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Chronic junk food intake and abstinence: sex-specific effects on gene expression in the mesolimbic reward system

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Abstract

Studies examining the impact of chronic palatable food intake on the mesolimbic reward system have been conducted almost exclusively in males. This study aimed to compare the effects of chronic junk food intake and subsequent abstinence between males and females on fat mass, food intake and key gene expression of the mesolimbic reward system. Albino Wistar rats were fed for 8 weeks on standard chow (Control, n=5 males, 5 females) or junk foods (JF; n=16 males, 16 females). Junk food was then removed from a subset of JF rats for 72 hours (JF-Withdrawal group, JF-W). Nucleus accumbens (NAc) was isolated and mRNA expression of tyrosine hydroxylase (TH), dopamine active transporter (DAT), D1 and D2 dopamine receptors, and μ-opioid receptor determined by qRT-PCR. Chronic junk food intake increased fat mass in all JF rats but junk food abstinence, reduced body weight and chow intake. In males, TH mRNA was reduced in JF and JF-W rats. D1 mRNA was reduced in JF and JF-W females, but increased in JF males, compared to Controls. μ-opioid receptor expression was reduced in JF and JF-W males but not females. These data highlight the importance of investigating sex differences in the neurobiological response to palatable foods.

Key words: reward, dopamine, food intake

Introduction

The incidence of obesity has reached epidemic proportions in industrialised and semi-industrialised nations across the globe. According to the World Health Organisation, over 300 million adults world-wide were classified as obese in 2005, and this figure has risen to 500 million in 2008 (WHO, 2011). In the context of this obesity epidemic, there is a growing emphasis on understanding the physiological mechanisms which may contribute to the dysregulation of food intake and energy expenditure, and thus promote weight gain and obesity.

The causes of obesity are multi-factorial. Whilst genetics clearly plays a role in determining obesity risk at an individual level, the marked increase in the incidence of obesity in populations around the world over the past 3 decades has occurred during a time when the gene pool has remained relatively stable, and this has led to suggestions that environmental, rather than genetic factors, are the major contributors (WHO, 2011). One of the most obvious environmental factors contributing to weight gain and fat deposition is the increased consumption of energy dense, high-fat, high-sugar 'junk foods'. The caloric intake of individuals in both industrialised and semi-industrialised nations has risen steadily over the past decade (EarthTrends, 2010) and, importantly, obese individuals are found to exhibit a stronger preference for junk foods than lean individuals (Drewnowski, Kurth, Holden-Wiltse, & Saari, 1992). These studies have raised questions on the ability of the appetite regulatory system, the system that controls food intake and energy homeostasis, to function optimally in an over-nourished state.

The phenomenon whereby animals and humans continue to consume high-fat, high-sugar foods despite their negative health consequences is analogous to behaviour displayed by drug addicts. Indeed, there have now been a number of studies which have shown that increased consumption of high-fat and high-sugar foods is associated with acute increases in opioid and dopamine synthesis and release, as is observed for drugs of abuse, and has led to the concept of food as a natural reward (Erlanson-Albertsson, 2005). The similarities between the effects of junk food and drugs of abuse has led to the suggestion that the overconsumption of junk foods could precipitate junk food addiction, leading people to seek out junk foods despite knowing their negative health consequences (Erlanson-Albertsson, 2005; Wang, Volkow, Thanos, & Fowler, 2004). However, studies which have examined the effects of chronic junk food feeding and subsequent junk food abstinence on the central reward pathway have been conducted almost exclusively in males, and whether comparable effects occur in females remains unclear.

Therefore, the aim of this study was to compare the effects of 8 weeks of junk food intake and subsequent abstinence on body weight, body fat mass, food intake and the expression of key components of the mesolimbic reward system in the nucleus accumbens (NAc), including tyrosine hydroxylase (TH), dopamine active transporter (DAT), D1 and D2 dopamine receptors, and the μ-opioid receptor in both male and female adult rats.

Materials and Methods

Animals and feeding

This study was approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science. Twenty one male and twenty one female Albino Wistar rats (200-300g) were used in this experiment. All rats were individually housed under a 12 hour light/12 hour dark cycle at a room temperature of 25°C. Rats were fed *ad libitum* on control chow and were allowed to acclimatise to the animal housing facility for one week prior to the start of the feeding protocol.

Male and female rats were randomly assigned to control (Control, n=10, 5 males, 5 females) or junk food (Junk Food, JF; n=32, 16 males, 16 females) groups. Control rats were given free access to standard rat chow (Specialty Feeds, Glen Forrest, Western Australia, Australia) while JF rats were provided with a cafeteria diet containing peanut butter, hazelnut spread, chocolate biscuits, savoury snacks, sweetened multi-grain breakfast cereal, ham and chicken flavoured processed meat and a mixture of lard and standard rat chow (Ong & Muhlhausler, 2011). The standard rat chow diet contained 67.1% carbohydrate, 21% protein and 11.9% fat whilst the cafeteria junk food diet contained 44.5% carbohydrate, 8.2% protein and 43.4% fat. Food and macronutrient intake was determined every 2 days throughout the duration of the experiment. In the JF group, the amount of each type of junk food remaining at the end of the 2 day period was individually weighed and subtracted from the original quantity provided. Bedding was searched through

thoroughly to ensure complete removal of all remaining foods. All rats had *ad libitum* access to water. Rats were weighed once a week throughout the experimental period. Rats in the Control and JF groups were maintained on their respective diets for 8 weeks to determine the chronic effects of continuous exposure to these junk foods. At the end of the 8 week period, a random sample of 11 male and 6 female rats in the JF group were euthanised and tissues collected. The remaining 5 male and 10 female rats had their junk food removed and replaced with control chow for a 72 hour period (Junk Food-withdrawal (JF-W) group). Both Control and JF-W rats were then euthanised and tissues collected.

Assessing the effect of junk food removal

In order to assess whether removal of junk food was associated with the onset of classic signs of withdrawal (piloerection, teeth-chattering, paw tremors and wet dog shakes), behaviour was observed every 24 hours for 72 hours following junk food removal in the JF-W rats and a corresponding group of Control rats which had never been exposed to junk food. The withdrawal period of 72 hours was chosen to allow identification of early withdrawal signs as a result of junk food removal, and the molecular adaptations associated with it within the mesolimbic reward system. Rats were removed from their home cage, placed in the clear plastic observational cage (20cm x 30cm x 50cm) and video recorded for 20 minutes. All rats were acclimatised to the environment and experimental procedure 1 day prior to the behavioural assessment. Body weight and food intake were recorded daily. All measurements were conducted by a single observer.

Post-mortem and tissue collection

All rats were weighed immediately prior to euthanasia and killed with an overdose of CO_2. Blood samples were collected by cardiac puncture into heparinsed tubes and spun at 3500g for 15 minutes at 4°C. Plasma samples were stored at -20°C for subsequent analyses of glucose, insulin, non-esterified free fatty acids (NEFA) and leptin concentrations. All internal and subcutaneous fat depots were dissected and weighed to provide an accurate measure of total body fat mass.

Determination of plasma glucose, NEFA, insulin and leptin concentrations

The plasma concentrations of glucose (Infinity Glucose Hexokinase kit, Thermo Electron, Pittsburgh, PA) and NEFA (Wako NEFA C kit, Wako Pure Chemical Industries Ltd, Osaka, Japan) were determined by enzymatic assay using the Konelab automated analysis system (Thermoscientific, Vantaa, Finland). Inter and intra-assay coefficients of variation (CoV) were <5%. Insulin and leptin concentrations were measured by radioimmunoassay with Rat Insulin and Rat Leptin Kits (Linco Research, St. Charles, MO) respectively, according to manufacturer's instructions. Inter and intra-assay CoV were <10%.

Nucleus Accumbens (NAc) isolation

The whole brain was carefully removed and placed on a glass dish that had been cooled on dry ice. The NAc was dissected from a coronal slice (bregma-0.24mm to 1.68mm) which spans from the optic chiasm to 2mm anterior to the optic chiasm as previously described (Ong & Muhlhausler, 2011). The section was immediately snap frozen in liquid nitrogen and stored at -80°C for subsequent determination of mRNA expression by qRT-PCR.

Determination of gene expression in the NAc

Methods for RNA extractions of the NAc and cDNA synthesis have been described elsewhere (Ong & Muhlhausler, 2011). Quantitative real time PCR was conducted using the SYBR Green system in an ABIPrism 7300 Sequence Detection System (PE Applied Biosystems, Foster City, CA). Rat β actin QuantiTect Primer Assay (Qiagen Pty Ltd, Doncaster, Australia) was used to determine β actin mRNA expression. All primers (TH, DAT, D1, D2 and μ-opioid receptor) have previously been sequenced and validated in our laboratory (Ong & Muhlhausler, 2011). Amplification efficiency of all primers was 0.997 – 0.999. A constant amount of cDNA (1μl) was used for each qRT-PCR measurement and three technical replicates were performed for each gene. Two quality controls were included on each plate in order to verify inter-plate consistency.

Each qRT-PCR reaction well (10μl total volume) contained 5μl of 2x SYBR Green Master Mix (PE Applied Biosystems, Foster City, CA); 1μl of each primer giving a final concentration of 60nM to 900nM, 2μl of molecular grade H_2O and 1μl of a 50 ng/μl dilution of the stock template. The cycling conditions consisted of 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The abundance of each mRNA transcript was measured and expression relative to that of β actin was calculated using Q-gene qRT-PCR analysis software (Muller, Janovjak, Miserez, & Dobbie, 2002).

Assessing the effect of chronic junk food feeding on subsequent food preferences

An additional 10 male and 10 female rats were given either the control (n=5 male, n-5 female) or the junk food (n=5 male, n=5 female) diet for a period of 8 weeks. At the end of the 8 week period, the rats were given free access to both the control and the junk food diet for 2 weeks and macronutrient intake recorded to assess the impact of prior chronic exposure to a junk food diet on subsequent junk food intake and preference. Macronutrient intake was analysed separately for week 1 and week 2 of the food preferences study.

Statistical analysis

Data are presented as the mean ± SEM. The effect of 8 weeks of junk food feeding on nutritional intake was determined separately in males and females using a Students' t-test. The JF group included all rats exposed to the junk food diet, irrespective of whether they were subsequently exposed to junk food abstinence.

The effect of junk food feeding and withdrawal on body weight, body fat mass, plasma hormones and metabolites, and gene expression was determined by two-way ANOVA, with sex and treatment groups as factors. Where a significant interaction between sex and junk food feeding was identified, data from male and female rats were analysed separately by one-way ANOVA. The effect of junk food withdrawal on food intake, body weight and behaviour in male and female rats was determined by a multifactorial ANOVA with repeated measures, with sex and time post-withdrawal as factors. Where the ANOVA identified a significant effect, the Duncan's multiple range test was used post-hoc in order to determine significant differences between values. All statistical tests were carried out

using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL) or STATA 10 (StataCorp LP, USA). A probability <5% ($P<0.05$) was accepted as statistically significant.

Results

Macronutrient and energy intake of male and female rats

Total energy and fat intakes were higher in the JF rats than Controls in both males and females (Table 1). In males only, carbohydrate intake was higher in the JF group (Table 1). Protein intake was lower in both male and female rats in the JF group when compared to Controls (Table 1).

Effect of chronic junk food feeding on body weight and body fat

There was no difference in body weight between the Control and JF groups before commencement of the control or junk food diet in either male or female rats (Males: Control, 278.7 ± 5.3g, JF, 268.4 ± 6.6g; Females: Control, 253.4 ± 4.7g, JF, 258.6 ± 5.8g). In female rats, body weight and percentage body fat were significantly higher in the JF and JF-W groups compared to Controls at the end of the feeding period (Table 2). In males, there was no significant increase in body weight in the JF and JF-W groups, however percentage body fat was higher in the JF and JF-W males compared to Controls (Table 2)**.**

Effect of chronic junk food feeding and abstinence on plasma hormones and metabolites

Continuous junk food intake resulted in elevated plasma leptin concentrations in both male and female rats compared to those consuming the standard rat chow; however plasma leptin concentrations were no longer different from Controls after 72 hours of junk food abstinence (Table 2). In females, but not in males, the JF and JF-W groups also had higher plasma NEFA concentrations compared to Controls (Table 2). Plasma glucose

concentrations were also higher in the female JF group at the end of the 8 week period of junk food feeding, but were no longer different to Controls after 72 hours of junk food abstinence. There was no effect of junk food intake on plasma glucose or NEFA concentrations in males or on plasma insulin concentrations in either males or females (Table 2).

Effect of forced junk food abstinence on body weight, food intake and behaviour in male and female rats

The body weight of the JF-W rats decreased significantly over the 72 hour period of forced junk food abstinence, where both male and female rats lost an average of between 1.5 and 3.4% of their initial body weight during this period (Figure 1A and 1B). Both male and female rats also consumed significantly less chow during the period of forced junk food abstinence compared to Control rats which had never been exposed to junk food (Figure 1C and 1D). No rats in either feeding group or of either sex exhibited classic signs of withdrawal during the period of forced junk food abstinence *(data not shown)*.

Effect of chronic junk food intake and abstinence on the mRNA expression in the NAc TH and DAT

There was a significant sex by treatment interaction for TH mRNA expression in the NAc ($P<0.01$). In male rats, TH mRNA expression was lower in both JF and JF-W groups when compared to Controls ($P<0.001$) (Figure 2A). TH mRNA expression in the male rats was also negatively correlated with total energy intake (r=-0.74; $P<0.01$) and fat intake (r=-0.82; $P<0.01$) during the 8 week feeding period. There was no effect of chronic junk food

feeding on TH mRNA expression in female rats (Figure 2B). DAT mRNA expression was not different between Control, JF and JF-W groups (Figure 2C, D).

D1 and D2 receptor

There was a significant interaction between sex and treatment for D1 expression in the NAc ($P<0.01$). In male rats, D1 mRNA expression was higher in the JF, but not JF-W rats, compared to Controls ($P<0.05$) (Figure 3A)**.** In female rats, however, D1 mRNA expression was lower in the JF and JF-W groups compared to the Controls ($P<0.01$) (Figure 3B). D1 mRNA expression in female rats was also negatively correlated with total energy intake (r=-0.71; $P<0.01$) and fat intake (r=-0.66; $P<0.01$) during the 8 week feeding period. There was no difference in D2 mRNA expression between Control, JF or JF-W groups in either males or females (Figure 3C, D).

μ-opioid receptor

The impact of chronic junk food feeding and subsequent junk food removal on the expression of the μ-opioid receptor was also different between male and female rats. In male rats, mRNA expression of μ-opioid receptor was lower in the JF and JF-W groups when compared to Controls ($P<0.01$) (Figure 4A). There was no difference in μ-opioid receptor mRNA expression between groups in female rats (Figure 4B).

Sex differences in mRNA expression in Control rats

In addition to the different responses of the male and female rats to the highly palatable diet, there were also sex differences between in the expression of TH and μ-opioid receptor

mRNA in the NAc in Control rats not exposed to junk foods, with higher expression in males than females (TH: Male, $1.1x10^{-3} \pm 2.2x10^{-4}$, Female, $2.4x10^{-4} \pm 4.5x10^{-5}$; μ-opioid receptor: Male, $5.9x10^{-3} \pm 6.7x10^{-4}$, Female, $2.9x10^{-3} \pm 6.5x10^{-4}$; $P<0.05$).

Effect of chronic junk food intake on subsequent food preferences

During the first week of the food preference study, both male and female Control rats with no prior exposure to junk food consumed significantly more total energy than JF rats ($P<0.01$) and derived a significantly higher proportion of their total energy intake from the junk food diet (Males: Control, 95 ± 1.6%, JF, 90 ± 1.7%; Females: Control, 99 ± 0.2%, JF, 97 ± 0.8%). Fat and protein intake in both males and females and carbohydrate intake in females were also higher in Control rats when compared to the JF rats ($P<0.05$) (Figure 5A, B). During the second week of the food preferences study, total energy and fat intake remained higher in the Control rats compared to the JF rats in both males and females ($P<0.05$), however protein and carbohydrate intake was no longer different between the Control and JF groups (Figure 5C, D). In both males and females, the percentage of energy derived from the junk food diet remained higher ($P<0.01$) in the Control rats compared to the JF rats in the second week of free access to the control and junk food diets (Males: Control, 98 ± 0.7%, JF, 91 ± 1.7%; Females: Control, 99 ± 0.1%, JF, 96 ± 1.7%).

Discussion

This has been the first study to directly compare the effects of chronic exposure to a palatable diet, and a subsequent period of abstinence in male and female adult rats. We have demonstrated that whilst the effects of chronic junk food intake on body fat mass, plasma leptin concentrations and the physiological response to acute abstinence were similar between sexes, the effects on gene expression in the dopaminergic and opioid systems within the mesolimbic reward centre were sexually dimorphic. These results suggest that the neurophysiological mechanisms which govern the response to chronic high-fat, high-sugar feeding are likely to differ between males and females, and highlights the need for further studies investigating the neurobiology of junk food addiction to include both males and females.

As expected, 8 weeks of continuous access to the palatable junk food diet resulted in increased fat deposition and elevated leptin concentrations in both males and females. Moreover, both males and females exhibited comparable behavioural responses to the 72 hour period of junk food abstinence after 8 weeks on the junk food diet. These responses, a significant reduction in standard rat chow intake and body weight loss, are consistent with those reported in previous studies in male rodents and suggest that females and males have comparable physiological responses to removal of junk food after a period of chronic exposure (Johnson & Kenny, 2010; Pickering, Alsio, Hulting, & Schioth, 2009; Teegarden & Bale, 2007). Importantly, the reduction in chow intake in the period following junk food removal occurred despite the lower plasma leptin concentrations in these animals compared

to rats consuming the junk food diet, which would normally be expected to stimulate appetite. This suggests that the compulsion to obtain more palatable food dampened normal physiological hunger signals during this period (Kalra et al., 1999).

In contrast to a previous study where removal of sucrose after an intermittent access schedule resulted in the development of classic signs of withdrawal similar to that of opiate withdrawal (Colantuoni et al., 2002), we did not observe any classic withdrawal signs following removal of the palatable diet in either males or females. This may be due to differences in the composition of the diet and/or the feeding schedule (i.e. intermittent vs *ad libitum* access). It has previously been reported that rats given *ad libitum* access to sucrose for one month did not exhibit signs of withdrawal upon removal of access to sucrose (Colantuoni, et al., 2002), whilst intermittent access to sucrose, but not fat or a combination of sucrose and fat, has been associated with the onset of signs of withdrawal when access to the diets was removed (Avena, Rada, & Hoebel, 2009). In contrast, it was reported that removal of a high-fat diet after a period of *ad libitum* access does not elicit classic withdrawal signs, but instead results in symptoms of heightened anxiety (Teegarden & Bale, 2007). The results of the present study suggest that 8 weeks of chronic exposure to a palatable diet containing both sucrose and fat does not result in classic symptoms of withdrawal during a 72 hour period of abstinence.

Despite the similar behavioural and metabolic outcomes in both male and female rats in response to chronic junk food intake and subsequent junk food removal, the effects of the dietary regimen on gene expression of key components of the mesolimbic reward system,

specifically the NAc, were distinct between sexes, thus suggesting that the responses are likely to be regulated by different underlying mechanisms.

In the dopamine system, we found sex-specific effects of chronic junk food intake on the expression of TH and D1 mRNA in the NAc. In males, TH mRNA expression in the NAc was lower following the 8 week period of junk food intake and remained lower after the period of abstinence, whilst TH mRNA expression was not affected by the same treatment in females. The lower TH expression at the NAc is indicative of lower dopamine synthesis and release in the male JF rats, which is supported by previous studies where chronic exposure to a high-fat diet reduced accumbens dopamine release in rats (Geiger et al., 2009; Rada, Bocarsly, Barson, Hoebel, & Leibowitz, 2010). This lower dopamine synthesis in the NAc may be directly mediated by fat and energy intake as demonstrated by our finding of a negative correlation between TH mRNA expression and average daily fat and energy intake in male rats. The lower TH expression during the period of junk food abstinence in male rats is also consistent with previous studies reporting reduced dopamine content within the NAc during withdrawal from sucrose and addictive drugs (Colantuoni, et al., 2002; Littleton, 1998; Schmidt et al., 2001). The absence of any effect of chronic junk food feeding on TH mRNA expression in female rats suggests that dopamine synthesis in the NAc may be less responsive to increased dietary fat and energy intake in females compared to males.

The impact of chronic junk food feeding and subsequent abstinence on the D1 dopamine receptor was also different between male and female rats. Male rats exhibited a significant

increase in D1 receptor expression after chronic exposure to the palatable diet, which may be a compensatory response to the reduction in dopamine synthesis (as indicated by lower TH mRNA expression). The results of our study differ from previous studies, in which D1 receptor mRNA and protein concentrations in the NAc of male rats were reduced after chronic exposure to a high-fat diet (Sharma & Fulton, 2012; Vucetic, Carlin, Totoki, & Reyes, 2012). These differences may be the result of the shorter period of exposure to the palatable diet in our study (8 weeks) compared to previous studies (12 weeks and 20 weeks). Interestingly, the increased D1 receptor expression was no longer present after 72 hours of junk food abstinence, suggesting a restoration of D1 mRNA expression following a relatively short period of removal of the junk food stimulus.

In females, however, the expression of the D1 receptor was significantly reduced at the end of the junk food feeding period, and remained significantly lower than Controls at the end of the 72 hour period of junk food abstinence. This study is the first to demonstrate changes in D1 receptor expression in females in response to high-fat, high-sugar feeding. The functional desensitisation of dopamine D1-like receptors has been implicated in the development of tolerance in both male and female chronic cocaine users (Hammer, Egilmez, & Emmett-Oglesby, 1997). Therefore, the reduction in D1 receptor expression after a highly palatable diet implies that the responsiveness to dopamine stimulation in the NAc in females was reduced by chronic exposure to a palatable diet. In addition, in contrast to males, it appears that the expression of D1 receptors in female rats is sensitive to increases in fat and energy intake, as shown by our finding of a negative correlation between D1 receptor mRNA expression and, fat and energy intake in the female rats.

We did not observe any effect of chronic junk food feeding or a subsequent period of junk food abstinence on the mRNA expression of D2 receptor in the NAc in either males or females. There are conflicting data from previous studies in relation to the effect of palatable diets on the expression of the D2 receptor in the NAc; one study reported an increase in expression following acute exposure to a high-fat diet (South & Huang, 2008), whilst expression was either reduced after 4 and 5 weeks of high-fat, high-sugar food intake (Alsio et al., 2010; Johnson & Kenny, 2010) or unchanged following 20 weeks of exposure to high-fat diets (Vucetic, et al., 2012). There was also no effect of the junk food diet or subsequent period of abstinence on the mRNA expression of DAT, the transporter responsible for clearing dopamine from the synapse, in either male or female rats. This is different from the reduced DAT binding reported after acute exposure to a high-fat diet in mice (South & Huang, 2008). It would therefore appear that whilst D2 and DAT mRNA expression in the NAc may be altered by acute exposure to a palatable diet, expression is restored during more extended periods of exposure.

The effects of the nutritional regimen on the opioid system were also different in male and female rats, with the expression of the μ-opioid receptor in the NAc reduced in male rats by chronic exposure to the palatable diet and remained lower following the period of junk food abstinence, but no changes in mRNA expression of the μ-opioid receptor seen in females. The results in male rats are consistent with previous studies in male mice, which reported similar reductions in μ-opioid receptor expression in the NAc when animals were fed on a high-fat diet for 20 weeks (Vucetic, Kimmel, & Reyes, 2011). Interestingly, differences in expression of the μ-opioid receptor in the NAc was not seen during shorter periods of

406 exposure (5 weeks) (Alsio, et al., 2010), suggesting that chronic exposure to an *ad libitum*
407 supply of the palatable diet are required to elicit the observed changes in μ-opioid receptor
408 expression. The reduction in μ-opioid receptor expression may be a response to the
409 increased synthesis and release of endogenous opioids, including β-endorphin (Dum,
410 Gramsch, & Herz, 1983), associated with intake of palatable foods (Blendy et al., 2005;
411 Kelley, Will, Steininger, Zhang, & Haber, 2003; Spangler et al., 2004). The results of the
412 present study therefore adds to a growing body of evidence that exposure to an extended
413 period of palatable food intake, at least in male rats, results in reduced sensitivity to opioid
414 stimulation (Buntin-Mushock, Phillip, Moriyama, & Palmer, 2005).
415
416 The findings of the present study identified sex-specific effects on the mesolimbic reward
417 system in response to chronic junk food feeding. Sex-specific effects on the reward system
418 have also been reported in studies of drugs of abuse (Walker, Ray, & Kuhn, 2006; Zhou,
419 Nazarian, Sun, Jenab, & Quinones-Jenab, 2009) and alcohol addiction (Blanchard,
420 Steindorf, Wang, & Glick, 1993), and may be related to the different basal dopaminergic
421 tone between males and females (Becker & Hu, 2008). We reported that female rats which
422 have never been exposed to the junk food diet expressed lower TH in the NAc compared to
423 naive males. The lower dopaminergic tone in female rats is postulated to be associated with
424 a low stimulatory threshold and therefore an increased susceptibility to addiction (Becker &
425 Hu, 2008). Our finding of lower μ-opioid receptor mRNA expression in naive females is
426 however in contrast with previous observations, where μ-opioid receptor binding and
427 density has been found to be higher in females than in males in various brain regions
428 (Hammer, 1990; Vathy, Šlamberová, Rimanóczy, Riley, & Bar, 2003; Zubieta, Dannals, &

Frost, 1999). The reason for this is unclear but may be due to the different methods of μ-opioid receptor quantification between studies. Nevertheless, given that the reward system is sexually dimorphic both at the basal and stimulated state, it is possible that the basal expression of key genes within the mesolimbic reward system could potentially influence the system's response to rewarding stimuli, thus explaining the sex-specific effects on the mesolimbic reward system in response to junk food intake.

Despite the differential adaptation of the mesolimbic reward system to chronic junk food feeding between sexes, the gene expression data of both male and female rats suggest reduced sensitivity of the reward system after 8 weeks of junk food feeding. The effect of chronic junk food feeding on subsequent food choices in a paradigm where rats were given free access to both standard chow and a range of junk foods provided evidence of the functional consequences of the changes in gene expression within the mesolimbic reward circuitry. We found that rats previously exposed to the junk food diet consumed less total energy and fat than naive rats when provided with free access to both control chow and a palatable diet throughout the 2 week test period. This is consistent with the molecular changes in the NAc of the junk food rats, which would be expected to be associated with reduced dopamine sensitivity in the mesolimbic reward system. Previous work has also demonstrated that rodents chronically fed on a palatable high-fat, high-sugar diet exhibited higher brain reward thresholds (Johnson & Kenny, 2010) and reduced reward sensitivity as indicated by reduced sucrose preference (Vucetic, et al., 2011) and high-fat food intake (Vucetic, et al., 2012).

Conclusion

We have demonstrated that continuous intake of a highly palatable high-fat, high-sugar diet has distinct effects on the expression of genes in the dopaminergic and opioid signalling pathways in the mesolimbic reward pathway in male and female rats. To our knowledge, this is the first study to investigate sex differences in the mesolimbic reward pathway both at baseline and in response to chronic intake of a palatable diet, and the clear differences observed between the sexes highlights the need for further comparative studies in other signalling pathways, other brain regions and/or other treatment paradigms. Interestingly, whilst there are different molecular changes within the dopamine system in response to a chronic palatable diet in male and female animals, both were consistent with a suppression of dopamine signalling, either via decreased dopamine synthesis or decreased dopamine receptors, and this may account for the similar behavioural responses. A major impetus for the present study was the strong male bias in existing animal studies of food reward (Eckel, 2011). Given the high incidences of eating disorders (Hilbert, de Zwaan, & Braehler, 2012; Hoek & van Hoeken, 2003) and susceptibility to addiction (Becker & Hu, 2008; Kosten, Gawin, Kosten, & Rounsaville, 1993; Zilberman, Tavares, & el-Guebaly, 2003) in women compared to men, there is an increasing need to identify sex differences and mechanisms underlying the differential responses towards food reward in males and females. Overall, the data from this study add to the growing body of evidence that palatable foods have comparable effects on the central reward pathway to those of characterised drugs of abuse, and that junk food addiction is a concept that is likely to be underpinned by sex-specific physiological mechanisms.

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589 848.
590
591

592 **Table 1.** Total energy, fat, protein and carbohydrate intake of Control and JF groups in
593 male and female rats.
594

	Male		Female	
	Control	JF	Control	JF
Total energy intake (kJ/day)	345 ± 10	557 ± 21**	367 ± 8	436 ± 14**
Fat intake (g/day)	0.91 ± 0.02	6.54 ± 0.28**	0.94 ± 0.03	5.90 ± 0.29**
Protein intake (g/day)	3.91 ± 0.10	2.57 ± 0.11*	3.98 ± 0.10	1.89 ± 0.11**
Carbohydrate Intake (g/day)	12.0 ± 0.32	16.3 ± 0.77*	12.2 ± 0.28	12.2 ± 0.58

595
596 Data expressed as mean ± SEM. * $P<0.05$, ** $P<0.01$.
597

598 **Table 2.** Body weight, percentage body fat, plasma leptin, NEFA, insulin and glucose
599 levels of male and female Control, JF and JF-W rats at the end of experimental period.
600

	Male			Female		
	Control	JF	JF-W	Control	JF	JF-W
Weight (g)	476.6 ± 4.4[a]	533.9 ± 22.6[a]	509.3 ± 32.3[a]	303.4 ± 5.4[a]	401.6 ± 22.7[b]	351.7 ± 11.3[c]
Fat mass (%)	9.2 ± 1.3[a]	15.3 ± 1.7[b]	18.0 ± 3.8[b]	9.4 ± 0.7[a]	20.7 ± 2.0[b]	14.0 ± 1.4[b]
Leptin (ng/ml)	18.7 ± 2.7[a]	47.6 ± 8.3[b]	31.7 ± 8.4[ab]	5.6 ± 1.0[a]	51.3 ± 8.0[b]	13.7 ± 2.3[a]
NEFA (meq/ml)	0.4 ± 0.1[a]	0.3 ± 0.0[a]	0.4 ± 0.1[a]	0.4 ± 0.0[a]	0.5 ± 0.0[b]	0.5 ± 0.0[b]
Insulin (μU/L)	3.0 ± 1.3[a]	2.8 ± 0.5[a]	3.9 ± 1.0[a]	1.6 ± 0.2[a]	2.0 ± 0.9[a]	1.6 ± 0.3[a]
Glucose (mmol/L)	13.1 ± 2.3[a]	12.4 ± 1.4[a]	13.6 ± 1.9[a]	11.2 ± 0.9[a]	17.3 ± 1.7[b]	9.8 ± 0.6[a]

601

602 Data expressed as mean ± SEM. Fat mass is presented as a percentage of total body weight.
603 Different superscript letters indicate significance within rows between Control, JF and JF-
604 W groups for male and female rats. $P<0.01$ for female body weight, fat mass, leptin and
605 glucose; $P<0.05$ for male fat mass and leptin.
606

Figure Legends

Figure 1: The effects of junk food removal on body weight (expressed as a percentage from weight prior to removal of junk food) (A, B) and chow intake (C, D) of Control (closed bar) and JF-W (open bar) groups in both male and female rats at 24, 48 and 72 hours post-junk food removal. Values are expressed as mean ± SEM. * $P<0.05$.

Figure 2: Normalised mRNA expression of TH (A, B) and DAT (C, D) in the NAc of Control (closed bar), JF (open bar) and JF-W (striped bar) groups at the end of the experimental period (n = 5-11 in each group). Values are expressed as mean ± SEM. Different superscript letters indicate values which are significantly different ($P<0.01$).

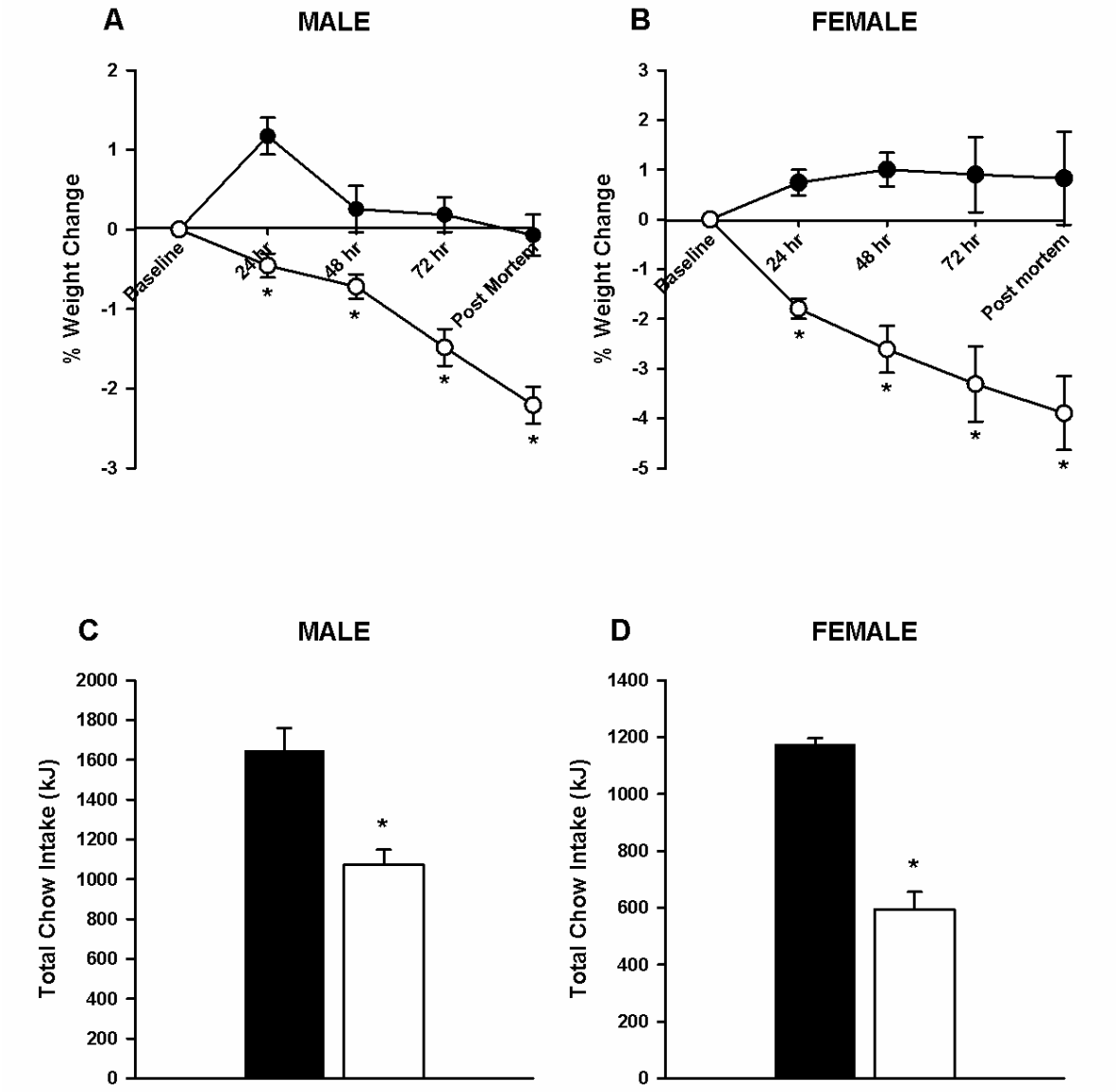
Figure 3: Normalised mRNA expression of D1 (A, B) and D2 (C, D) in the NAc of Control (closed bar), JF (open bar) and JF-W (striped bar) groups at the end of the experimental period (n = 5-11 in each group). Values are expressed as mean ± SEM. Different superscript letters indicate values which are significantly different ($P<0.05$).

Figure 4: Normalised mRNA expression of μ-opioid receptor in the NAc of Control (closed bar), JF (open bar), and JF-W (striped bar) groups in both male (A) and female (B) rats (n = 5-11 in each group). Values are expressed as mean ± SEM. Different superscript letters indicate values which are significantly different ($P<0.05$).

Figure 5: Total fat, protein, carbohydrate (CHO) and energy intake of both male and

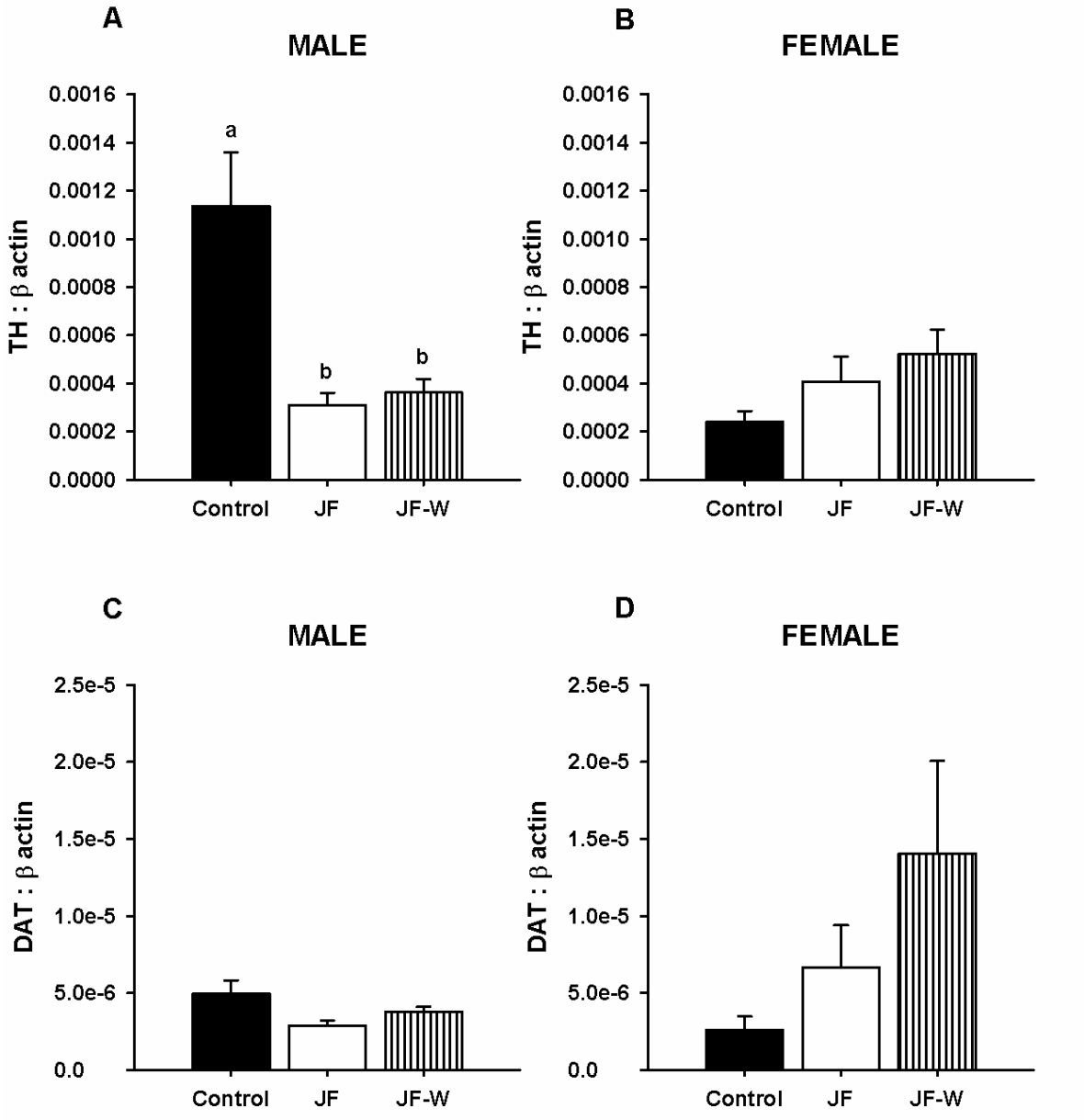
630 female Control (closed bar) and JF (open bar) rats (n = 5 in each group) during week 1 (A,
631 B) and week 2 (C, D) of the food preference study. Values are expressed as mean ± SEM.
632 * $P<0.05$, ** $P<0.01$.
633

FIGURE 1


A
MALE
% Weight Change
Baseline
24 hr
48 hr
72 hr
Post Mortem
B
FEMALE
% Weight Change
Baseline
24 hr
48 hr
72 hr
Post mortem
C
MALE
Total Chow Intake (kJ)
D
FEMALE
Total Chow Intake (kJ)

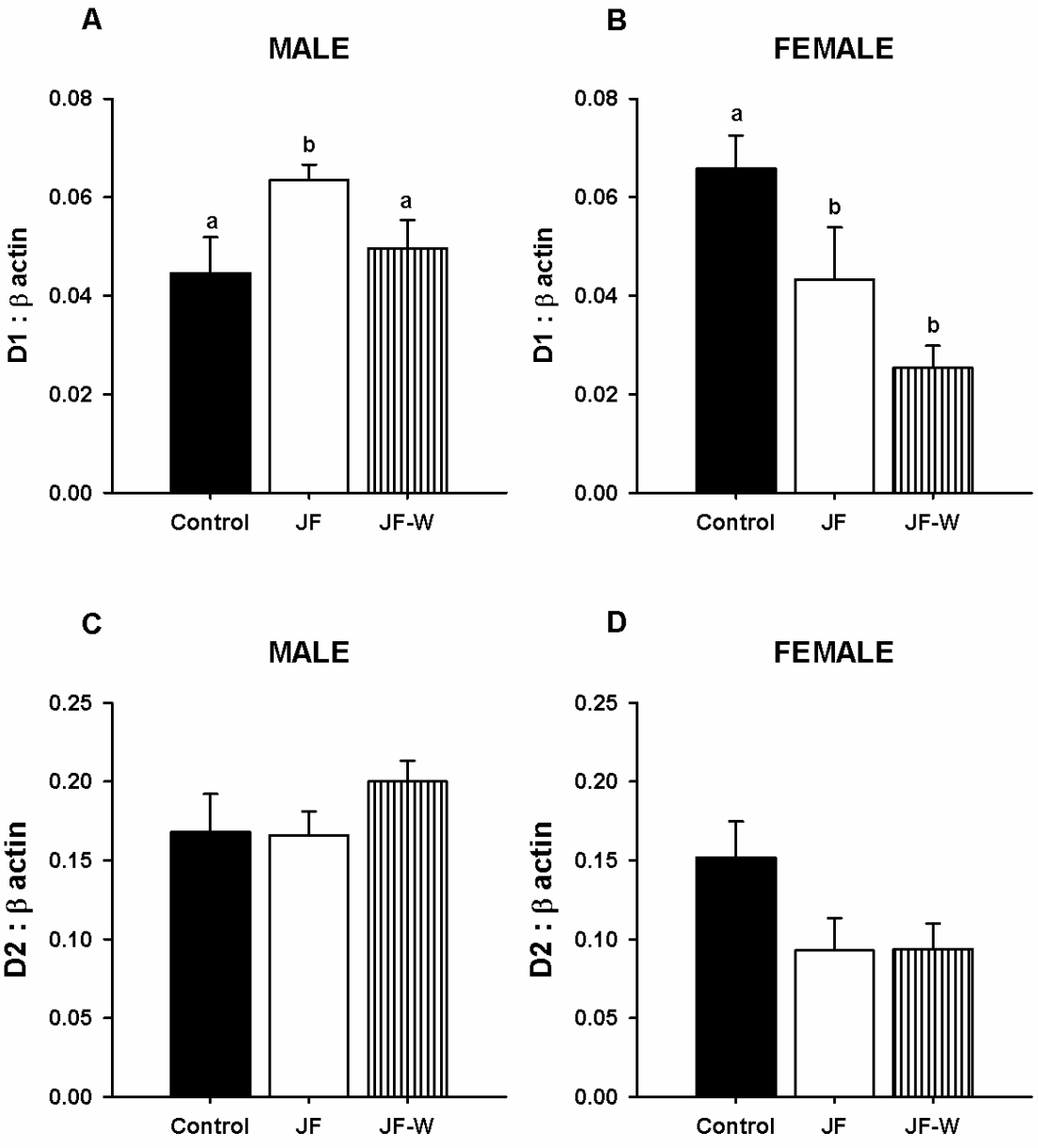
634

FIGURE 2


A
MALE
TH : β actin
0.0016
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
a
b
b
Control
JF
JF-W
B
FEMALE
TH : β actin
0.0016
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
Control
JF
JF-W
C
MALE
DAT : β actin
2.5e-5
2.0e-5
1.5e-5
1.0e-5
5.0e-6
0.0
Control
JF
JF-W
D
FEMALE
DAT : β actin
2.5e-5
2.0e-5
1.5e-5
1.0e-5
5.0e-6
0.0
Control
JF
JF-W

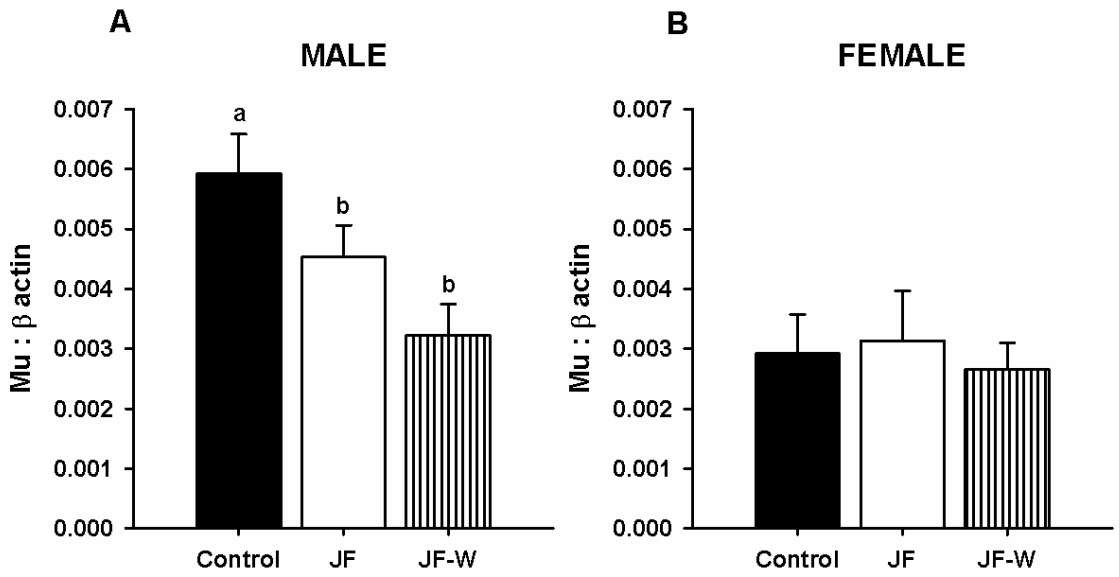
635

FIGURE 3


A
MALE
D1 : β actin
0.08
0.06
0.04
0.02
0.00
a
b
a
Control
JF
JF-W
B
FEMALE
D1 : β actin
0.08
0.06
0.04
0.02
0.00
a
b
b
Control
JF
JF-W
C
MALE
D2 : β actin
0.25
0.20
0.15
0.10
0.05
0.00
Control
JF
JF-W
D
FEMALE
D2 : β actin
0.25
0.20
0.15
0.10
0.05
0.00
Control
JF
JF-W

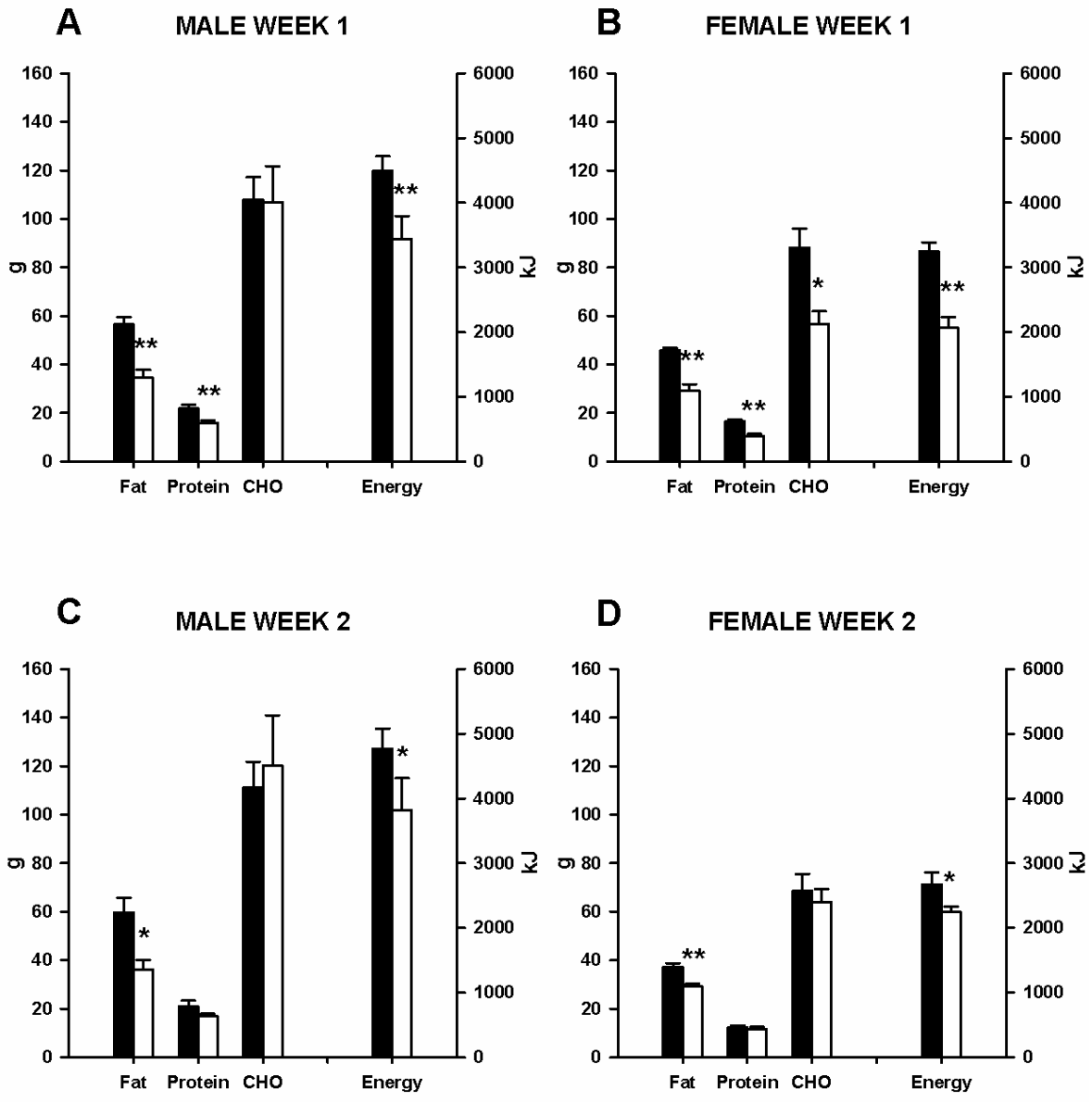
636

FIGURE 4


A
MALE
B
FEMALE
Mu : β actin
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
a
b
b
Control
JF
JF-W

637

FIGURE 5


A
MALE WEEK 1
B
FEMALE WEEK 1
C
MALE WEEK 2
D
FEMALE WEEK 2
g
kJ
0
20
40
60
80
100
120
140
160
0
1000
2000
3000
4000
5000
6000
Fat
Protein
CHO
Energy
*
**

638