
Initial body fat (g)	92.21	64.90	91.83	67.08	79.19	72.35	81.63	61.29
	±45.83	±30.38	±31.46	±23.24	±9.59	±31.67	±21.83	±14.65
Final body fat (g)	105.76	60.17	167.09	106.32	124.57	104.57	94.06	56.84
	±51.16	±17.09	±35.30	±37.05	±19.15	±45.70	±29.16	±8.89
Adiposity (g)	13.55	-4.73	75.25	39.24	45.38	32.22	12.43	-4.45
	±5.44	±14.20	±21.96	±16.37	±10.65	±16.10	±8.69	±6.23
Food intake (g)	635.43	409.58	504.00	365.90	417.00	320.28	383.32	251.05
	±90.80	±63.84	±61.84	±51.66	±16.79	±47.89	±29.39	±28.03
Energy intake (kcal)	1969.82	1269.68	2484.72	1803.89	2049.31	1575.93	1797.78	1185.63
	±281.49	±197.89	±304.89	±254.69	±118.34	±194.38	±137.85	±118.05

Table 2 continued

	CHOW		HFD-LCT		HFD-COCO		HFD-MCT	
	Male	Female	Male	Female	Male	Female	Male	Female
BDNF (relative optical density)	1.39	1.05	0.71	1.32	0.73	0.61	1.34	0.90
	±0.11	±0.73	±0.39	±0.39	±0.53	±0.27	±0.57	±0.20

Note. All values represent *mean* ± *SD*. *n* = 4/group. One HFD-COCO male died before study completion, and therefore this subject's data was excluded here and from further analyses.



Note. Pearson's correlation coefficients are located above the diagonal, histograms are located on the diagonal, and bivariate scatter plots are located below the diagonal and fit with a linear regression line.
* indicates $p < .05$. ** indicates $p < .01$. *** indicates $p < .001$.

Figure 1. Scatter plot matrix of, adiposity, energy intake, and hippocampal BDNF.

Table 3. Hierarchical Regression Results Using Energy Intake as the Criterion

Predictor	*b*	*b* 95% CI [LL, UL]	sr^2	Fit	Difference
Step 1:					
(Intercept)	1458.78***	[1292.51, 1625.05]			
Sex	618.36***	[379.34, 857.39]	.49		
					R^2 = .49***
Step 2(a):					
(Intercept)	1308.79***	[1139.07, 1478.50]			
Sex	621.93***	[467.91, 775.95]	.50		
HFD-LCT	524.55***	[310.67, 738.44]	.18	Reference: CHOW	
HFD-COCO	203.48	[-18.19, 425.15]	.03		
HFD-MCT	-128.04	[-341.93, 85.84]	.01		
Step 2(b):					
(Intercept)	1883.34	[1663.62, 2003.06]			
Sex	621.93	[467.91, 775.95]	.50		
CHOW	-524.55	[-738.44, -310.67]	.18	Reference: HFD-LCT	
HFD-COCO	-321.07**	[-542.74, -99.41]	.06		
HFD-MCT	-652.60***	[-866.48, -438.71]	.28		

Table 3 continued

Predictor	*b*	*b* 95% CI [LL, UL]	sr^2	Fit	Difference
Step 2(c):					
(Intercept)	1512.26***	[1337.63, 1686.90]			
Sex	621.93***	[467.91, 775.95]	.50		
CHOW	-203.48	[-425.15, 18.19]	.03	Reference: HFD-COCO	
HFD-LCT	321.07**	[99.41, 542.74]	.06		
HFD-MCT	-331.52**	[-553.19, -109.86]	.07		
Step 2(d):					
(Intercept)	1180.74***	[1011.02, 1350.46]			
Sex	621.93***	[467.91, 775.95]	.50		
CHOW	128.04	[-85.84, 341.93]	.01	Reference: HFD-MCT	
HFD-LCT	652.60***	[438.71, 866.48]	.28		
HFD-COCO	331.52**	[109.86, 553.19]	.07		
					R^2 = .81*** ΔR^2 = .32***

Note. b represents the unstandardized regression weights. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval, respectively. sr^2 represents the semi-partial correlation squared. A significant *p*-value indicates the beta-weight and semi-partial correlation are significant.
* indicates p < .05. ** indicates p < .01. *** indicates p < .001.

The second hierarchical regression predicted adiposity from sex, diet, and energy intake. These results are displayed in Table 4. Sex independently predicted adiposity, $F(1, 29) = 27.99$, $p = .045$, $R^2 = 0.13$, such that males gained more body fat than females ($t = 2.09$, $p = .045$). Diet type predicted adiposity over and above sex, $F(3, 26) = 60.93$, $p < .001$, $\Delta R^2 = 0.67$, such that HFD-LCT ($t = 7.70$, $p < .001$) and HFD-COCO ($t = 4.92$, $p < .001$), but not HFD-MCT ($t = -0.06$, $p = .952$) gained more body fat than CHOW. Furthermore, HFD-COCO ($t = -2.51$, $p = .019$) and HFD-MCT ($t = -7.76$, $p < .001$) gained less body fat than HFD-LCT, and HFD-MCT gained less body fat than HFD-COCO ($t = -4.98$, $p < .001$). Energy intake uniquely predicted adiposity over and above sex and diet type, $F(1, 25) = 28.71$, $p < .001$, $\Delta R^2 = 0.11$, such that more energy consumed resulted in greater body fat gain ($t = 5.36$, $p < .001$). As illustrated in Figure 2, the effect of sex on adiposity was eliminated after controlling for energy intake, suggesting complete mediation. This finding was supported by bootstrapping for causal mediation analysis. Furthermore, the effect of each significant diet type comparison on adiposity was reduced but not eliminated after controlling for energy intake, suggesting partial mediation. These findings were supported by bootstrapping for causal mediation analysis for all diet comparisons except HFD-COCO relative to CHOW.

Table 4. Hierarchical Regression Results Using Adiposity as the Criterion

Predictor	*b*	*b* 95% CI [LL, UL]	sr^2	Fit	Difference
Step 1:					
(Intercept)	15.57**	[1.63, 29.51]			
Sex	20.50*	[0.46, 40.54]	.13		
					R^2 = .13*
Step 2(a):					
(Intercept)	-6.28	[-17.47, 4.91]			
Sex	21.38***	[11.22, 31.53]	.14		
HFD-LCT	52.84***	[38.73, 66.94]	.45	Reference: CHOW	
HFD-COCO	34.98***	[20.36, 49.60]	.18		
HFD-MCT	-0.42	[-14.52, 13.68]	.00		
Step 2(b):					
(Intercept)	46.56***	[35.37, 57.75]			
Sex	21.38***	[11.22, 31.53]	.14		
CHOW	-52.84***	[-66.94, -38.73]	.45	Reference: HFD-LCT	
HFD-COCO	-17.86*	[-32.47, -3.24]	.05		
HFD-MCT	-53.26***	[-67.36, -39.15]	.46		

Table 4 continued

Predictor	*b*	*b* 95% CI [LL, UL]	sr^2	Fit	Difference
Step 2(c):					
(Intercept)	28.70***	[17.19, 40.22]			
Sex	21.38***	[11.22, 31.53]	.14		
CHOW	-34.98***	[-49.60. -20.36]	.18	Reference: HFD-COCO	
HFD-LCT	17.86*	[3.24, 32.47]	.05		
HFD-MCT	-35.40***	[-50.01, -20.78]	.19		
Step 2(d):					
(Intercept)	-6.70	[-17.89, 4.50]			
Sex	21.38***	[11.22, 31.53]	.14		
CHOW	0.42	[-13.68, 14.52]	.00	Reference: HFD-MCT	
HFD-LCT	53.26***	[39.15, 67.36]	.46		
HFD-COCO	35.40***	[20.78, 50.01]	.19		
Step 3(a):					
(Intercept)	-69.37***	[-94.85, -43.89]			
Sex	-8.61	[-22.13, 4.92]	.01		
HFD-LCT	27.55***	[13.72, 41.37]	.06	Reference: CHOW	

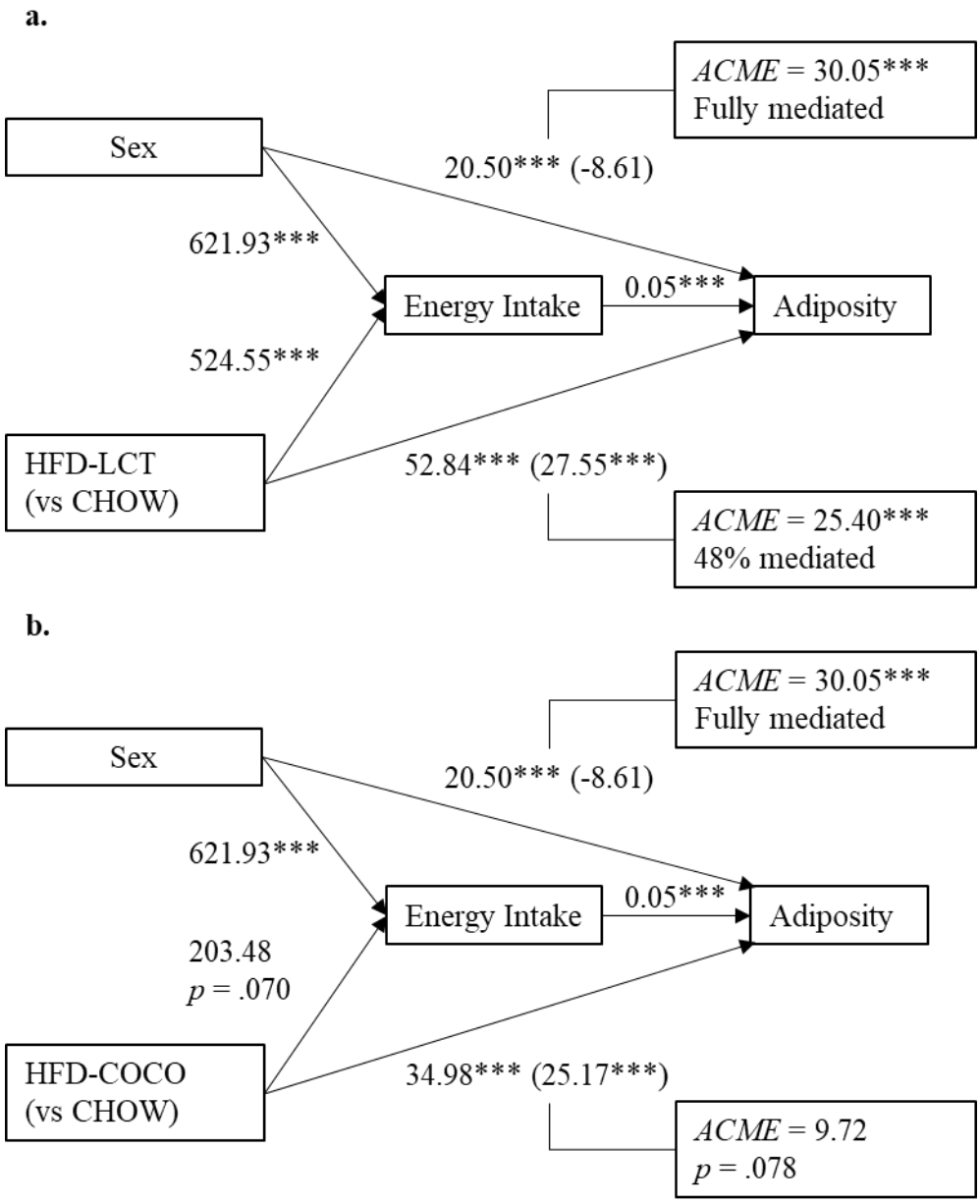
Table 4 continued

Predictor	*b*	*b* 95% CI [LL, UL]	sr^2	Fit	Difference
HFD-COCO	25.17***	[14.30, 36.03]	.08		
HFD-MCT	5.75	[-4.36, 15.57]	.01		
Energy Intake	0.05***	[0.03, 0.07]	.11		
Step 3(b):					
(Intercept)	-41.82*	[-76.68, -6.97]			
Sex	-8.61	[-22.13, 4.92]	.01		
CHOW	-27.55***	[-41.37, -13.72]	.06	Reference: HFD-LCT	
HFD-COCO	-2.38	[-14.18, 9.42]	.00		
HFD-MCT	-21.79**	[-37.38, -6.21]	.03		
Energy Intake	0.05***	[0.03, 0.07]	.11		
Step 3(c):					
(Intercept)	-44.20**	[-73.35, -15.05]			
Sex	-8.61	[-22.13, 4.92]	.01		
CHOW	-25.17***	[-36.03, 14.18]	.08	Reference: HFD-COCO	
HFD-LCT	2.38	[-9.42, 14.18]	.00		
HFD-MCT	-19.42**	[-31.31, -7.52]	.04		

Table 4 continued

Predictor	*b*	*b* 95% CI [LL, UL]	sr^2	Fit	Difference
Energy Intake	0.05***	[0.03, 0.07]	.11		
Step 3(d):					
(Intercept)	21.13**	[7.89, 34.37]		Reference: HFD-MCT	
Sex	-8.61	[-22.13, 4.92]	.01		
CHOW	-5.75	[-15.87, 4.36]	.01		
HFD-LCT	21.79**	[6.21, 37.38]	.03		
HFD-COCO	19.42**	[7.52, 31.31]	.04		
Energy Intake	0.05***	[0.03, 0.07]	.11		
				R^2 = .91***	ΔR^2 = .10***

Note. b represents unstandardized regression weights. *LL* and *UL* indicate the lower and upper limits of the 95% confidence interval, respectively. sr^2 represents the semi-partial correlation squared. A significant *p-value* indicates the beta-weight and semi-partial correlation are significant.
* indicates $p < .05$. ** indicates $p < .01$. *** indicates $p < .001$.



Note. The path analyses show associations between each diet type comparison and adiposity, and between sex and adiposity, as mediated by energy intake. All values represent unstandardized linear regression coefficients unless specified otherwise. In parentheses are the coefficients that correspond to the model while controlling for energy intake. *ACME* indicates the bootstrapped average causal mediation effect, i.e. the indirect effect, through energy intake. * indicates $p < .05$. ** indicates $p < .01$. *** indicates $p < .001$.

Figure 2. Path analysis models of associations between each diet type comparison and adiposity.

Figure 2 continued

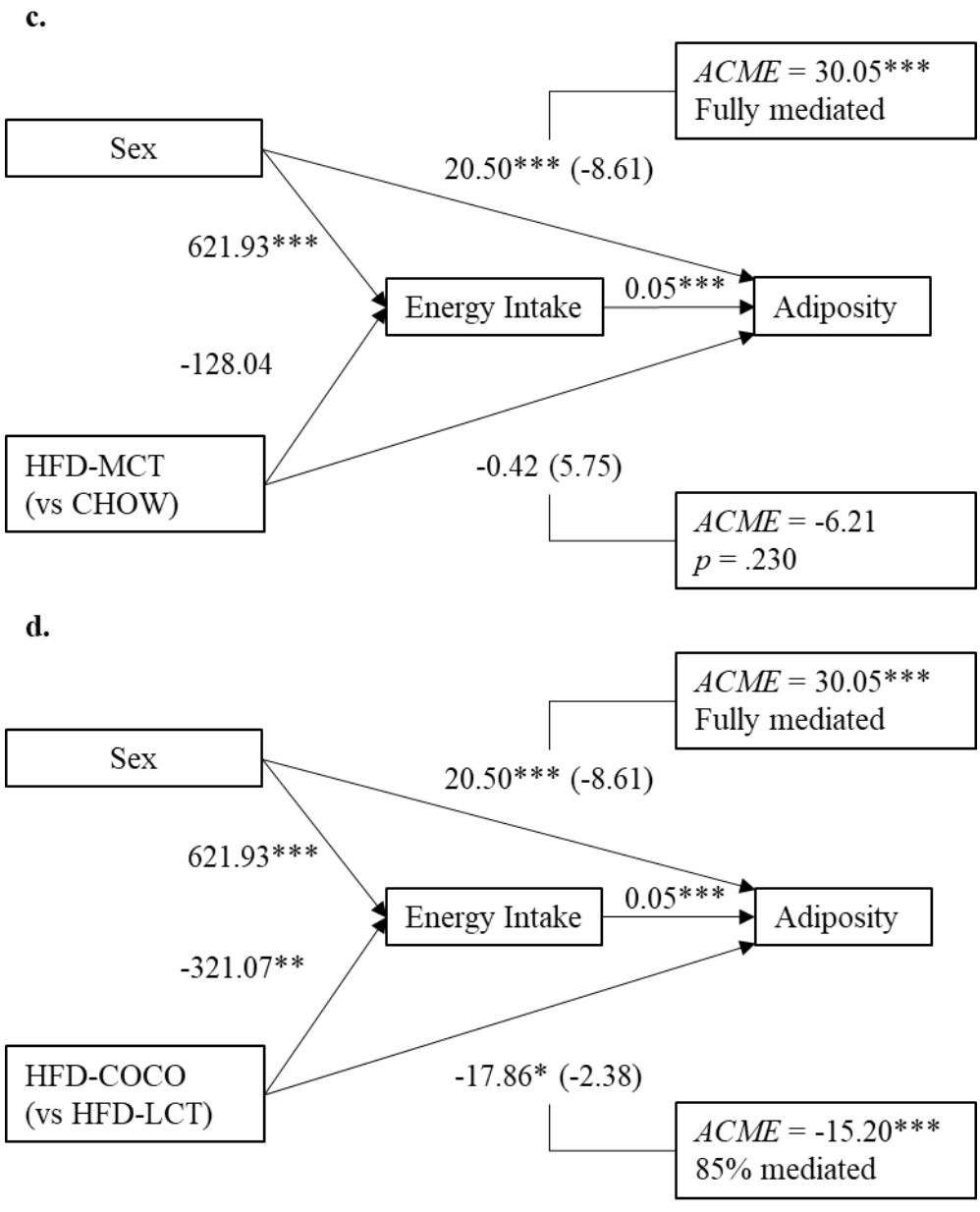
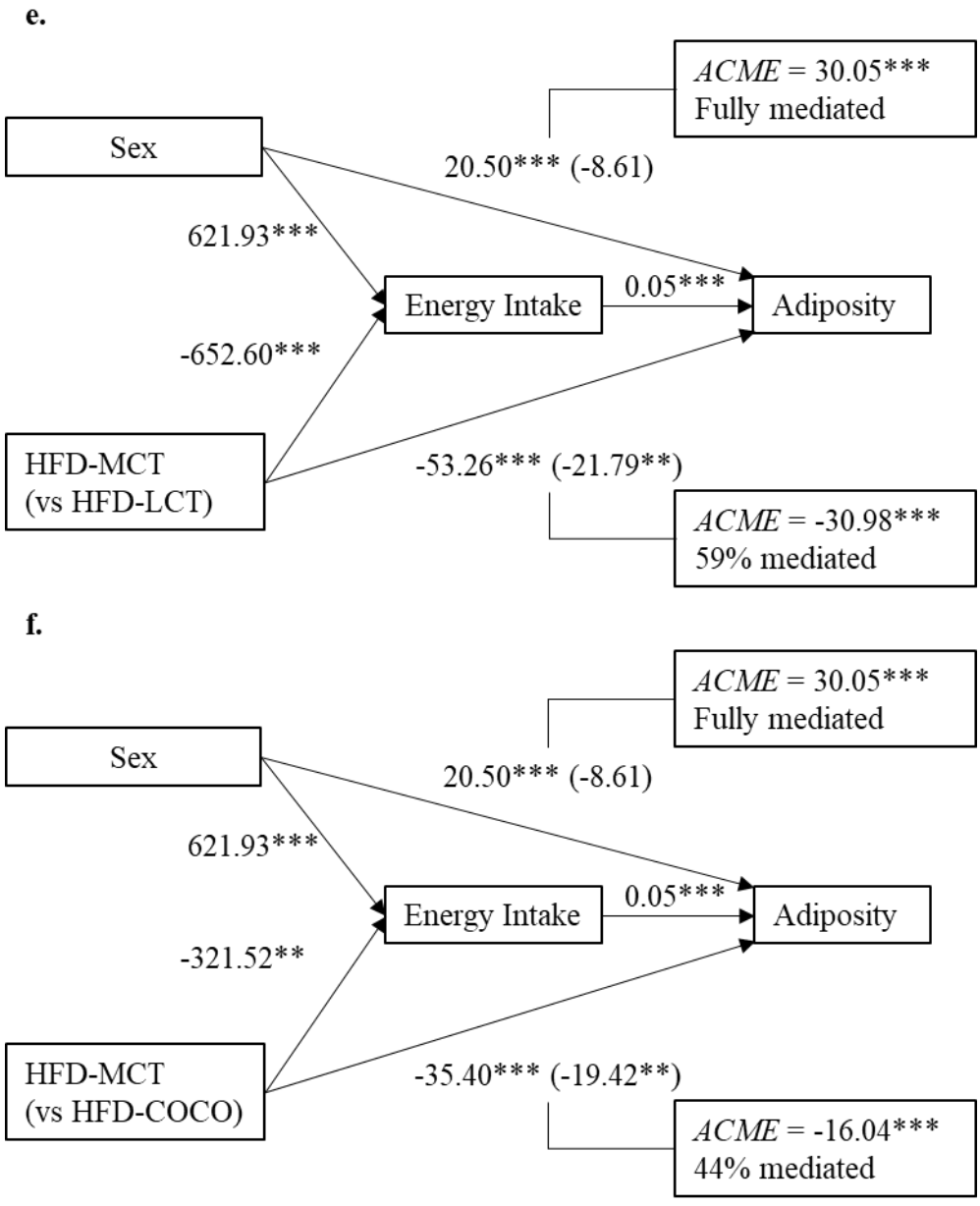


Figure 2 continued



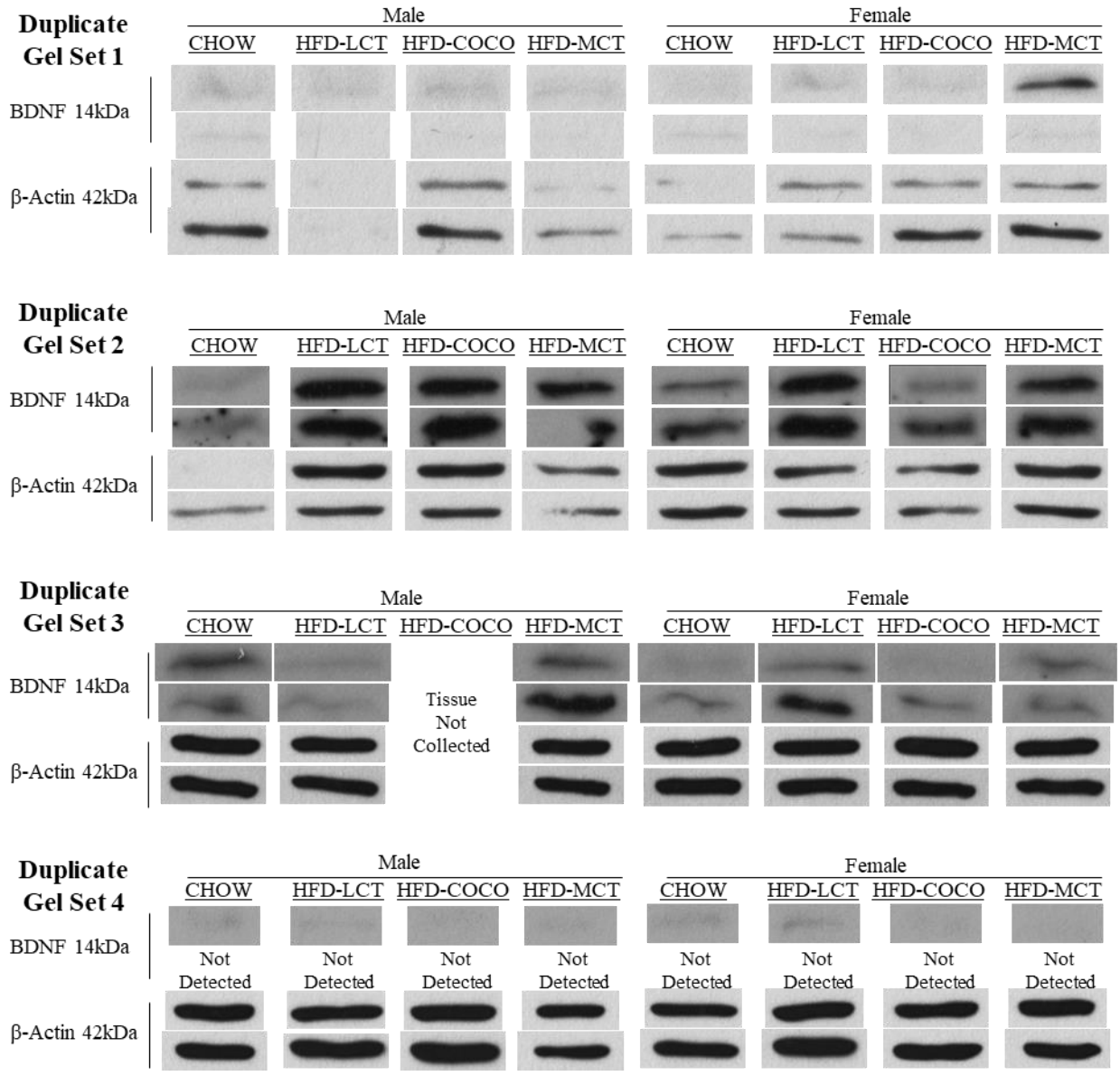
BDNF

To explore the effects of sex and diet on hippocampal BDNF, a two-way between-subjects ANOVA was performed. These results are illustrated in Figure 3. There was not a significant main effect of sex, $F(1, 23) = 0.37$, $p = .557$, $\eta_p^2 = 0.09$, diet type, $F(3, 23) = 2.17$, $p = .120$, $\eta_p^2 = 0.22$, or an interaction, $F(3, 23) = 2.32$, $p = .102$, $\eta_p^2 = 0.23$. These results indicate that different HFD consumption has no effect on hippocampal BDNF.

Discussion

This experiment aimed to examine the relationship between different HFD compositions, hyperphagia, and adiposity in an *ad libitum* environment. These results are consistent with previous findings demonstrating that *ad libitum* consumption of a HFD composed of LCT induces hyperphagia and adiposity (Hariri & Thibault, 2010). In addition, these data provide evidence that the two outcomes are causally related, such that HFD-induced adiposity is partially mediated by hyperphagia. Furthermore, these results are consistent with previous findings demonstrating that the replacement of LCT with MCT suppresses HFD-induced hyperphagia and adiposity (Ferreira et al., 2014). In addition, these data provide evidence that the two outcomes are causally related, such that the effect of MCT consumption on suppressing HFD-induced adiposity is partially mediated by suppressed hyperphagia.

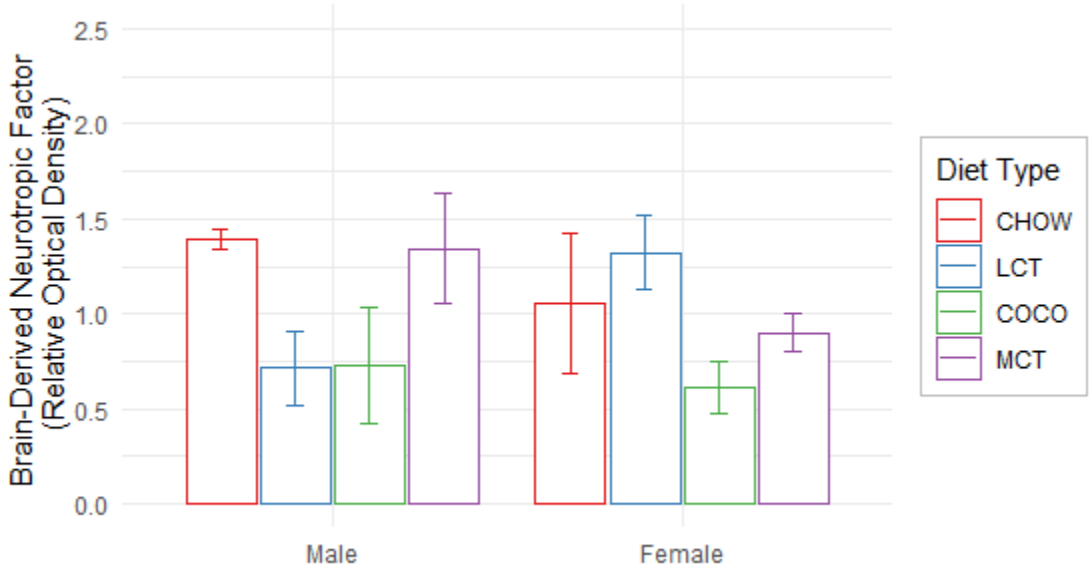
This experiment also aimed to explore the effects of different HFD compositions on BDNF levels in the hippocampus. We failed to detect an effect of HFD consumption on hippocampal BDNF, and therefore our findings did not support or oppose the central hypothesis that MCT consumption attenuates HFD-induced BDNF deficiency. However, the effect sizes were large and in the expected direction, so it is likely that the experiment was underpowered. Future studies should therefore increase sample size to adequately test this hypothesis.



Note. Western blot analysis of Hippocampal BDNF. Error bars represent standard error measurements.

Figure 3. Hippocampal BDNF grouped by sex and diet type.

Figure 3 continued



EXPERIMENT 2

Method

Subjects

30 adult male (n = 18) and female (n = 12) Long Evans rats aged 24 weeks were housed in the same conditions and given the same chow diet as Experiment 1. All procedures were approved by the Purdue Animal Care and Use Committee (PACUC).

Dietary Intervention

Subjects were given the same diet assignments as in Experiment 1. However, here, only CHOW subjects were allowed *ad libitum* access to diet. All HFD subjects were pair-fed to the chow group to prevent overeating (Ellacott et al., 2010). Caloric allowance for each animal assigned to a HFD group was matched to the average caloric intake of the chow group on the previous day, adjusting for body weight. All animals were exposed to their assigned diet for a total of three weeks before euthanasia

Energy Intake

Food intake and energy intake were recorded and calculated as in Experiment 1.

Body Weight and Body Fat

Body weight, body fat, and adiposity were recorded and calculated as in Experiment 1, except body weight was measured daily instead of thrice per week.

Perfusion

Subjects were perfused and hippocampi were extracted and processed as in Experiment 1.

Western Blot (WB)

BDNF was semi-quantified with the same WB procedure as in Experiment 1.

Statistical Analysis

All statistical analyses were conducted with the same statistical software and packages as Experiment 1. All significance tests set $\alpha = 0.05$.

Results

Assumptions

Descriptive statistics of these data are displayed in Table 5. Normality and homoskedasticity of energy intake, adiposity, and BDNF were tested using Shapiro-Wilks Test and Bartlett's Test for Homogeneity of Variances, respectively. Energy intake was not normally distributed ($W = 0.90$, $p = .011$), but instead appeared bimodal, each peak likely corresponding to a sex. This is illustrated in Figure 4. Therefore, normality and homoskedasticity of male and female data were tested separately. Energy intake was normally distributed (Male: $W = 0.96$, $p = .619$; Female: $W = 0.94$, $p = .514$) and homoscedastic (Male: $K^2 = 1.61$, $df = 3$, $p = .658$; Female: $K^2 = 0.78$, $df = 3$, $p = .855$) across diet groups; adiposity was normally distributed (Male: $W = 0.95$, $p = .424$; Female: $W = 0.99$, $p = .999$) and homoscedastic (Male: $K^2 = 0.75$, $df = 3$, $p = .860$; Female: $K^2 = 1.35$, $df = 3$, $p = .712$) across diet groups; and BDNF was normally distributed (Male: $W = 0.92$, $p = .144$; Female: $W = 0.97$, $p = .938$) and homoscedastic (Male: $K^2 = 2.33$, $df = 3$, $p = .508$; Female: $K^2 = 1.90$, $df = 3$, $p = .595$) across diet groups. Therefore, the subsequent analyses performed on male and female data separately are appropriate.

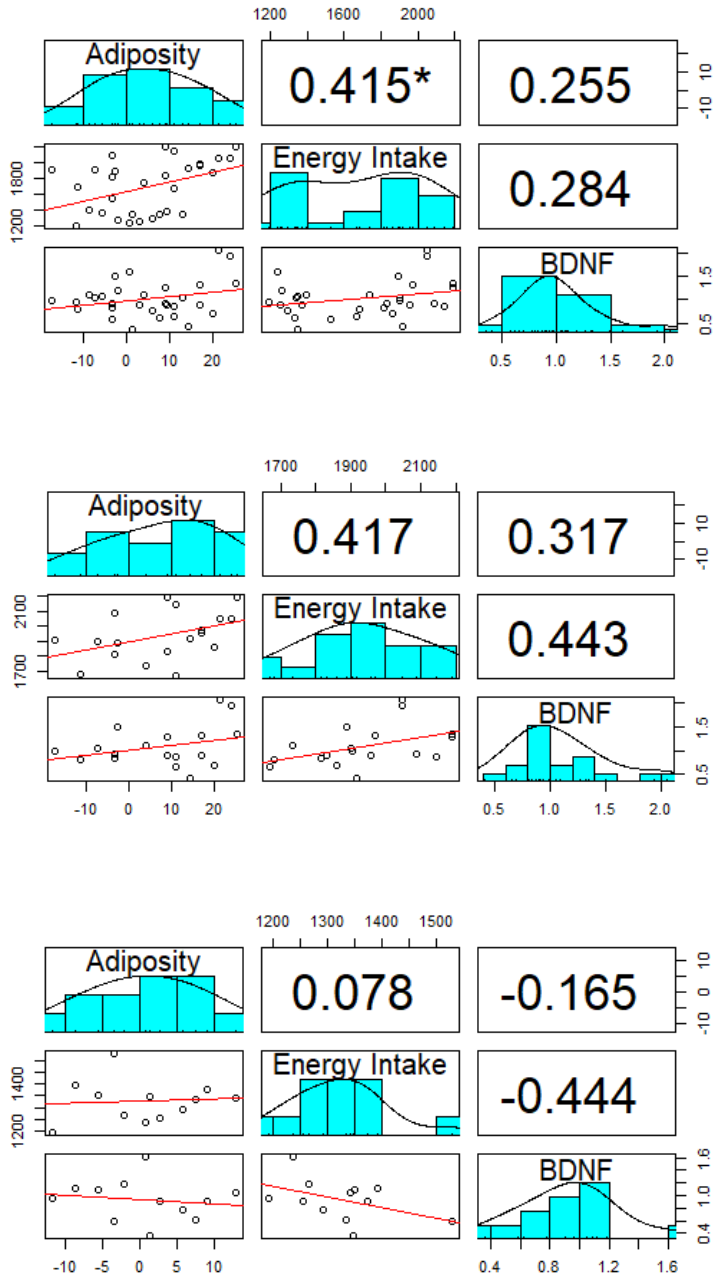
Table 5. Descriptive Statistics

	CHOW		HFD-LCT		HFD-COCO		HFD-MCT	
	Male	Female	Male	Female	Male	Female	Male	Female
Group size (*n*)	4	3	5	3	4	3	5	3
Initial body weight (g)	554.75	297.20	544.78	278.57	529.28	292.03	546.60	284.80
	±32.41	±49.90	±45.75	±15.22	±34.39	±25.16	±35.14	±16.18
Final body weight (g)	559.28	301.83	557.44	283.27	532.58	298.90	548.18	297.00
	±35.96	±44.59	±42.84	±31.45	±47.40	±17.04	±40.25	±11.26
Change in body weight (g)	4.53	4.63	12.66	4.70	3.30	6.87	1.58	12.20
	±11.65	±5.43	±8.51	±18.07	±14.81	±9.03	±20.73	±8.64
Initial body fat (g)	73.48	48.95	71.91	57.13	69.99	56.92	90.90	60.68
	±25.49	±12.32	±19.56	±17.71	±22.27	±7.77	±6.10	±12.58
Final body fat (g)	83.48	49.03	84.85	57.98	83.31	56.96	86.78	62.79
	±28.30	±6.81	±14.35	±29.74	±29.54	±5.40	±13.87	±17.38
Adiposity (g)	10.01	0.08	12.94	0.85	13.31	0.05	-4.12	2.11
	11.76	±7.96	±12.40	±12.33	±7.62	±7.61	±12.05	±4.94
Food intake (g)	669.45	457.53	402.20	262.27	388.20	274.77	392.52	272.73
	54.30	±31.57	±23.21	±17.90	±38.04	±15.10	±21.00	±10.61
Energy intake (kcal)	2075.30	1418.35	1982.85	1292.97	1885.13	1313.05	1840.92	1279.12
	±168.34	±97.86	±114.44	±88.27	±181.78	±72.18	±98.49	±49.75

Table 5 continued

	CHOW		HFD-LCT		HFD-COCO		HFD-MCT	
	Male	Female	Male	Female	Male	Female	Male	Female
BDNF (relative optical density)	1.12	0.86	1.09	0.79	1.28	0.93	0.91	1.14
	±0.22	±0.25	±0.49	±0.38	±0.58	±0.17	±0.39	±0.40

Note. All values represent *mean* ± *SD*. *n* = 3-5/group.

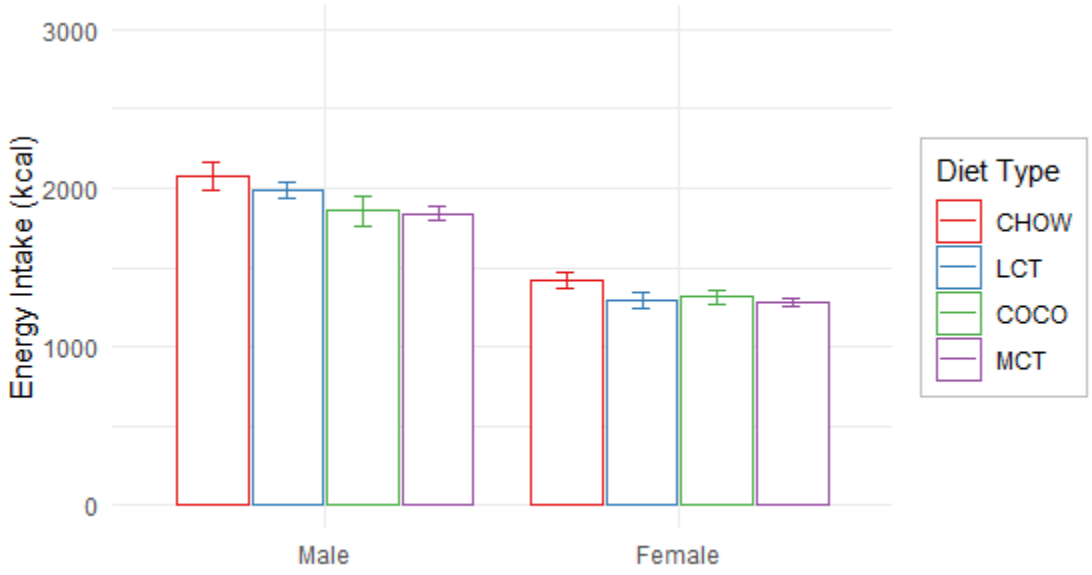


Note. Pearson's correlation coefficients are located above the diagonal, histograms are located on the diagonal, and bivariate scatter plots are located below the diagonal and fit with a linear regression line. The top plot uses male and female data combined, the middle plot uses only male data, and the bottom plot uses only female data.
* indicates $p < .05$; ** indicates $p < .01$; *** indicates $p < .001$.

Figure 4. Scatter plot matrix of adiposity, energy intake, and hippocampal BDNF.

Energy Intake

To evaluate the effectiveness of the pair-feeding protocol used in this sample, we performed a one-way between-subjects ANOVA of energy intake with diet as the factor. These results are illustrated in Figure 5. There was not a significant effect of diet on energy intake in males, $F(3, 14) = 2.71$, $p = .085$, $\eta^2 = 0.37$, or females, $F(3, 8) = 1.91$, $p = .206$, $\eta^2 = 0.42$. These results suggest that the pair-feeding protocol was effective at prohibiting hyperphagia across diet groups.

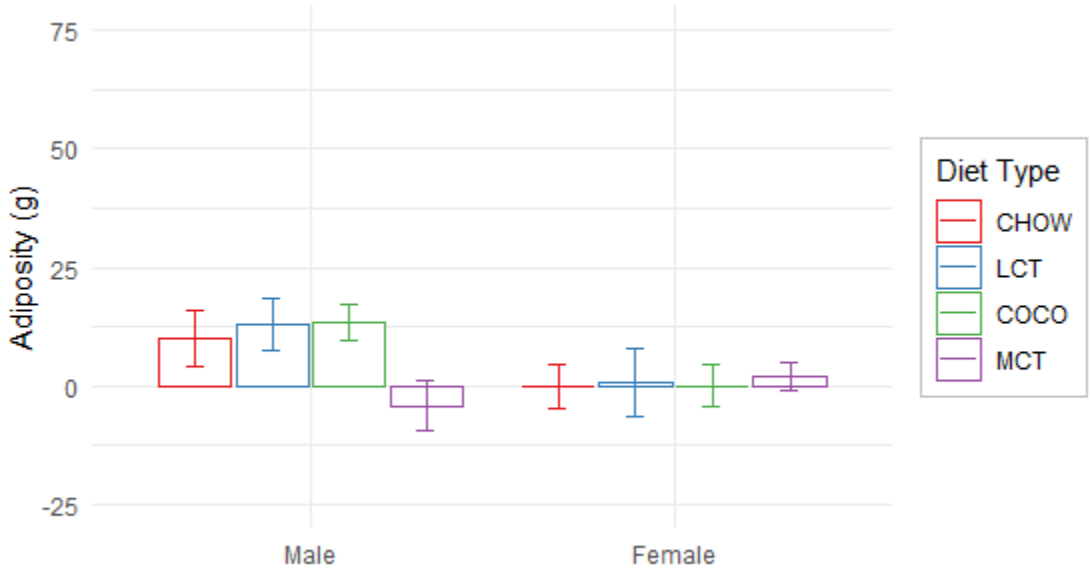


Note. Energy intake of males and females are displayed for each diet type. Error bars represent standard error measurements.

Figure 5. Energy intake grouped by sex and diet type.

Adiposity

To evaluate the effect of different HFD consumption on adiposity under the pair-feeding protocol used in this sample, we performed a one-way between-subjects ANOVA of adiposity with diet as the factor. These results are illustrated in Figure 6. There was not a significant effect of diet on adiposity in males, $F(3, 14) = 2.57$, $p = .096$, $\eta_p^2 = 0.36$, or females, $F(3, 8) = 2.81$, $p = .989$, $\eta_p^2 = 0.01$. These results suggest that different HFD consumption has no effect on adiposity when hyperphagia is prohibited.

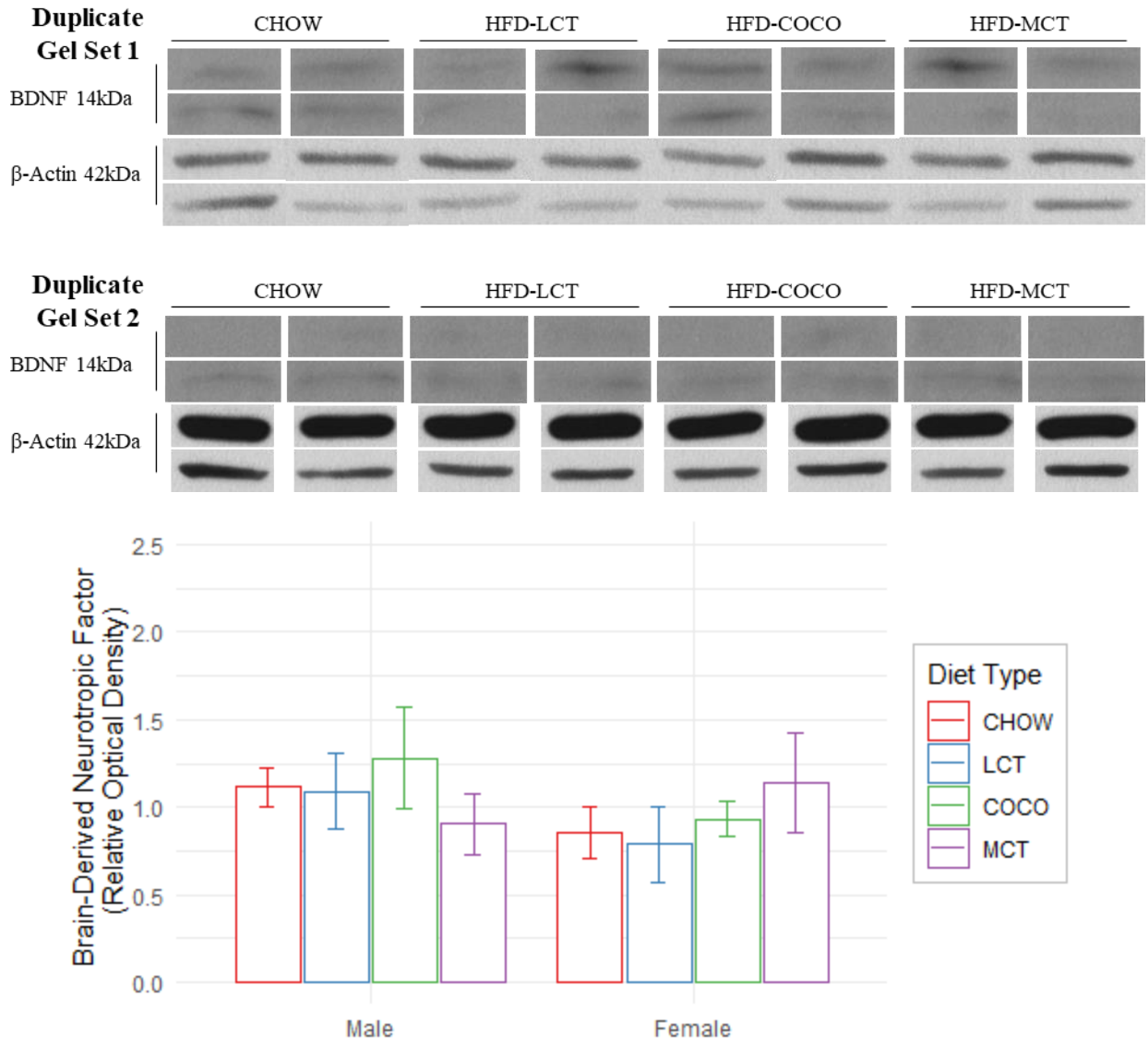


Note. Adiposity of males and females are displayed for each diet type. Error bars represent standard error measurements.

Figure 6. Adiposity grouped by sex and diet type.

BDNF

To explore the effect of different HFD consumption on hippocampal BDNF under the pair-feeding protocol used in this sample, we performed a one-way between-subjects ANOVA of BDNF with diet as the factor. These results are illustrated in Figure 7. There was not a significant effect of diet on BDNF in males, $F(3, 14) = 0.54$, $p = .661$, $\eta^2 = 0.10$, or females, $F(3, 8) = 0.57$, $p = .648$, $\eta_p^2 = 0.18$. These results suggest that different HFD consumption has no effect on hippocampal BDNF when hyperphagia is prohibited.



Note. Western Blot Analysis of Hippocampal BDNF. Error bars represent standard error measurements.

Figure 7. Hippocampal BDNF grouped by sex and diet type.

Discussion

This experiment aimed to extend the findings from Experiment 1 by examining the effect of consuming different HFD mixtures on adiposity when hyperphagia is prohibited. We predicted the effect of HFD-induced adiposity to be reduced. However, HFD-induced adiposity was undetected in this sample. Therefore, these results suggest that hyperphagia is necessary for HFD-induced adiposity. However, although insignificant, the effect size of diet on adiposity in males, but not females, was large and in the expected direction. Therefore, it is likely that this experiment was underpowered. Future studies investigating residual HFD-induced adiposity when hyperphagia is eliminated should therefore increase sample size. Moreover, although the effect of diet on energy intake was insignificant, the effect size was large in both males and females, suggesting that all HFD groups may consume less energy than the control group when pair-fed, possibly due to increased but undetected spillage. Therefore, insignificant reductions in energy intake by HFD groups may have suppressed HFD-induced adiposity beyond what was expected. Future studies that investigate the effects of these diet mixtures may want to adjust the pair-feeding protocol or the spillage collection method.

This experiment also aimed to explore the effects of different HFD compositions on BDNF levels in the hippocampus when hyperphagia is eliminated. We failed to detect an effect of HFD consumption on hippocampal BDNF, and therefore our findings did not support or oppose the central hypothesis that MCT consumption attenuates HFD-induced BDNF deficiency.

EXPERIMENT 3

Method

Subjects

Adult female (N = 16) Long Evans rats aged 10-11 weeks were housed in the same conditions and given the same chow diet as Experiment 2. All procedures were approved by the Purdue Animal Care and Use Committee (PACUC).

Dietary Intervention

Subjects were given the same diet assignments and subjected to the same pair-feeding protocol as in Experiment 2.

Energy Intake

Food intake and energy intake were recorded and calculated as in Experiment 2.

Body Weight and Body Fat

Body weight, body fat, and adiposity were recorded and calculated as in Experiment 2.

Perfusion

Subjects were perfused and hippocampi were extracted and processed as in Experiment 2.

Western Blot (WB)

BDNF was semi-quantified with the same WB procedure as in Experiment 2.

Statistical Analysis

All statistical analyses were conducted with the same statistical software and packages as Experiment 2. All significance tests set $\alpha = 0.05$.

Results

Assumptions

Descriptive statistics of these data are displayed in Table 6. Normality and homoskedasticity of energy intake, adiposity, and BDNF were tested using Shapiro-Wilks Test and Bartlett's Test for Homogeneity of Variances, respectively. Energy intake was normally distributed ($W = 0.89$, $p = .063$), and homoscedastic ($K^2 = 0.35$, $df = 3$, $p = .951$) across diet groups; adiposity was normally distributed ($W = 0.95$, $p = .542$) and homoscedastic ($K^2 = 2.63$, $df = 3$, $p = .452$) across diet groups; and BDNF was normally distributed ($W = 0.94$, $p = .386$) and homoscedastic ($K^2 = 0.16$, $df = 3$, $p = .985$) across diet groups. These assumptions are visualized in Figure 8. Therefore, the subsequent analyses are appropriate for these data.

Energy Intake

To evaluate the effectiveness of the pair-feeding protocol used in this sample, we performed a one-way between-subjects ANOVA of energy intake with diet as the factor. These results are illustrated in Figure 9. There was not a significant effect of diet on energy intake, $F(3, 12) = 1.29$, $p = .323$, $\eta_p^2 = 0.24$. These results suggest that the pair-feeding protocol was effective at prohibiting hyperphagia across diet groups.

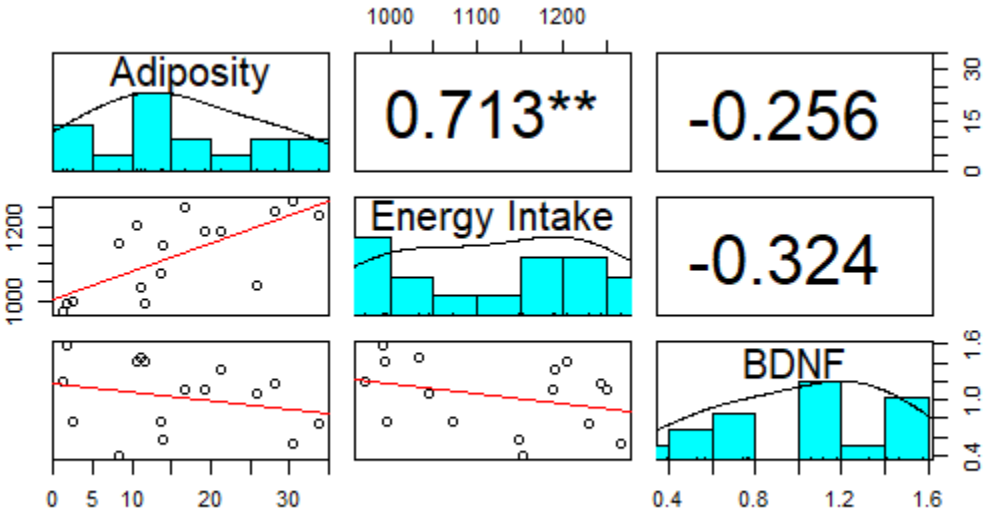
Adiposity

To evaluate the effect of different HFD consumption on adiposity under our pair-feeding protocol, we performed a one-way between-subjects ANOVA of adiposity with diet as the factor. These results are illustrated in Figure 10. There was not a significant effect of diet on adiposity, $F(3, 12) = 2.22$, $p = .138$, $\eta_p^2 = 0.36$. These results suggest that different HFD consumption has no effect on adiposity when hyperphagia is prohibited.

Table 6. Descriptive Statistics

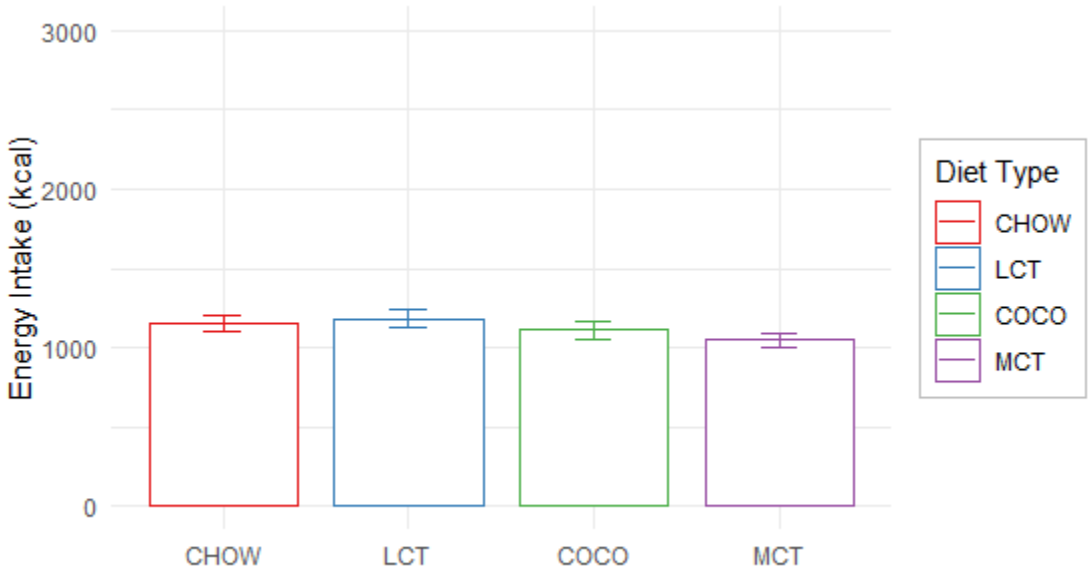
	CHOW	HFD-LCT	HFD-COCO	HFD-MCT
Initial body weight (g)	232.35	231.07	230.48	229.25
	±19.14	±16.04	±14.77	±15.28
Final body weight (g)	252.05	256.88	250.55	239.77
	±19.84	±27.49	±24.47	±17.23
Change in body weight (g)	19.70	25.80	20.08	10.52
	±10.46	±12.24	±9.95	±5.72
Initial body fat (g)	27.45	29.55	27.71	25.68
	±7.29	±5.84	±3.22	±4.01
Final body fat (g)	37.03	52.27	47.85	35.78
	±12.37	±14.14	±16.52	±6.34
Adiposity (g)	9.58	22.72	20.13	10.10
	±5.81	±8.67	±13.60	±6.05
Food intake (g)	383.55	239.57	233.43	216.95
	±35.83	±20.40	±23.39	±15.76
Energy intake (kcal)	1151.18	1183.08	1113.22	1046.81
	±110.67	±104.64	±113.52	±80.83
BDNF (relative optical density)	0.92	1.12	1.12	0.98
	±0.44	±0.41	±0.35	±0.39

Note. All values represent *mean* ± SD. n = 4/group.



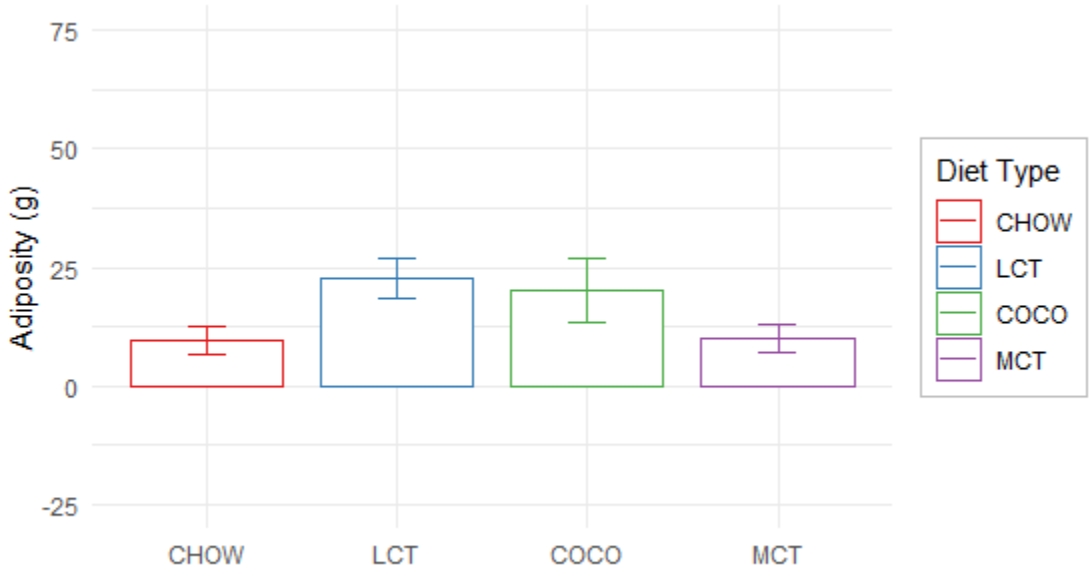
Note. Pearson's correlation coefficients are located above the diagonal, histograms are located on the diagonal, and bivariate scatter plots are located below the diagonal and fit with a linear regression line.
* indicates $p < .05$. ** indicates $p < .01$. *** indicates $p < .001$.

Figure 8. Scatter plot matrix of adiposity, energy intake, and BDNF.



Note. Energy intake is displayed for each diet type. Error bars represent standard error measurements.

Figure 9. Energy intake grouped by diet type.

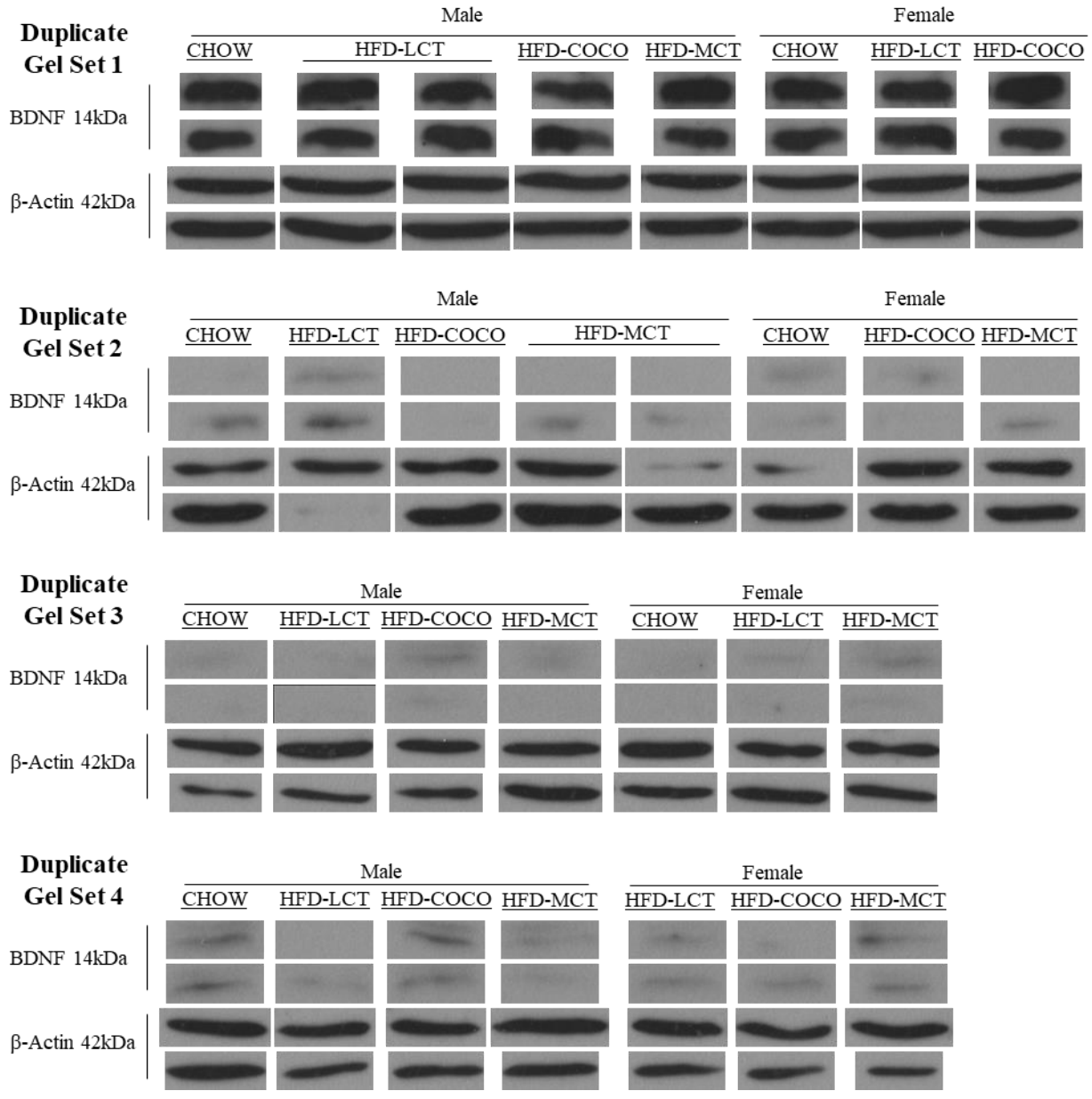


Note. Adiposity is displayed for each diet type. Error bars represent standard error measurements.

Figure 10. Adiposity grouped by diet type.

BDNF

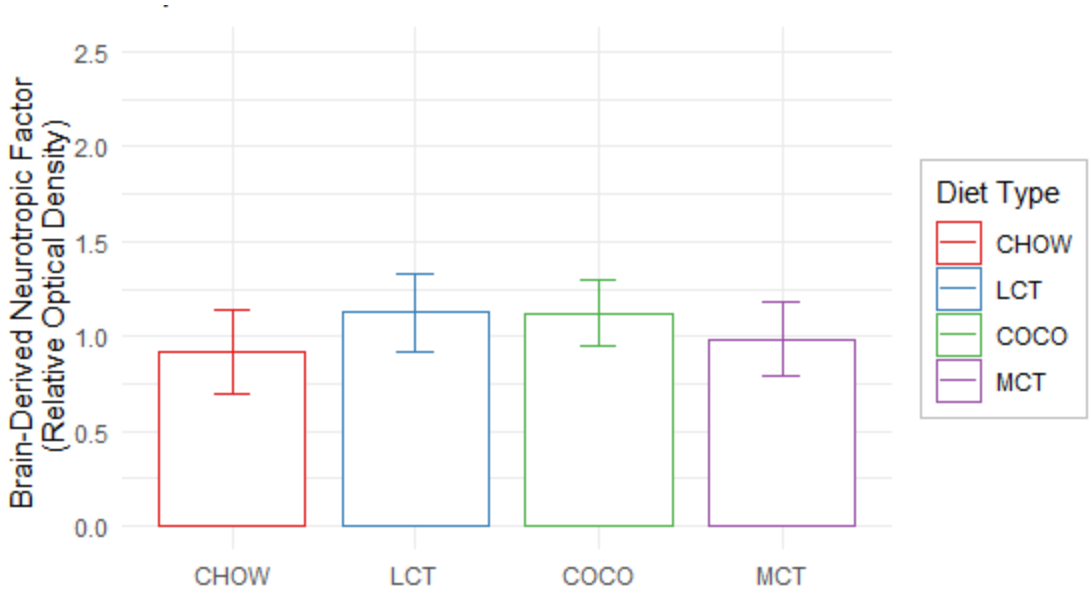
To explore the effect of different HFD consumption on hippocampal BDNF under the pair-feeding protocol used in this sample, we performed a one-way between-subjects ANOVA of BDNF with diet as the factor. These results are illustrated in Figure 11. There was not a significant effect of diet on BDNF, $F(3, 12) = 0.27$, $p = .843$, $\eta_p^2 = 0.06$. These results suggest that different HFD consumption has no effect on hippocampal BDNF when hyperphagia is prohibited.



Note. Western Blot Analysis of Hippocampal BDNF. Error bars represent standard error measurements.

Figure 11. Hippocampal BDNF grouped by diet type.

Figure 11 continued



Discussion

This experiment aimed to extend the findings from Experiment 2 by examining the effect of consuming different HFD mixtures on adiposity when hyperphagia is prohibited in a younger sample. Similar to Experiment 2, we predicted the effect of HFD-induced adiposity to be reduced. However, HFD-induced adiposity was undetected in this sample. Therefore, these results suggest that hyperphagia is necessary for HFD-induced adiposity. However, although insignificant, the effect size of diet on adiposity, was large and in the expected direction. Therefore, it is likely that this experiment was underpowered. Future studies investigating residual HFD-induced adiposity when hyperphagia is eliminated should therefore increase sample size.

This experiment also aimed to explore the effects of different HFD compositions on BDNF levels in the hippocampus when hyperphagia is prohibited in a younger sample. We failed to detect an effect of HFD consumption on hippocampal BDNF, and therefore our findings did not support or oppose the central hypothesis that MCT consumption attenuates HFD-induced BDNF deficiency.

GENERAL DISCUSSION AND CONCLUSIONS

In summary, the present experiments utilized different approaches to address two aims: first, to evaluate the relationships between MCT consumption, HFD-induced hyperphagia, and HFD-induced adiposity, and second, to explore the effects of MCT consumption on BDNF levels in the hippocampus. Regarding the first aim, these data support the existing literature by demonstrating that the replacement of LCT with MCT suppresses HFD-induced hyperphagia and adiposity. Furthermore, using both a statistical mediation model and an experimental pair-feeding manipulation, these data expand upon previous works by demonstrating that these effects are causally related to each other in both adult and young adult rats. However, a major limitation of these experiments, particularly Experiments 2 and 3, is small sample sizes. Future investigations attempting to replicate these findings should utilize larger sample sizes to appropriately evaluate these conclusions. Another major limitation of these experiments is that we did not measure energy expenditure. Other studies have demonstrated that MCT consumption suppresses HFD-induced adiposity without suppression of HFD-induced hyperphagia. For example, Murata et al. (2019) reported that MCT consumption suppresses HFD-induced adiposity in wild-type mice without concomitant suppression of energy intake, but rather with a concomitant increase in energy expenditure. Similarly, Rial et al. (2020) reported that MCT consumption both suppresses HFD-induced adiposity in lean mice and reduces adiposity in HFD-induced obese mice without concomitant differences in energy intake, but rather with concomitant improvements in metabolic health markers. Therefore, future studies are warranted that examine the causal relationships between MCT consumption, hyperphagia, decreased energy expenditure, and adiposity.

Regarding the second aim, we failed to detect an effect of HFD consumption on hippocampal BDNF in any of the three experiments. Therefore, our findings did not support or oppose the central hypothesis that MCT consumption attenuates HFD-induced BDNF deficiency. However, the effect size observed in experiment 1 was large and in the hypothesized direction, so it is likely that the experiment was underpowered. Some methodological limitations may also have contributed to these findings. We failed to detect reduced BDNF in our rats after 21 days of HFD, but Park et al. (2010) reported reduced BDNF in mice after 49 days of HFD, and Kanoski et al. (2007) reported reduced BDNF in rats after 90 days of HFD. Therefore, perhaps our dietary intervention was too short to noticeably reduce BDNF. Additionally, Kanoski et al. (2007) reported

that BDNF is reduced in the ventral, but not dorsal, hippocampus. Therefore, we may have diluted the effect of HFD on reducing BDNF by measuring in the whole hippocampus. Future studies should consider increasing sample size, increasing the length of HFD exposure, and/or measuring BDNF only in the ventral hippocampus to adequately test this hypothesis.

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