Report Research Project: The effect of diet on sucrose water intake in female Wistar rats

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Abstract
Previous research has shown that exposure to a high-fat diet leads to increasing reward deficits and reward hyposensitivity, as well as a decreased dopamine turnover in rats. This has been suggested to lead to compensation through a compulsive increase in the intake of high-fat containing food. In this study, rats were put on a high-fat/sugar diet and measurements of sucrose water intake were taken in order to answer the research question "what is the effect of diet on the sucrose water intake of female Wistar rats?" and to subsequently provide evidence for the above-mentioned potential compensation. Our results have shown that, contrary to the hypotheses, rats on a high-fat diet showed a decreased sucrose water intake and a lower initial intake rate compared to rats on a LF diet. These contradicting findings might be explained by other studies indicating potential sex differences in food intake in response to a high-fat diet, since this study included female rats whereas the above-mentioned studies on reward sensitivity and dopamine turnover included male rats. In conclusion, female Wistar rats on a HF diet decrease the intake of sucrose water in addition to diet consumption compared to rats on a LF diet, which might be explained by the suggestion that total food intake is reduced during estrous.

Introduction

Obesity is a growing problem in present-day society and is defined as the presence of an excessive level of fat in adipose tissue that might exert negative consequences on a person's health (Ofei, 2005). However, the development of obesity is not limited to humans but also occurs in other species (Bastías-Pérez et al., 2020; Warwick & Schiffman, 1992). Specifically, rodents have shown to be effective models for obesity-related studies since weight gain can quickly be induced via a high-fat diet in these animals (Bastías-Pérez et al., 2020; Van Heek et al., 1997). At the current age, several commercially available diets containing high fat and sugar levels have shown to be effective in inducing obesity in these rodents (Speakman, 2019). These high fat and sugar containing diets have been shown to elevate, not limited to but including, body weight, fat mass, and plasma leptin levels in rat models (Marques et al., 2016). The (high fat) diet-induced obesity observed is suggested to lead to changes in the brain, for example in a study by Johnson & Kenny (2010) that showed that diet-induced obesity in rats that had an prolonged access to a high fat diet was associated with an increasing reward deficit.

The mesolimbic dopaminergic system is known to be greatly involved in reward (Baik, 2020). Regarding this dopamine system, a study has shown that exposure to a high-fat diet leads to a decrease in dopamine turnover in the nucleus accumbens of rats (Davis et al., 2008). Furthermore, reduced levels of dopamine D2 receptors have been observed in human individuals with obesity compared to non-obese individuals (Wang et al., 2001). This relates to another experiment in the aforementioned study by Johnson & Kenny (2010) in which knocking out dopamine D2 receptors resulted in an increased reward threshold in the rats that had prolonged access to a high fat diet, compared to rats that either had no access or a restricted access to the high fat diet (Johnson & Kenny, 2010). The authors of the study by Wang et al. (2001) postulate that the observed deficits in dopamine D2 receptors might lead to increased food intake to compensate for the reward deficits. The reward hyposensitivity observed in diet-induced obesity might thus actually be compensated for by a compulsive consumption of high fat/sugar-containing food items (Wang et al., 2001; Wang et al., 2002; Johnson & Kenny, 2010).

In addition to the above-mentioned effects of a high-fat diet on reward sensitivity and dopamine turnover (Johnson & Kenny, 2010; Davis et al., 2008), studies have also suggested that diet has an effect on satiety (Covasa & Ritter, 1998; Savastano & Covasa, 2005). Namely, the findings of these studies showed that rats being fed a high-fat diet show a decrease in the sensitivity to signals of satiety compared to rats on a low-fat diet, which might subsequently lead to an increased food consumption in these rats on a high-fat diet (Covasa & Ritter, 1998; Savastano & Covasa, 2005).

Taking the aforementioned findings into account, this research project therefore tests the hypotheses that 1) rats being fed a high fat/sugar (HF/S) diet show a compulsively increased sucrose water intake to compensate for the potential reward hyposensitivity induced by this HF/S diet compared to rats being fed a

standard chow, low-fat diet (LF); and 2) rats on a high-fat diet will again show an increased intake when exposed to sucrose water for a second time after a first exposure compared to rats on a LF diet. The data obtained in testing these hypotheses will subsequently aid in answering the research question "what is the effect of diet on the sucrose water intake of female Wistar rats?" and give more insight into how diet affects the subsequent intake of palatable food items. This study investigated the effects of diet on the total sucrose water intake of rats. Furthermore, the effect of diet on the initial sucrose water intake rate of rats was examined. Finally, we investigated the effect of diet on satiety through a second exposure to sucrose water.

Methods

Animals & housing

This project included 20 female Wistar rats of around 3 months old. All rats were housed in duos in large cages of 80 x 55 x 50 cm (2 rows of 5 cages) and rats were randomly assigned to a cage. The cages were located in an enclosed ventilated room **(**~21℃) with a light-dark cycle of 12:12, with lights-off starting at 1PM. The duos in each cage were separated at 12:30PM for a maximum of 3 hours using a transparent divider that restricted movement to the other half of the cage while still allowing the rats to see and smell each other. Furthermore, a starvation period was induced by removal of all food hoppers 3 hours before the start of each experiment (10AM-1PM). The food hoppers furthermore remained absent throughout the experiment. Food hoppers were placed back immediately after the end of the experimental procedures. Water bottles were only removed during the experimental procedures.

Diet

All rats were fed a low fat (LF) diet for 7 days during the baseline week. At the start of the second week, half of the experimental group (10 rats) were changed to a high fat/sugar (HF/S) diet, whereas the other half (10 rats) remained on the LF diet. Distribution of the diets among the cages/rats is shown in table 1. The HF/S diet consisted of: RMH-B food pulverized (4072 g), sucrose (1500 g), casein (1000 g), vegetable oil (666 g), lard (2000 g), salt-mix (236 g), vitamin-mix (155 g), and arabic gum (500 g); the LF diet consisted of regular chow (maintenance diet, Altromin International).

Injections

Due to scheduling reasons, all repetitions of this experiment took place on injection days. On each of these days, all rats received a subcutaneous injection of either a cholecystokinin (CCK) solution (CCK, 1.5 µg in 0.3 ml saline) or saline (0.3 ml). During experiments 1-5, 10 rats received the CCK injection, and 10 rats received the Saline injection. During experiment 6, all 20 rats received saline as a baseline. Table 1 shows which type of injection every cage received during each experiment. Since the data from experiment 1 was later excluded, all rats received a saline injection 3 times in total (experiment 2-6). Due to the nature of this project, only the data obtained through saline-injected rats or non-injected rats was used.

Behavioral experiments

1.1 Sucrose drinking: first exposure (injections)

To determine potential differences in sucrose drinking between the HF/S and LF group, intake of sucrose water was measured during 6 experimental repeats divided over a period of 4 weeks. Shortly before the start of each experiment, all water bottles were removed from the cages. At 1:10PM, the experiment started with injections of all rats in the 5 cages in the top row. Within a given cage, there was a 30 second interval between the injection of the 2 rats. Rats were left alone for 5 minutes after injection to largely prevent stress from potentially interfering with the data. 5 minutes after injection, a rat was given access to a buret containing 14 ml of 10% sucrose water for 20 minutes, during which the current volume of the buret was noted each minute. However, measurements were only taken up to 10 ml since the metal drinking tip held 4 ml, and did not allow for precise measurements. Therefore, the experiment ended for a rat after 10 ml were consumed. After 20 minutes of access, the buret was removed. After all measurements were taken for all rats in the top row, the full procedure was repeated for the rats in the bottom row.

Table 1. Distribution of the High Fat / Sugar (HF/S) diet and the Low Fat (LF) diet among the cages and rats included in the experiments, furthermore including an overview on which type of injection (CCK or Saline) every cage received during each of the 6 experiments (denoted as Exp.)

Diet	Cage	Rat Nr.	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
HF/S	2	1 & 2	CCK	Saline	CCK	Saline	CCK	Saline
LF	3	11 & 12	Saline	CCK	Saline	CCK	Saline	Saline
LF	5	3 & 4	CCK	Saline	CCK	Saline	CCK	Saline
HF/S	6	13 & 14	Saline	CCK	Saline	CCK	Saline	Saline
HF/S	8	5 & 6	CCK	Saline	CCK	Saline	CCK	Saline
HF/S	9	15 & 16	Saline	CCK	Saline	CCK	Saline	Saline
HF/S	11	7 & 8	CCK	Saline	CCK	Saline	CCK	Saline
LF	12	17 & 18	Saline	CCK	Saline	CCK	Saline	Saline
LF	14	9 & 10	CCK	Saline	CCK	Saline	CCK	Saline
LF	15	19 & 20	Saline	CCK	Saline	CCK	Saline	Saline

1.2 Sucrose drinking: second exposure (no injections)

From experiment 4 onward (experiment 4-6), a second exposure to sucrose water was introduced after the initial first exposure (section 1.1) to determine potential diet-induced differences in satiety. During this procedure, rats were given access to a new buret containing 14 ml of 10% sucrose water for 20 minutes, with a 30 second interval between the 2 rats in each cage. However, rats received no injections and the 5-minute break before measurements was removed.

Statistical Analysis

Total sucrose water intake measures have been expressed as means. Data comparisons between HF and LF groups, including average total sucrose water intake, average decrease in total sucrose water intake, and average drinking onset time, have been analyzed using independent two-sample T-tests. However, the average slopes (all experiments combined) were analyzed using Mann-Whitney U tests, since these specific data-sets did not follow a normal distribution. Slopes of each graph were calculated over the range of minutes that provided the R^2-value closest to 0.95. Furthermore, all within-group comparisons, including the comparison of total sucrose water intake between first and second exposure, have been analyzed using paired T-tests. Lastly, changes in average intake over the weeks have been analyzed using repeated measures one-way ANOVA tests. For all statistical tests performed, a significance level of $P < 0.05$ was assumed. Analysis was performed using SPSS Statistic 26.

Results

Sucrose water intake: first exposure

HF/S diet reduced sucrose water intake compared to a LF diet

To determine potential diet-induced differences in sucrose drinking behavior between rats being fed a HF/S diet and rats being fed a LF diet, sucrose water intake was measured over 6 experimental trials in female Wistar rats. However, due to measurement errors and leaking burets, all data from experimental trial 1 was excluded from further calculations. When rats were exposed to a buret of sucrose water for 20 minutes, the rats on the HF/S diet on average showed a significantly lower total intake of 10% sucrose water compared to rats on a LF diet ($P < 0.001$), as shown in Figure 1. The average sucrose water intake for both diet groups per individual experiment is shown in Appendix A (Figure A1-A5).

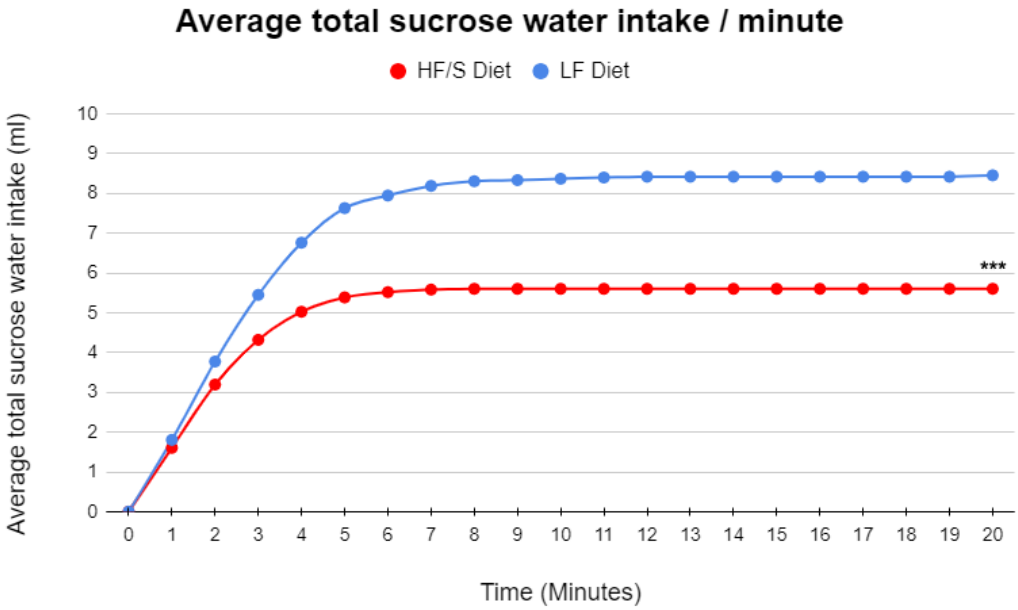

Figure 1. Total sucrose water intake over 20 minutes expressed as a cumulative average for both the HF/S and LF diet group separately. The graph shows the intake during the first exposure to sucrose water for experiment 2-6 averaged. An independent T-test revealed a significantly decreased intake in the HF/S diet group compared to the LF diet group ($P < 0.001$).

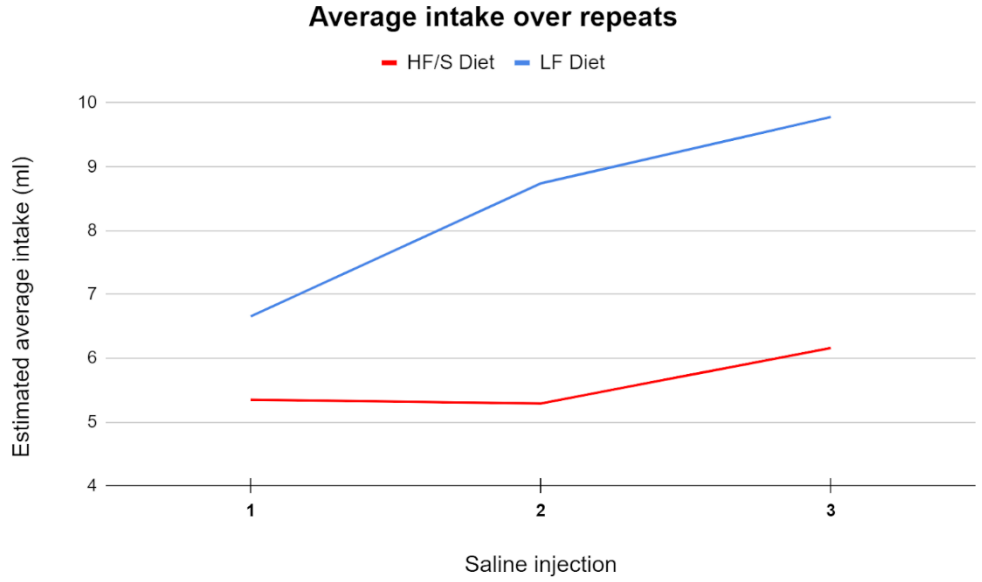

Figure 2. Comparison of the average total sucrose water intake value for each of the 3 times all rats received a saline injection. Injection 1 represents experiment 2 & 3, injection 2 represents experiment 4 & 5, and injection 3 represents experiment 6, corresponding to the first, second, and third time all 20 rats received a saline injection, respectively. Repeated measures one-way ANOVA tests revealed no significant changes in average intake for the HF/S group. However, significant positive changes in average intake were found in the LF group between injection 1 and 3 ($P < 0.01$) and between injection 2 and 3 ($P < 0.05$).

Furthermore, potential changes in the average total sucrose water intake over the three times a rat got saline were determined by comparing the average total intake values of experiment 2&3 (saline injection 1), experiment 4&5 (saline injection 2), and experiment 6 (saline injection 3) for both diet groups separately using a repeated measures one-way ANOVA (Note that 10 rats received a saline injection during experiment 2, 4, and 6 and the other 10 rats received a saline injection during experiment 3, 5, and 6, as described in the methods section). The analysis showed that there were no significant changes in the average total sucrose water intake over experimental repeats for the HF/S group, however a significant positive change was found in the LF group between saline injection 1 and saline injection 3 ($P < 0.01$) and between saline injection 2 and saline injection 3 ($P < 0.05$). The

change in average intake for both diet groups is shown in Figure 2. Furthermore, however, the difference in intake between the HF/S and LF group did not significantly change over experimental repeats.

HF/S diet reduced initial intake rate of sucrose water compared to a LF diet

In order to get better insight into potential differences between the diet groups in the initial 'likeability' of the sucrose water, the average initial intake rate for both diet groups was determined. This was done by combining all individual measurements for a given rat into an average and plotting this average intake over 20 minutes for each rat separately, after which the slope of the linear part of each average graph was calculated. Graphs displaying the individual measurements for each rat separately are shown in Appendix A (Figure A6-A26). To ensure that the slope of each graph was determined as accurate as possible, all slopes were calculated over the range of minutes that resulted in the R^2-value closest to 0.95 (note that, due to the fact that observations were only made per full minute, meaning that no data was obtained between these time points, an R^2-value of exactly 0.95 could not be consistently realized). Averaging of these slopes for both the rats in the HF/S diet group and the rats in the LF diet group separately, and subsequent analysis through a Mann-Whitney U test revealed a significantly lower average slope for the HF/S diet group compared to the LF diet group ($P = 0.016$), indicating a lower initial intake rate of sucrose water in this HF/S diet group. Furthermore, no significant differences in the average onset time of sucrose drinking after introduction of the buret were observed between the diet groups.

Sucrose water intake: second exposure

Both HF diet and LF diet lead to decreased intake after second exposure to sucrose water

After the first exposure in experimental trial 4-6, rats were exposed to a second buret containing again 14 ml of 10% sucrose water to determine potential diet-induced differences in satiety. The results show that the HF/S diet group showed a significantly decreased total sucrose water intake compared to the LF diet group during the second exposure ($P < 0.001$), as shown in Figure 3. Finally, comparison of the average total intake after the first exposure to that of the second exposure for both diet groups separately showed that both the HF/S diet group and the LF diet group showed a significant decrease in the average total sucrose water intake in the second exposure compared to the first exposure (HF/S: $P < 0.001$; LF: $P < 0.001$), as shown in Figure 4. Finally, further comparison showed that the decrease in intake from exposure 1 to exposure 2 was significantly lower for the HF/S diet group compared to the LF diet group ($P < 0.05$), as shown in Appendix A (Figure A-27).

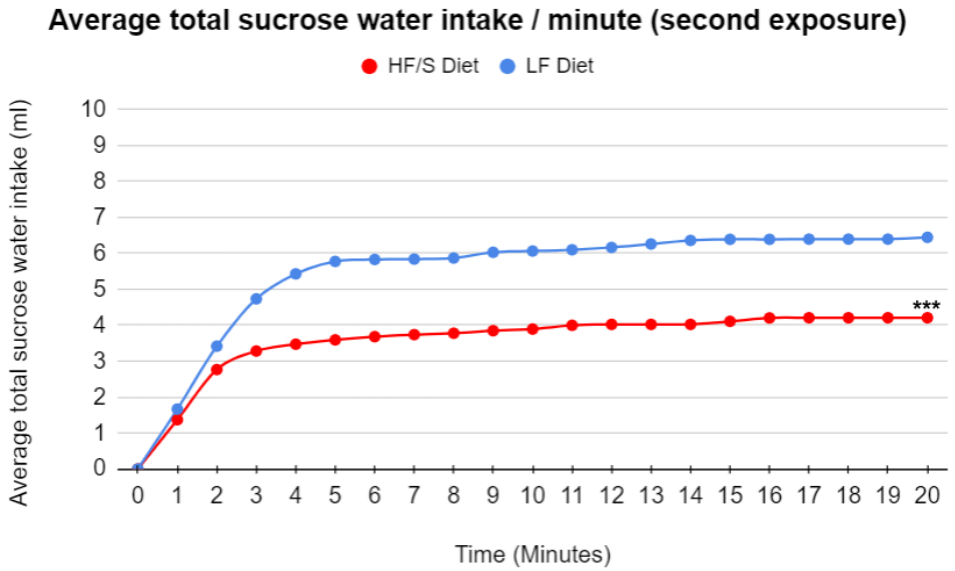

Figure 3. Total sucrose water intake over 20 minutes expressed as a cumulative average for both the HF/S and LF diet group separately. The graph shows the intake during the second exposure to sucrose water for experiment 4-6 averaged. An independent T-test revealed a significantly decreased intake in the HF/S diet group compared to the LF diet group ($P < 0.001$).

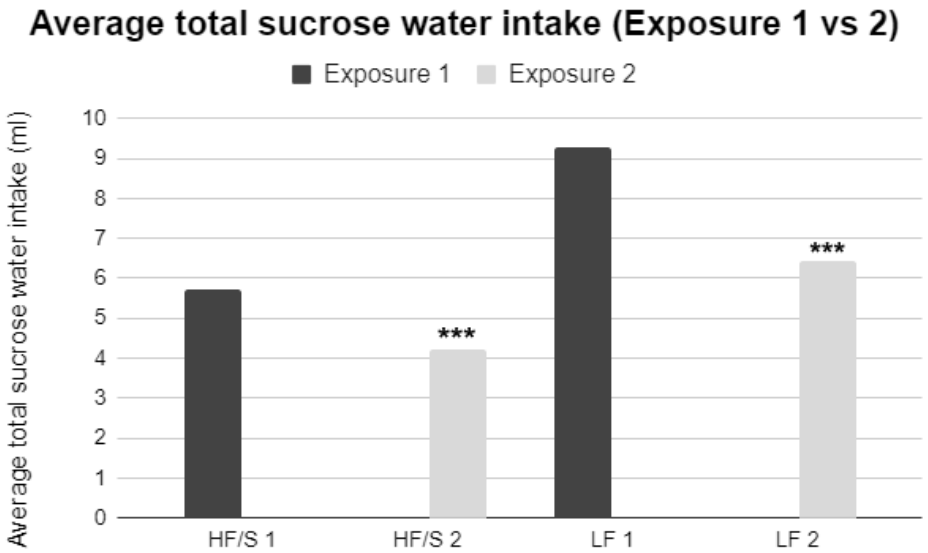

Figure 4. Comparison of the average total sucrose water intake between first and second exposure for both the HF/S and LF diet groups for experiment 4-6. Paired T-tests revealed a significant decrease for both the HF/S diet group ($P < 0.001$) and the LF diet group ($P < 0.001$).

Discussion

It has been shown that access to a high-fat diet leads to reductions in the sensitivity to signals associated with satiety, which is thought to subsequently lead to an increased intake of food (Covasa & Ritter, 1998; Savastano & Covasa, 2005). In addition, studies using rodent models have revealed that exposure to a high-fat diet led to reward deficits and a decreased dopamine turnover in the Nucleus Accumbens, thereby also influencing the reward aspects of food intake (Johnson & Kenny, 2010; Davis et al., 2008). This reward hyposensitivity has then been suggested to lead to compulsive compensation through an increased food intake (Wang et al., 2001; Wang et al., 2002). Taking all these aforementioned findings into account for this study, the expectation would clearly be that the rats on the HF/S diet included in these experiments would show both a higher initial intake rate and an increased total intake across both sucrose water exposures. However, the findings of this study show a complete contrast to the expectations described in the hypotheses. Rats being fed a HF/S diet actually show a decreased average total intake of sucrose water compared to rats on a LF diet, a finding that remains consistent throughout both the first and second exposure to sucrose water. Furthermore, the rats on the HF/S diet actually show a decreased initial intake rate of the sucrose water compared to the rats of the LF diet.

Although these results are not in line with the hypotheses in any way, these contradicting findings might actually find a possible explanation when taking into account the sex of the rats. Whereas the current study included female rats, many of the studies that formed the theoretical basis for these experiments have actually included male rats (Johnson & Kenny, 2010; Davis et al., 2008; Marques et al., 2016; Covasa & Ritter, 1998; Savastano & Covasa, 2005). And indeed, several studies investigating the effect of the estrous cycle on food intake have shown that total food intake is reduced during proestrus (Blaustein & Wade, 1976; Ter Haar, 1972), or during estrous (Wurtman & Baum, 1980; Eckel et al., 2000). This has been suggested to be caused by a reduction in meal size without a subsequent significant increase in meal frequency as compensation (Blaustein & Wade, 1976). These findings may provide an explanation for the observed reduction in sucrose water intake in rats on a HF/S diet, since the rats in the HF/S diet group already take in a high number of calories through the provided diet. Therefore, via this reasoning, the rats on the HF/S diet would then be expected to take in less fat or sugar containing food items in addition to the calorie-dense diet provided. On the other hand, the rats on the LF diet generally consume less calories through the standard chow diet, and therefore these rats would then be expected to take in more additional fat or

sugar containing food items such as, in the case of this study, sucrose water.

As is the case for most scientific research, this study also doesn't come without limitations. First, the type of light that was used during the dark phase (1PM-1AM) of the rats was switched throughout the experiment. In experiment 1 and 2, a dim light was used, whereas in the successive experiments, the room was lit using a darker red light to mimic the dark phase better. Furthermore, this study was part of a larger research project course, in which 8 different experimental projects were run concomitantly throughout a period of 4 weeks, some of which might have provided interference with the results of this project. Finally, as mentioned in the methods section, the experiments of this project took place during injection days, on which rats received either a CCK or saline injection. However, this project did not take into account CCK effects, and so only data obtained through saline-injected rats was used. This means that the injections were not required for this experiment, and might have only provided interference through injection-induced stress. Therefore, a potential future repetition of this experiment should exclude the injections given during these experiments. Taken together, a significant role of confounding factors in the explanation of the results can therefore not be ruled out.

In conclusion, we have shown that exposure to a HF/S diet actually decreased sucrose water intake compared to exposure to a regular low-fat chow diet in female Wistar rats, in contrast to the expectations formulated in the first hypothesis. Additionally, in contrast to the first hypothesis, we showed that exposure to a HF/S diet led to a decreased initial intake rate of sucrose water compared to exposure to a LF diet, suggesting that the expected compulsive consumption of sucrose water in the HF/S diet group was not observed. Finally, we showed that exposure to a HF/S diet also led to a decreased sucrose water intake after second exposure compared to exposure to a LF diet, in contrast to the expectations described in the second hypothesis. However, even though the findings show to be in full contrast to the initial expectations, we have shown that the effect of diet on food intake may show sex differences, opening up opportunities for future research to elucidate to what extent potential differences between male and female rats in the effect of diet on sucrose water intake in Wistar rats exist.

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Appendix A

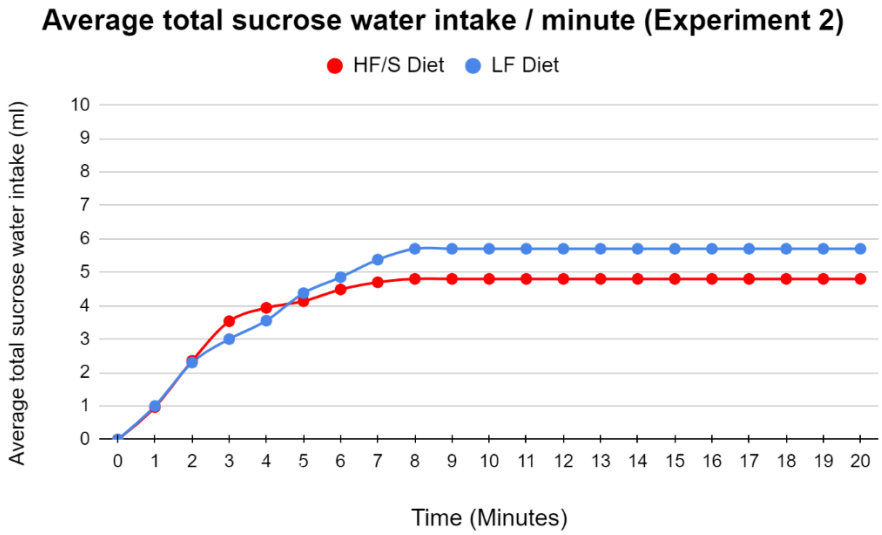

Figure A-1. Average total sucrose water intake after first exposure for experiment 2.

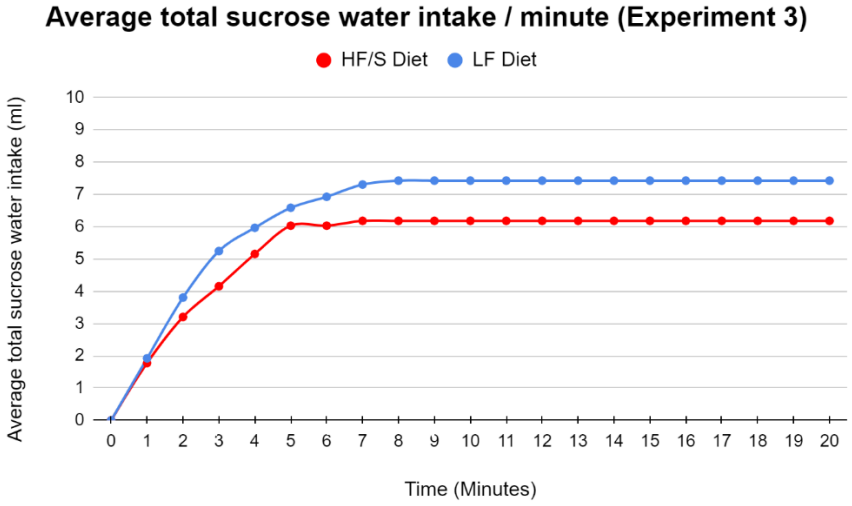

Figure A-2. Average total sucrose water intake after first exposure for experiment 3.

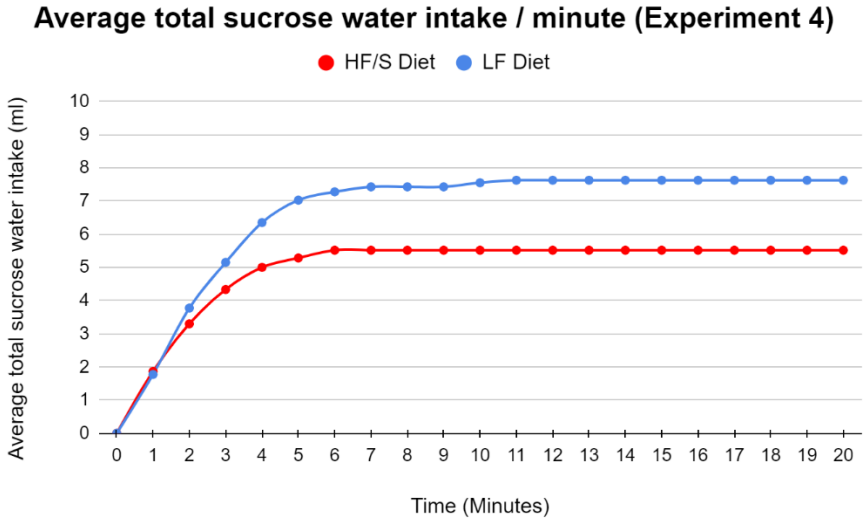

Figure A-3. Average total sucrose water intake after first exposure for experiment 4.

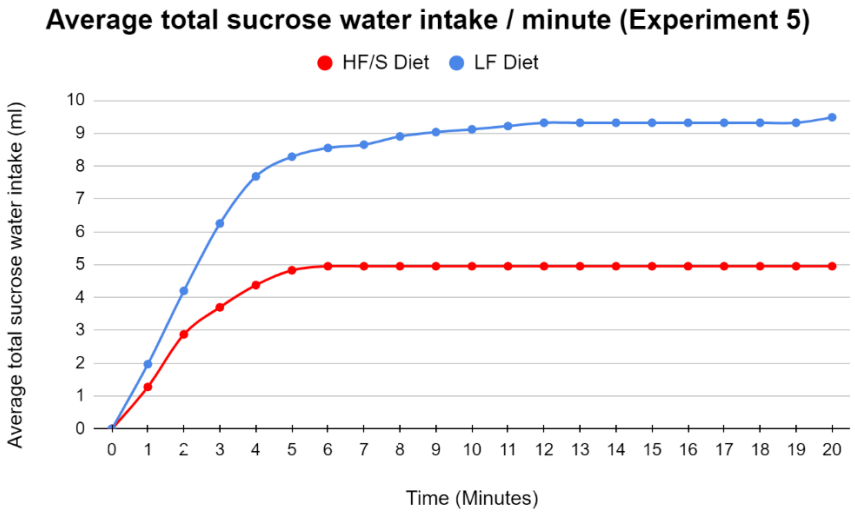

Figure A-4. Average total sucrose water intake after first exposure for experiment 5.

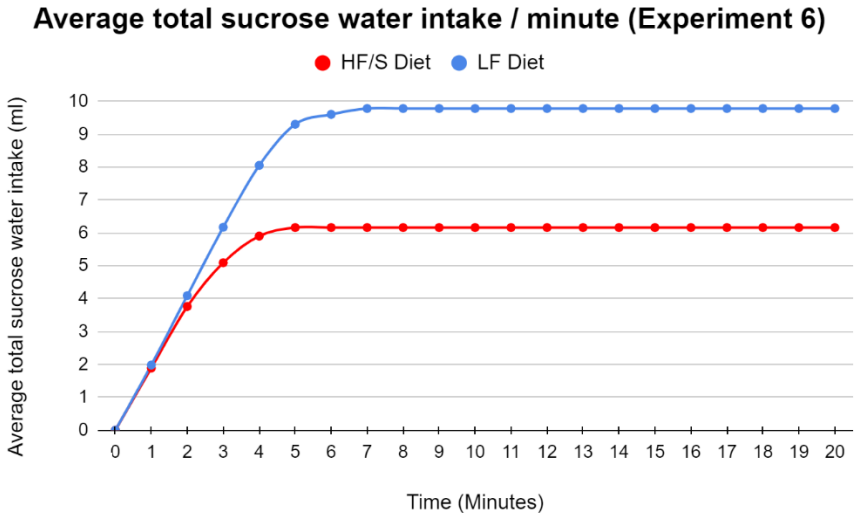

Figure A-5. Average total sucrose water intake after first exposure for experiment 6.

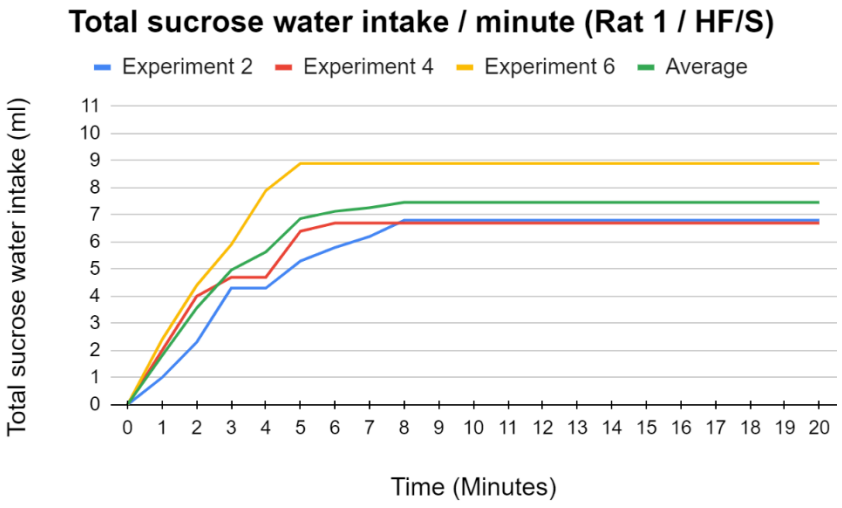

Figure A-6. For rat 1, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

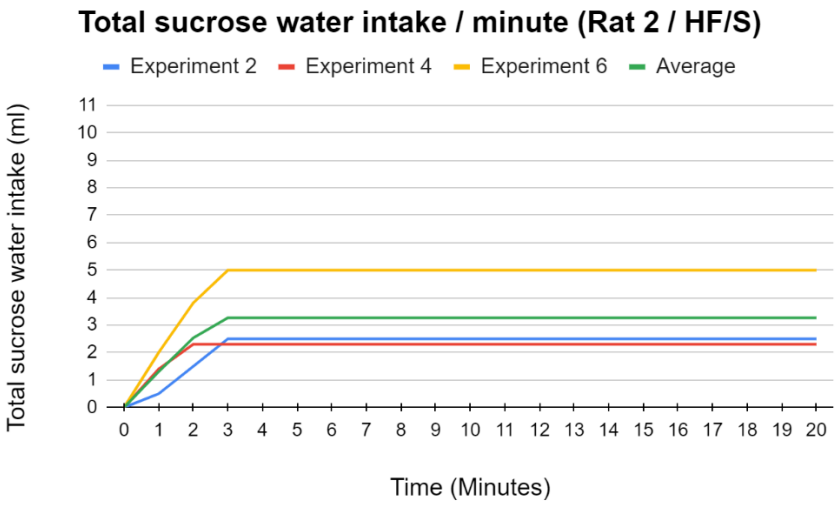

Figure A-7. For rat 2, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

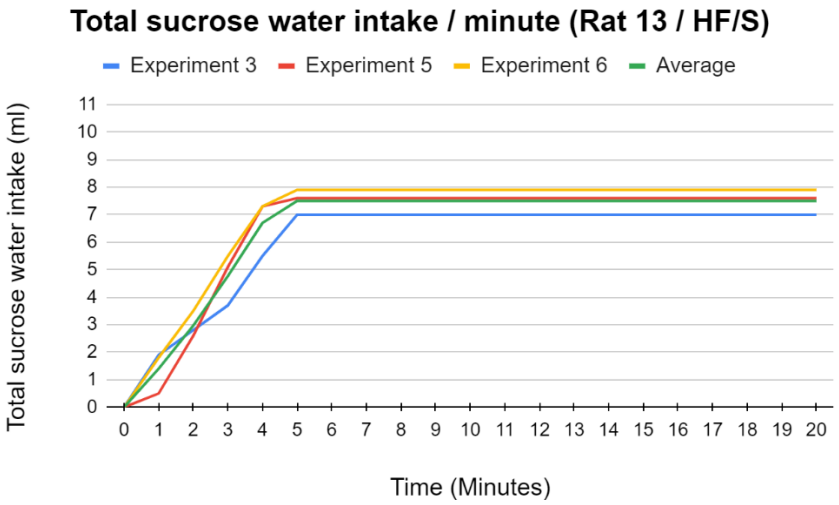

Figure A-8. For rat 13, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

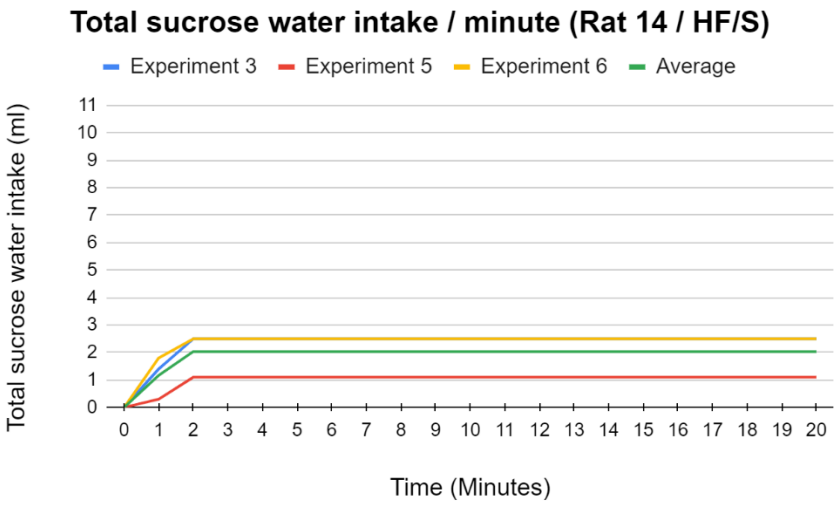

Figure A-9. For rat 14, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

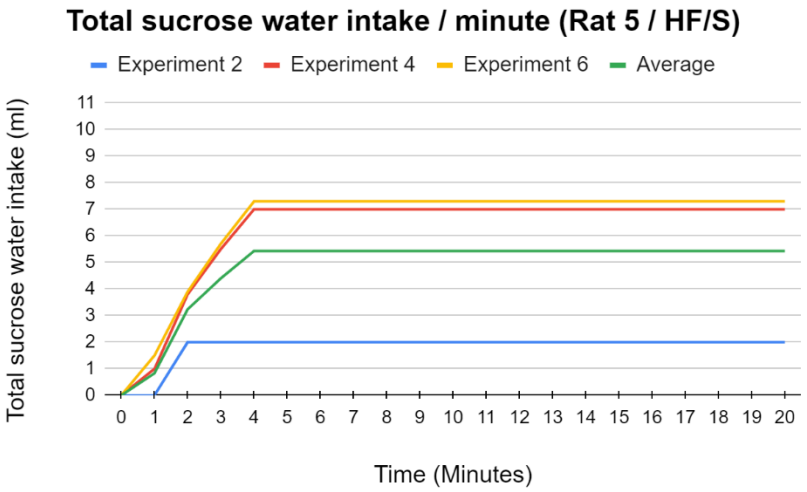

Figure A-10. For rat 5, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

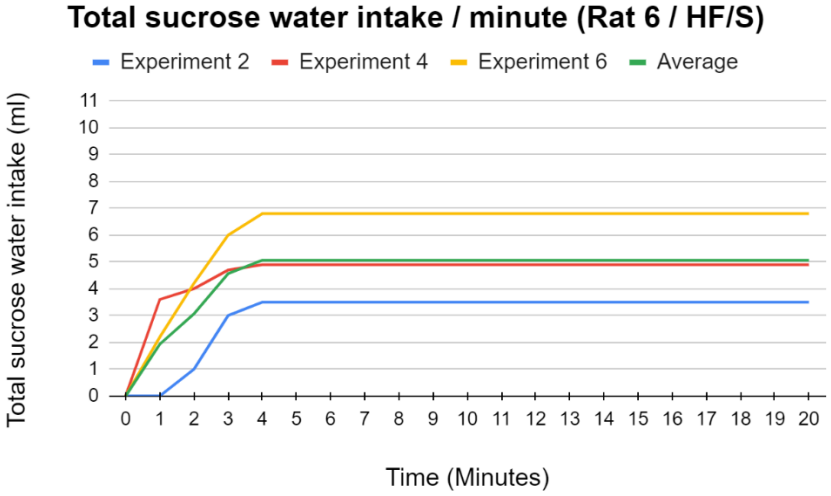

Figure A-11. For rat 6, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

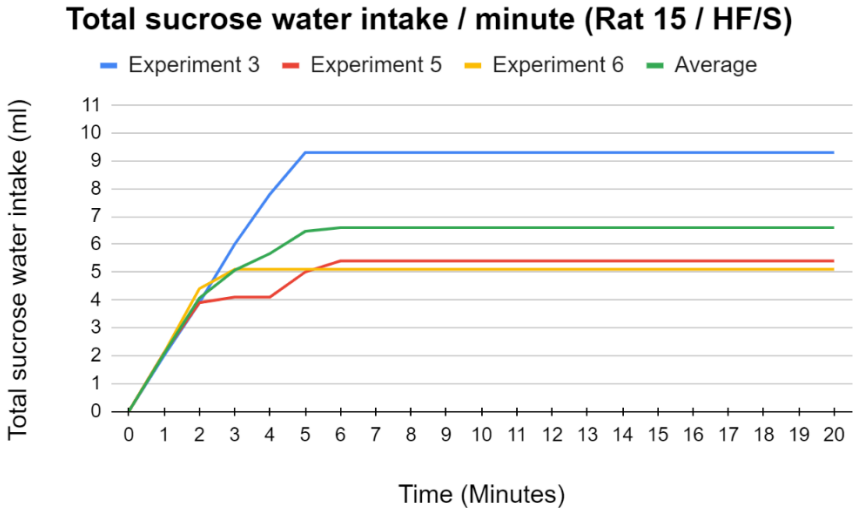

Figure A-12. For rat 15, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

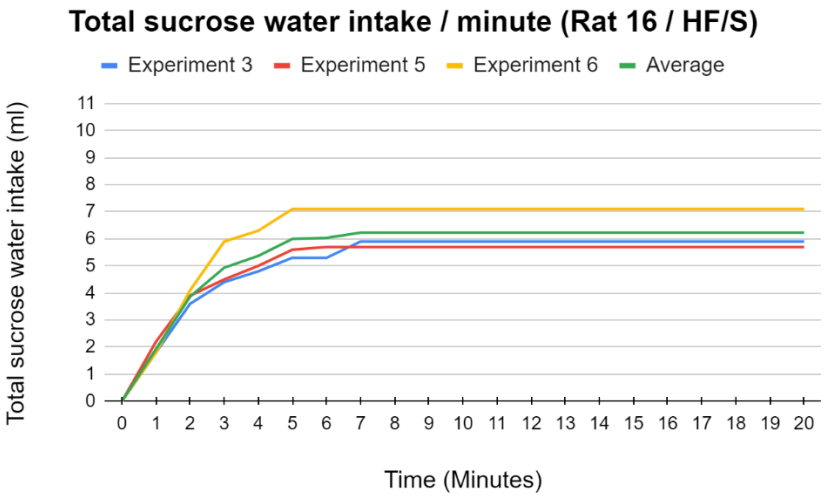

Figure A-13. For rat 16, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

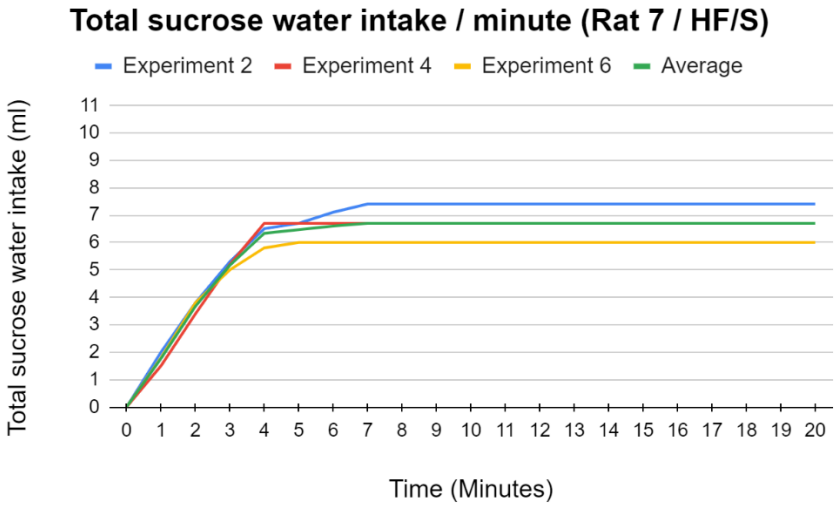

Figure A-14. For rat 7, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

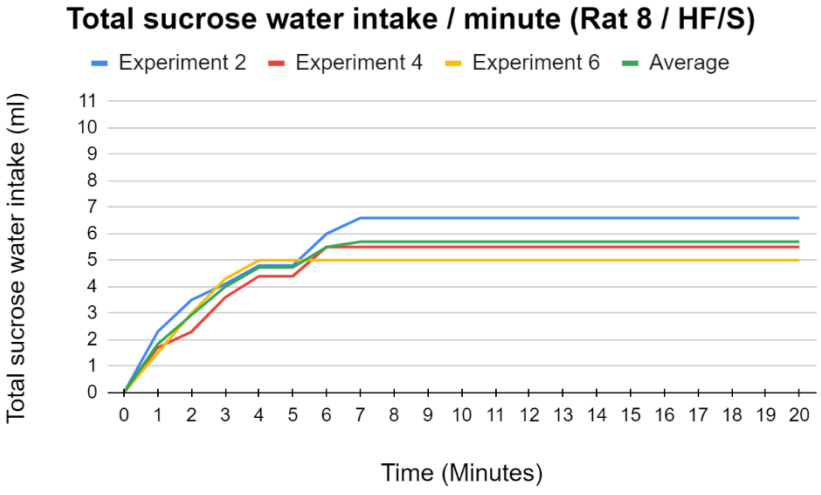

Figure A-15. For rat 8, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

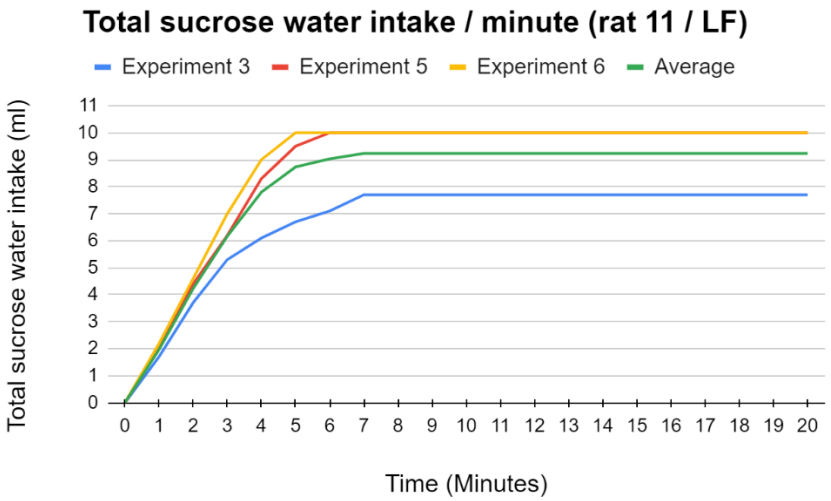

Figure A-16. For rat 11, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

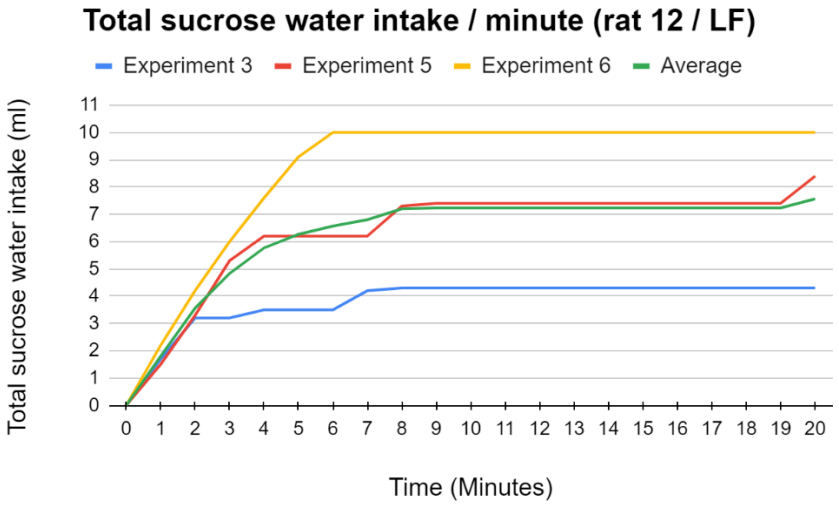

Figure A-17. For rat 12, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

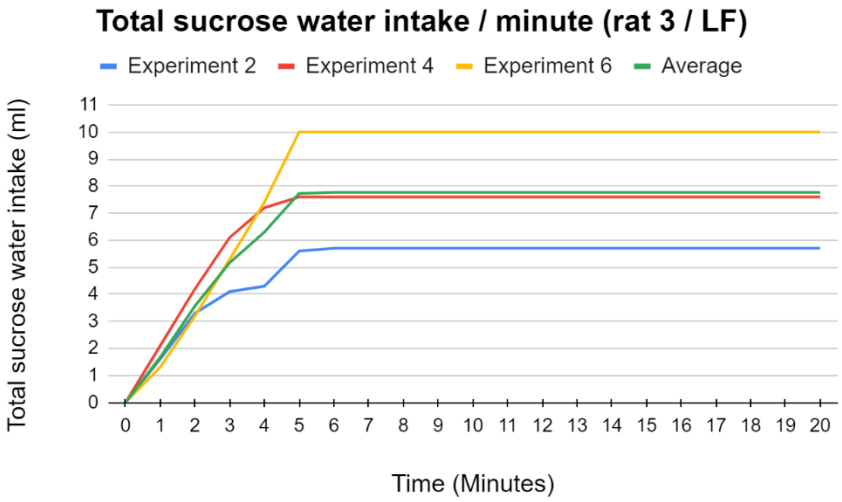

Figure A-18. For rat 3, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

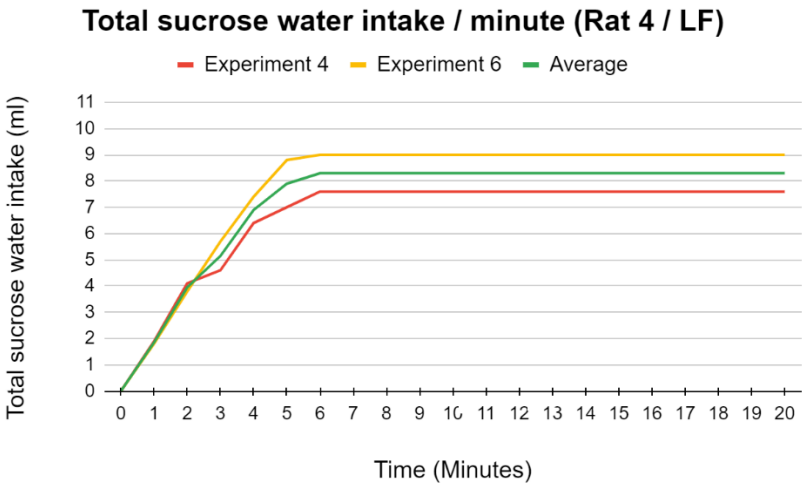

Figure A-19. For rat 4, the separate measurements of sucrose water intake over 20 minutes for experiment 4 and 6. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

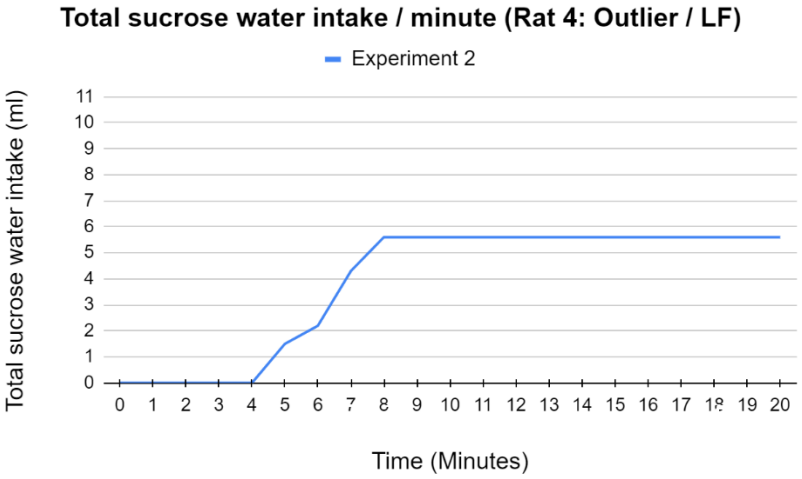

Figure A-20. For rat 4, the outlier in terms of onset time of drinking. The slope of this measurement was calculated separately from the average intake curve of rat 4 shown in Figure A-19.

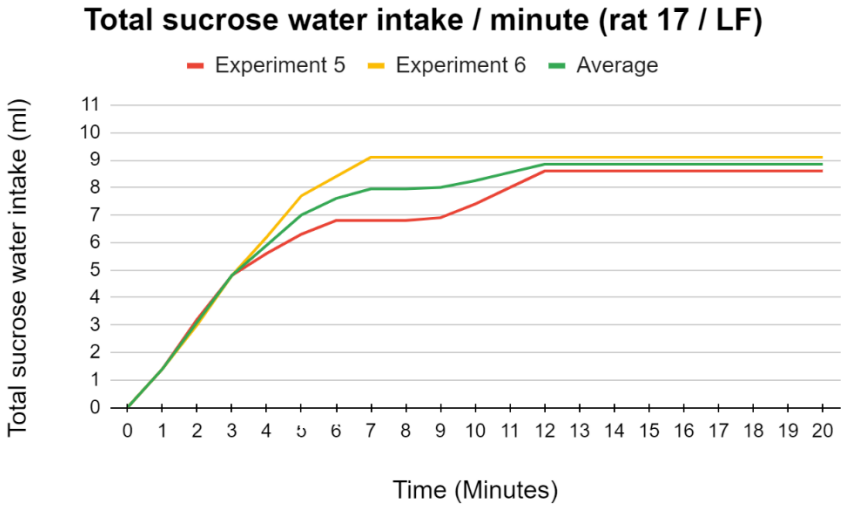

Figure A-21. For rat 17, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope (Note: rat 17 only has 2 measurements, the third was lost due to error.

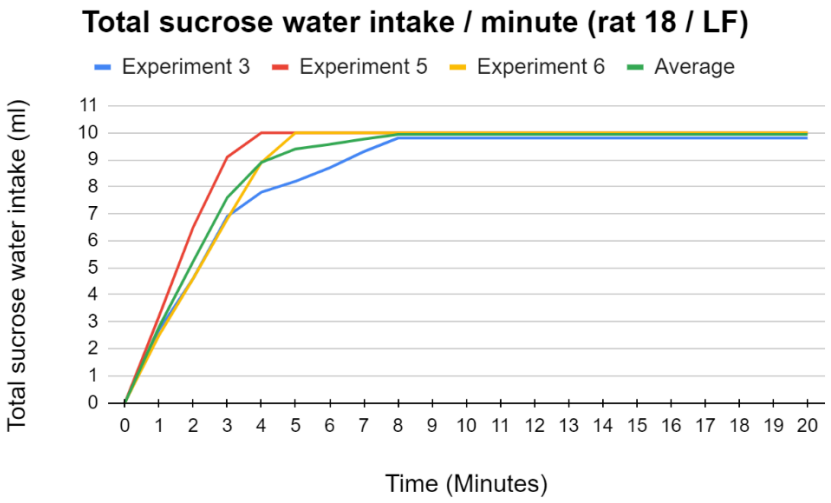

Figure A-22. For rat 18, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

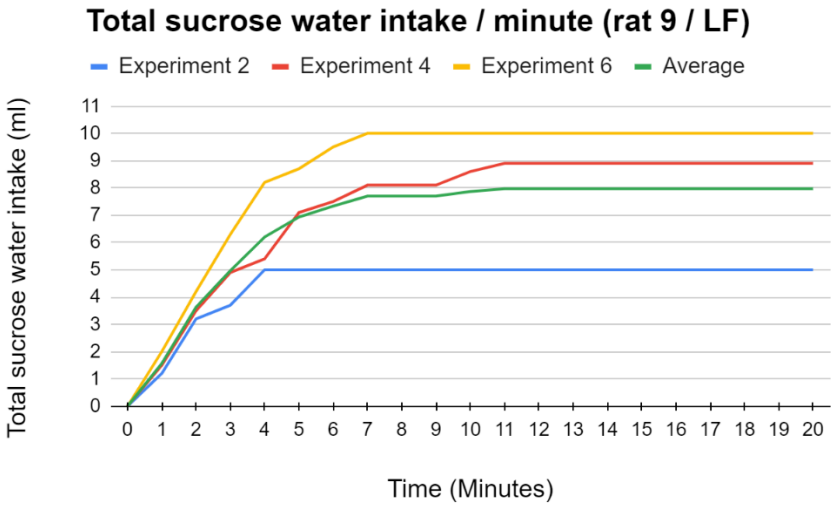

Figure A-23. For rat 9, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

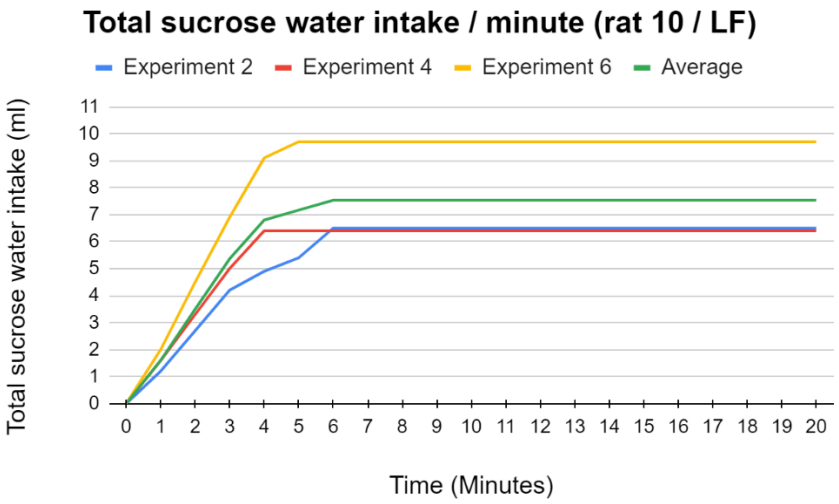

Figure A-24. For rat 10, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

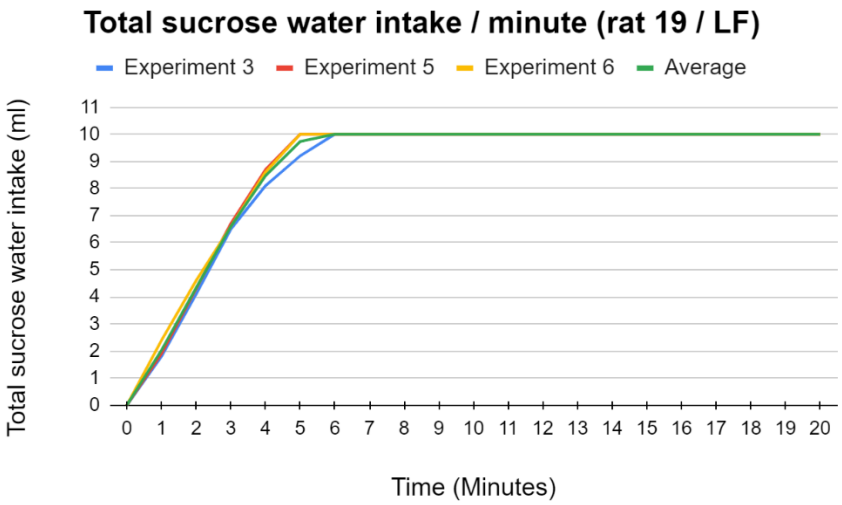

Figure A-25. For rat 19, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

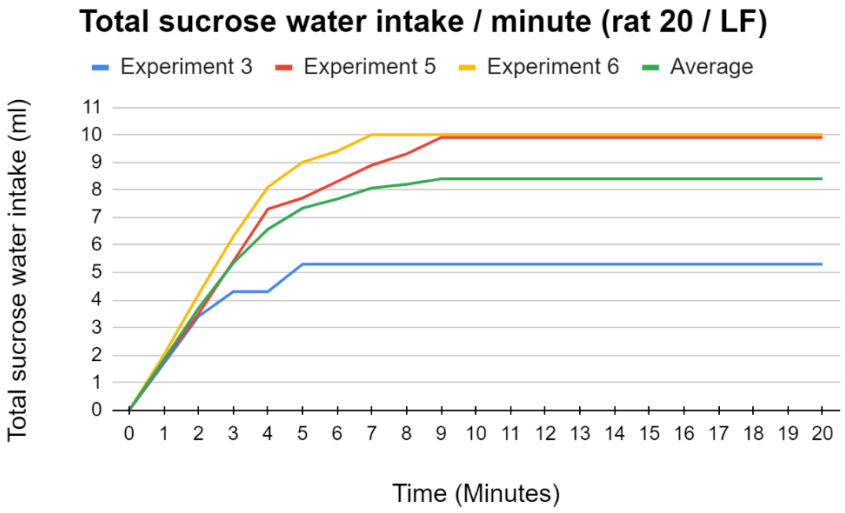

Figure A-26. For rat 20, all separate measurements of sucrose water intake over 20 minutes. The average curve displays the combination of the all separate measurements. This average curve (in green) was used to calculate the slope.

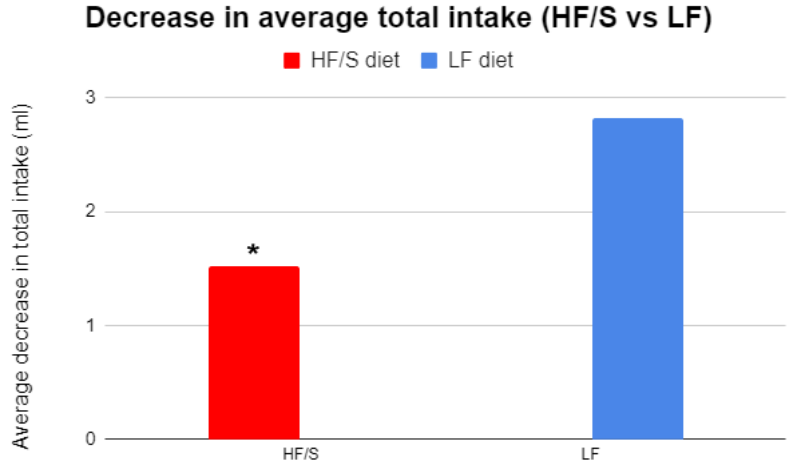

Figure A-27. Comparison of the average within-diet decrease in total sucrose water intake in exposure 2 compared to exposure 1 for both the HF/S diet group and the LF diet group. Analysis using an independent T-test revealed a significantly lower decrease in intake for the HF/S diet group compared to the LF diet group ($P < 0.05$).