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Interactions between Dietary Niacin and Sucrose Intake on Body Weight and Food Intake in Mice

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Abstract

Several sources of data suggest that obesity rates differ between countries that fortify their foods with niacin (also known as vitamin B3) and those that do not, which leads to the question whether niacin is involved with the metabolic disorder. Niacin plays a role in the breakdown of carbohydrates and nowadays people in many countries receive an excessive amount of niacin through their diet. Correlations between niacin intake and obesity rates have already been observed, but no causation has been established yet. In this study, we aimed to determine whether excessive dietary niacin leads to increased weight gain and food intake compared to a diet containing the minimal niacin requirement, when mice are presented with 30% sucrose access. Firstly, the minimal required niacin for mice was established by stepwise lowering niacin supplementation when mice were on a niacin-free diet. From this, we concluded that, under the circumstances of our experiment, mice do not need any niacin in their diet as they are able to convert tryptophan into niacin. For the second part of the experiment, half of a new cohort of mice received a control diet and the other half received a niacin-deficient diet. Half of these mice had access to a 30% sucrose solution. Once every 3 days, body weight, food intake, and, if applicable, sucrose intake were measured. Additionally, at the beginning and the end of the experiment, lean mass and fat mass were measured with echoMRI. Two-way ANOVA and repeated-measures ANOVA revealed no effect of dietary niacin, sucrose or the interaction between the two in females for any of the measured factors. In males, only excessive niacin independently of sucrose intake resulted in a higher body weight. However, the 30% sucrose solution failed to significantly increase body weight in both sexes. Further studies are required to investigate if the observed pattern persists in the presence of a diet that is successful at inducing weight gain.

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Introduction

The importance of vitamins in the diet becomes ever so clear when they are lacking. One of the diseases that results from a shortage of vitamins in the diet, in this case vitamin B3, is pellagra. This disease, in 1753 first described by Gaspar Casal of Oviedo, was later named after one of its main symptoms by Frapolli: "*pelle"* and *"agre",* rough skin. Besides the aforementioned dermatitis, diarrhoea, dementia, and death complete the 4 D's by which pellagra is characterised (Oldham & Ivkovic, 2012). From the early 1900s, pellagra was not merely a disease endemic to regions in Europe such as Northern Italy, it also occurred in southern regions of the United States. It was not until 1937, that the compound responsible for pellagra was identified: vitamin B3, also known as niacin or niacinamide (Makarov et al., 2018; Mariani-Costantini & Mariani-Costantini, 2007). Foods rich in niacin include poultry, beef, and legumes (NIH, 2022), which the corn-based diet of the poor at that time was glaringly lacking. In response to this discovery, the Italian government urged its citizens to shift their diet from corn-based to one containing more niacin-rich foods. In the case of Italians, this meant to drink more unfiltered wine and to eat more cheap protein such as rabbit (Schatzker, 2022). The U.S. government, however, took a different approach to eradicating dietary niacin deficiency. They fortified grain products such as bread, flour, and grits with niacin. The fortification was initially voluntary but later became mandated (Clay et al., 2019).

Now, almost a century later, pellagra has been almost entirely eradicated. Many countries followed the U.S.'s suit and implemented mandatory niacin fortification, for instance Kuwait, Qatar, and Saudi Arabia (Al Jawaldeh et al., 2019). Save for severely malnourished people such as those struggling with alcohol use disorder, pellagra is no longer common in the general population (Oldham & Ivkovic, 2012). Instead, the pellagra outbreaks are replaced by a different one: the obesity pandemic. Worldwide, around 2.8 million people per year die from the consequences of obesity (WHO, 2021). What is highly remarkable, is the stark difference between the two previously described regions, Northern Italy and Southern United States. The U.S. has a 41.9% obesity rate in adults (Trust for America's Health, 2023). Northern Italy, on the other hand, has merely a rate of around 8-9% (Azzolini & Ricciardi, 2019). This pattern is not only contained to the countries struggling with pellagra in the past. Countries such as Finland and Norway where niacin fortification is prohibited have relatively low child obesity rates around 2.5%. Kuwait and Saudi Arabia on the other hand, have increased rates by 14.6% and 6-6.7%, respectively (Zhou, 2014).

Multiple factors have been suggested to contribute to this difference in obesity rates between countries, such as the consumption of ultra-processed foods (Juul et al., 2018). It has also been hypothesised that niacin is another factor involved. Niacin and its similar compounds are precursor molecules of NAD+ and NADP+, which play an important role in cellular redox reactions. Hereby, one of the main processes for which redox reactions are essential is energy metabolism. NAD+ is a crucial factor in glycolysis, whilst NADP+ is vital for fatty acid synthesis (Xiao et al., 2018). Therefore, the

niacin we get through our diet has an influence on our fat and sugar metabolism. For instance, niacin influences glucose tolerance. It stimulates insulin release and is involved with the induction of insulin resistance (Kahn et al., 1989; Miettinen et al., 1969). In addition to this, niacin also promotes adipogenesis (Fujimori & Amano, 2011).

As becomes clear from the pellagra cases, these functions of niacin are a requirement for optimal health. What is less well known, however, are the consequences of an excess of dietary niacin, instead of a lack. As a consequence of the mandatory B3 fortification, U.S. citizens receive an excess of vitamin B3 in their diet. The recommended dietary allowance for adults is 16 mg of niacin (or a niacin equivalent) per day for men, and 14 mg for women (NIH, 2022). In the early 1900s, the average niacin intake was around 17-18 mg niacin per day, with individuals below this range developing pellagra. Nowadays, the average U.S. citizen ingests around 32 mg of niacin per day, which is twice the amount that is recommended (Zhou, 2014). Combining this knowledge with that of the functions of niacin, leads us to the intriguing question as to whether people have become too efficient during carbohydrate metabolism and whether this ultimately contributes to the development of obesity.

This rationale prompts us to look at the general role of niacin on metabolism and weight. It is already known that adding niacin to the diet stimulates growth. For example, in farm animals, where niacin supplementation has already proven to result in a significantly increased body weight, compared to animals that do not receive the supplementation. In chickens, for instance, it has been found that fortification of the food with nicotinic acid results in maximal average daily gain of body weight (Jiang et al., 2011). Similar patterns are observed in pigs (Ivers & Veum, 2012; Real et al., 2002). In a comparable manner, niacin also stimulates growth when added to baby formula. Formula is supplemented with multiple B-vitamins, including niacin, in an excessive amount and significantly higher than in breast milk. In some brands, the added niacin is up to 4 times as much as the recommended amount to be supplemented (Zhou, 2014). What is more, babies being formula-fed increase faster in weight than breast-fed babies during the first year (Dewey, 1998). The correlation persists also later in life, with formula-fed babies being more prone to developing obesity (Arenz et al., 2004). It is hypothesised that the high levels of niacin in the formula contribute to these findings (Li et al., 2013).

When looking at the effects of niacin in adults, it becomes clear that niacin intake is associated with their growth as well. Taking vitamin B supplements in general causes an increase in appetite that is additionally associated with an increase in BMI (Othman et al., 2023). For niacin specifically, correlations have been observed between the per capita daily intake and the obesity rates of a country. In U.S. adults, niacin consumption for both men and women is correlated with obesity prevalence in the U.S, as found by the study Zhou et al. (2010). In 1974, niacin fortification legislation became stricter, which resulted in a higher per capita intake of niacin. With a lag of 10 years, the obesity prevalence increased starkly. The niacin intake from the years 1978-1994 was significantly correlated with the obesity prevalence from 1988-2004. In a similar manner with a 16-year lag, diabetes prevalence was

found to be correlated with the per capita niacin intake. In a separate study focussing on U.S. children, this same pattern has been found, with again a lag of 10 years between increasing per capita niacin intake and a consequent surge in obesity cases (Li, 2010). Besides the U.S., this correlation has been additionally observed in Saudi Arabia, where niacin fortification started in 1978 (WHO, n.d.). By the 1980s and 1990s, obesity rates in children were vastly increased (Zhou, 2014).

However, the aforementioned epidemiological studies are all correlational. To our knowledge, no studies have investigated a causal relationship between excessive dietary niacin intake and body weight gain. The current study therefore aims to investigate this relationship between niacin intake and weight gain in both male and female C57BL/6NRj mice. This study's main hypothesis was that the mice with access to sucrose and excessive niacin in the diet, would gain more weight at a faster pace than those who did not have sucrose access, but whose diet had the minimal niacin requirements. Firstly, the minimal niacin requirement of the mice was established by stepwise lowering the niacin concentration in drinking water. The control diet used in this experiment contained 30 mg/kg niacin, twice the estimated daily requirement for rats (National Academies Press (US), 1995), which acted as the baseline level. Secondly, after having established the minimum dietary niacin level, half of the mice received their regular diet, whilst the other half received a diet containing the minimal required niacin concentration. Additionally, half of the mice underwent a protocol that induced obesity. That is, they were given access to 30% sucrose solution.

Materials and Methods

Experiment 1: Establishing Minimum Niacin Requirements

Sixteen male and sixteen female C57BL/6NRj mice aged 6-8 weeks were purchased from Janvier (France). Upon arrival, the mice were housed in a temperature- and humidity-controlled room, maintained on a 12:12 light:dark cycle, with lights on at 8:00. Two mice of the same sex shared a cage, with a perforated divider that allowed visual, olfactory, and auditory communication while maintaining physical separation. Mice were allowed 5 days of habituation to the facility, after which they were put on a control diet for a week (Research Diets; D11051801, for specifications see Table S1). Food and water were provided ad-libitum. During this week, the 12:12 light:dark cycle shifted to lights on at 0:00. Furthermore, starting this week, body weight, food intake, and water intake were measured daily between 9:00 and 11:00. After a week on the control diet, half of the mice were, based upon their bodyweight and baseline food- and water-intake, attributed to the "experimental" group and put on a niacin deficient diet (Research Diets; D2308240, for specifications see Table S2). Additionally, this group received 30 mg/L niacin (in the form of nicotinic acid; N4126, Sigma-Aldrich) in their drinking water. Mice were kept under these conditions for one week, after which the niacin-restricted group was subjected to stepwise decreasing of the niacin concentration in the drinking water. That is, niacin

concentrations were subsequently 15 mg/L, 7.5 mg/L, 4 mg/L, 2 mg/L, 1 mg/L and 0 mg/L. They were maintained on these levels of concentration for 3 days, allowing their body weight to stabilise. If, after 3 days, body weight did not decrease, lowering of the niacin concentration to the next tier occurred. On the other hand, if the body weight of an individual mouse decreased with a maximum of 10%, then niacin levels would immediately be increased. Concurrently, the control group remained on the control diet and received 0 mg/L niacin through their drink water.

Experiment 2: Effect of Excess Dietary Niacin on Body Weight

24 male and 24 female C57BL/6NRj mice (aged 8 weeks, from Janvier) arrived at the animal facility and habituated for 2 weeks, after which half of them were put on a control diet or on a niacin deficient diet. Housing conditions and diet were equal to the first cohort. Before the start of the experiment, the lean- and fat mass of the mice was established using an EchoMRI™-500 scanner. After 7 days on the diet, half of the mice of each group received additional ad-libitum access to a 30% sucrose solution (white sugar fine granulated, Dansukker), which was changed every 3 days. Group allocation was based on body weight and body composition, with n = 6 per group. Once every 3 days at the end of the light phase (09:00 - 12:00) body weight, food intake, and sucrose intake were measured for the duration of 12 weeks. At the end of these weeks, body composition was again measured with the EchoMRI scanner, after which the animals were sacrificed. Due to compulsive self-grooming, one female mouse (control + no sucrose group) had to be excluded from the experiment.

Data analysis

To limit the number of experimental animals, the variable criteria sequential stopping rule (vcSSR) was applied as described by Fitts (2017). This rule allows us to work with a minimal amount of mice. If the p-values were not yet significant but there seemed to be a trend, the sample size per group can be expanded by adding more mice without influencing the type I error rate. Based on an initial group size of n=6 and the option of adding 6 and 12 additional mice, the threshold values for our p-values were 0.3 (upper bound) and 0.026 (lower bound). This means that when the p-value of the comparison most of interest (bodyweight difference of control + sucrose mice versus niacin deficient + sucrose mice) is > 0.3, the experiment is stopped and the null hypothesis is accepted (there is no difference in body weight gain due to dietary niacin). If the p-value is < 0.026 the null hypothesis is rejected. For all other comparisons, a p-value of 0.05 or less is considered to be a significant effect, as suggested by Fitts (2017).

The production of graphs and the preprocessing of the data were conducted using Python (version 3.11.5) via Jupyter Notebook (version 6.5.4). All statistical tests were done with JASP version 0.18.3.0. If no significant interaction with sex was found, then the sexes were pooled. If significant interactions or a main effect of sex were identified, then analysis proceeded with each sex individually.

Data from the first experiment was analysed with repeated measures ANOVA, with time being the within-subject factor and diet the between-subjects factor. When the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. The post-hoc Holm test was used to investigate the effect of time on body weight.

The second experiment has been pre-registered in Open Science Framework before the start of data analysis. From the body weight measurements, the delta body weight was calculated by taking the average of the last 3 days of measurements of the experiment and subtracting the average of the first 3 days. Delta bodyweight was analysed with a two-way ANOVA, with the independent variables being sucrose access and diet. Post-hoc Tukey tests were conducted when applicable. Additionally, the difference in lean mass and fat mass between the two echoMRI measurements was calculated. This was done by calculating the % of fat weight of the total body weight and then subtracting the final % from the initial %. Consequent statistical testing proceeded in a similar manner as for delta body weight. For all ANOVAs, assumptions were checked by plotting a QQ-plot and conducting Levene's test.

The calorie intake due to food intake and sucrose intake was calculated separately and a ratio of calories from sucrose:calories from food was computed, as well as the total number of calories ingested. These factors were averaged and binned per 3-week period. A repeated measures ANOVA was conducted, with time being the within-subject factor and diet the between-subjects factor. For the total calories, sucrose was an additional between-subjects factor. When the sphericity assumption was not met, the Greenhouse-Geisser correction was again employed. Further, Levene's test was conducted to check for the homogeneity of variances. When this assumption was not met, data transformation was attempted. The following data transformations were conducted and checked for meeting the assumption: square root, cubicle root, logarithm both 10 and e, x ^ e, and 1/x. For the factor of sucrose ratio in the females, data transformation failed to correct for the homogeneity of variances violation. These data therefore ought to be interpreted with caution.

For all measurements, missing data points were removed from the dataset. When food intake was < 1 g per day and sucrose and water intake was < 1 ml per day, a measurement error was assumed, and the data points were removed.

Results

Experiment 1

The aim of the first experiment was to determine the minimal niacin requirements. Starting at day 0, half of the mice switched to a diet lacking niacin. For the first 7 days, mice got 30 mg/L niacin supplemented in their drinking water. This concentration resulted in an approximately equal intake of niacin compared to the control group, based upon baseline water intake and food intake measurements. Thereafter, once every 3 days the drinking water niacin concentration was halved. As is clear from

Figure 1, during the entirety of the experiment, the body weight of both the male and female mice undergoing this decrease in stepwise niacin concentration did not differ from their control counterparts. This prompted the next decreasing step. Ultimately, the concentration of 0 was reached at day 27 and remained stable for 7 days. In these 7 days, body weight of the niacin-deficient groups did not change. A repeated-measures ANOVA per sex and consequent post hoc Holm test were conducted to verify these observations and to justify the decrease of niacin supplementation. For both the males and the females, the average body weight during the transition of the baseline to the 30 mg/kg niacin concentration proved to be significant. Additionally, significant body weight changes during the transition from 30 mg/kg to 15 mg/kg were found. When looking at the graph, it becomes clear that the found significance is due to an increase and not a decrease of body weight. Therefore, lowering niacin concentration was justified. After all further transitions in both sexes, no significant body weight changes were observed. All p-statistics can be found in Table S3.

Simultaneously to body weight, food intake and water drinking were measured daily. The switch from chow being the niacin source to supplementing drinking water with it instead, did not prompt a change in eating or drinking behaviour. Similarly, lowering the niacin concentration up until a concentration of 0 mg/L did not alter this behaviour either, as can be observed in Figures S1 and S2.

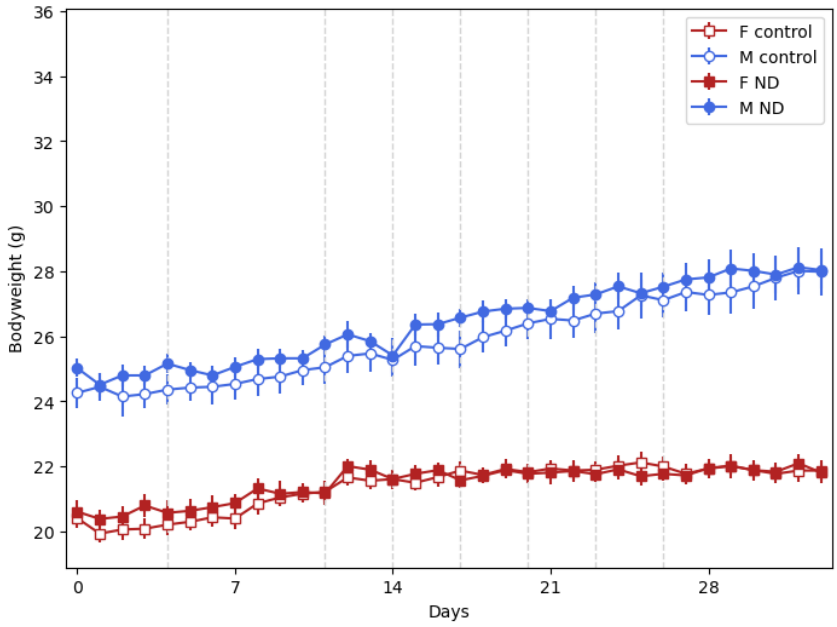

Figure 1. *Body weight course during experiment 1*. For both sexes, the minimal niacin requirement seems to be 0 mg/kg, as body weight did not significantly decrease over the duration of the experiment. Body weight is shown each day for male (blue) and female (red) mice on either control diet (open circles) or niacin-deficient (ND) diet (filled circles). The first dotted line indicates the switch from food as the niacin source to supplementing. Subsequent dotted lines indicate days on which niacin concentration is halved. Data are mean ± standard error of the mean.

Experiment 2

The main outcome measurement is body weight, which was measured once every 3 days. In Figure S3 the progress/track of body weight is displayed. More specifically, of main interest was the difference in the animal's body weight from the beginning of the experiment compared to the end of the experiment. This is displayed in Figure 2, with the body weight data of the first three days averaged and subtracted from the average body weight of the last three days of the experiment. This difference in body weight can be referred to as delta body weight. An initial 3-way ANOVA revealed a significant effect of sex on delta body weight ($F_{1,39}$ = 22.215, $p < 0.001$). The subsequent two-way ANOVA for females revealed no significant effects of sucrose ($F_{1,19}$ = 3.382, p = 0.082), diet ($F_{1,19}$ = 1.695, p = 0.209), or the interaction between the two ($F_{2,19}$ = 1.213, p = 0.285). In males, no main effect of sucrose ($F_{1,20}$ = 0.289, p = 0.597) and of the sucrose x diet interaction ($F_{2,20}$ = 0.156, p = 0.697) was found. However, diet independently showed a significant effect on body weight change ($F_{1,20}$ = 4.941, p = 0.038) with male mice on niacin-deficient diet having gained less weight relative to mice on the control diet. Thus, for males, dietary niacin seems to be involved with body weight.

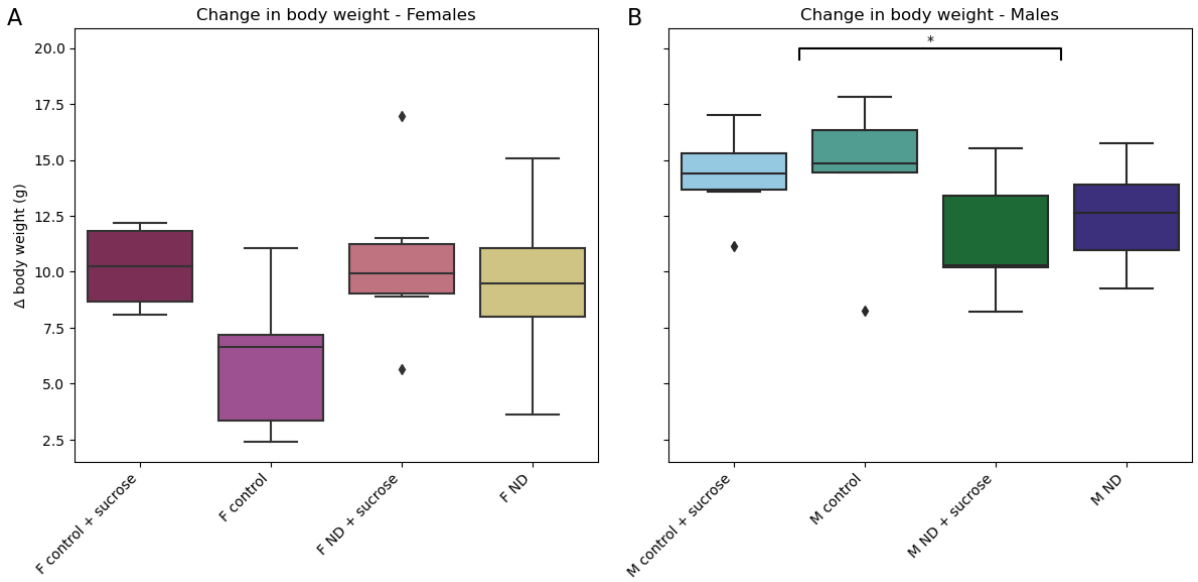

Figure 2. *Body weight gain.* A) No effect of sucrose access, dietary niacin, or the interaction has been found in females. B) In males, sucrose and the interaction of sucrose with niacin has no effect on delta body weight. Excessive niacin in the diet results in a higher delta body weight. * = p <0.05.

When comparing the mice's body composition at the beginning of the experiment with the end, the fat percentage was significantly increased (paired-samples t-test, $p < 0.001$). The difference between fat % over the experiment is displayed in Figure 3A and 3B for females and males, respectively. The initial 3-way ANOVA shows a significant effect of sex on fat gain ($F_{1,39}$ = 12.687, $p < 0.001$). Therefore, for each sex separately, a 2-way ANOVA was conducted. For females, no significant effect of either

diet ($F_{1,19}$ = 3.051, p = 0.097), sucrose ($F_{1,19}$ = 1.185, p = 0.290) or a diet * sucrose interaction ($F_{2,19}$ = 1.438, p = 0.245) was found. The same was observed in the male group, with no main effect of diet ($F_{1,20}$ = 2.108, p = 0.162), sucrose ($F_{1,20}$ = 0.016, p =0.902), or the interaction ($F_{2,20}$ = 3.092, p = 0.094). These patterns were identical when investigating lean mass (Figure S4).

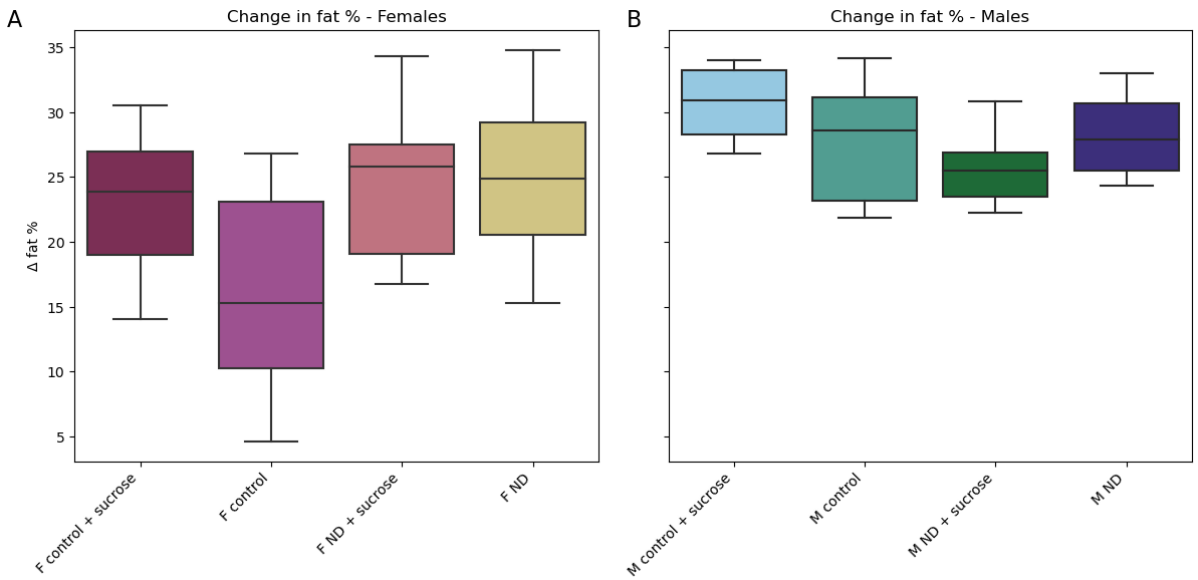

Figure 3. *Change of fat mass.* A) In females, no effect of sucrose, niacin, or the interaction of the two has been found on delta fat over the experiment. B) In males, the same insignificant findings are observed.

In addition to body weight and composition, food intake was measured for the duration of the experiment, as well as sucrose consumption. Firstly, in both sexes only sucrose access resulted in a significantly altered food intake. That is, mice with access to sucrose lowered their food intake (Figure S5). Secondly, in females diet did not influence the sucrose intake (Figure S6). On the other hand, male mice on control diet consumed more sucrose than mice on niacin-deficient diet, as shown by the main effect of diet ($F_{1,20}$ = 0.906, p = 0.018).

From both food intake and sucrose intake, the number of calories gained from these sources can be calculated, as well as the total calorie consumption (Figure 4). The initial repeated-measures ANOVA showed a significant effect of sex on the total calorie intake ($F_{1,39}$ = 5.610, p = 0.023) with males consuming more than females. Consequent repeated-measures ANOVA revealed for both males ($F_{1,20}$ = 14.039, p = 0.001) and females a significant effect of sucrose access on daily calorie intake ($F_{1,19}$ = 63.569, p < 0.001). That is, mice with 30% sucrose access had a higher total calorie intake compared to the mice that did not have this access. No effect of dietary niacin on calorie intake in both males and females was found ($F_{1,20}$ = 3.119, p = 0.093 and $F_{1,19}$ = 0.248, p = 0.624 respectively). Further, no effect of the interaction between sucrose was present for both males ($F_{2,20}$ = 0.068, p = 0.796) and females ($F_{2,19}$ = 0.005, p = 0.942). Therefore, only sucrose access results in a higher total calorie intake.

For the mice to whom it is applicable, a ratio can be calculated from the amount of calories the animals got from their food relative to their sucrose consumption. Overall, females obtained more of their total calories from sucrose than males, but no effect of diet was found on the origin of the calorie source. For details, see Figure S7.

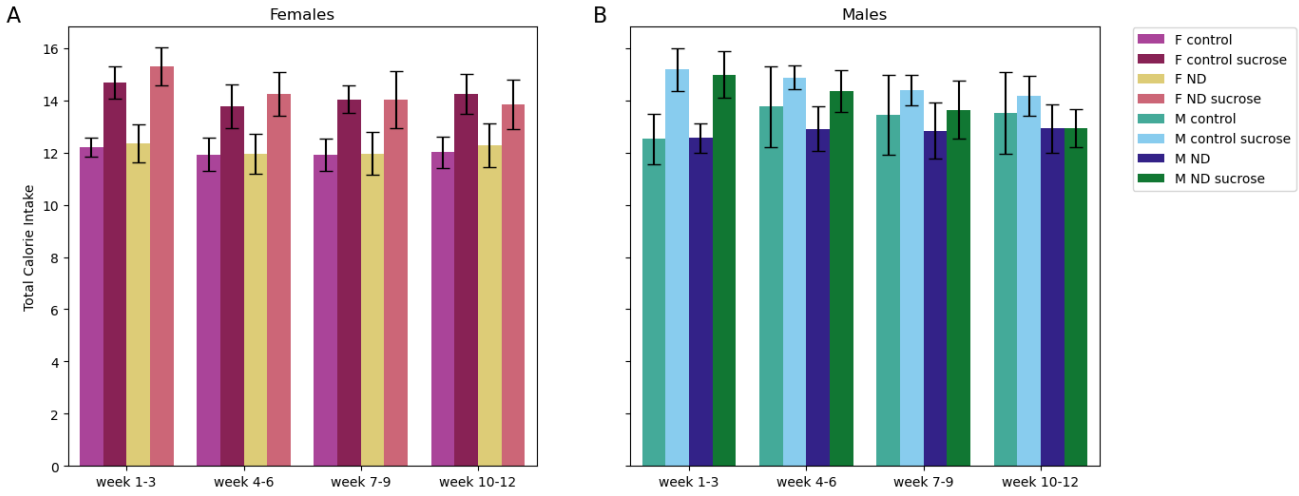

Figure 4. *Total Calorie Intake.* A) In females, sucrose access results in a higher total calorie intake, but dietary niacin is not influential. B) In males, the same patterns are found as for females. In both figures A and B, daily calorie intakes of 3 weeks are binned together. Data are mean ± standard deviation of the mean. The error bars display the standard deviation of the mean.

Discussion

Experiment 1

The first experiment aimed to establish the minimal niacin requirement of mice. As is clear in Figure 1, mice from the control condition and mice from the niacin-deficient condition did not significantly differ in body weight during the course of the experiment. Consequently, niacin concentrations in the drinking water were stepwise halved and ultimately were set at 0 mg/L. At this concentration, there still was not a significant difference in body weight. From these findings, it was concluded that mice do not seem to require niacin in their diet at all under the present conditions. This is in line with previous findings from Natsumi et al. (2020) and Shi et al. (2019). This is hypothesised to be due to the presence of tryptophan in the diet, a phenomenon that also has been shown by Terakata and colleagues (2013). Whilst humans are also able to produce niacin from tryptophan (around 60 mg of L-tryptophan for 1 mg of niacin, Horwitt et al. (1956)), mice perform this conversion at a higher rate (Krehl et al., 1945). The diet used in this study contains 20% casein, which is known to be tryptophan-rich (Stevens & Henderson, 1956). Thus, in the present study, mice are able to produce niacin from tryptophan, and their minimal niacin requirement in the diet is 0 mg/kg.

What must be mentioned is the duration of the stepwise decrease in niacin concentration. In this experiment, 3 days was deemed a sufficient amount of time to observe possible weight differences due to niacin deficiency. However, literature suggests that niacin deficiency takes longer to develop although precisely how long is still conflicting. Knockout mice completely lacking KMO (kynurenine 3-monooxygenase, an enzyme involved with niacin production from tryptophan) fed a niacin deficient diet, reported lower body weight and NAD blood and liver levels after one month (Mizutani et al., 2023), whilst others report three weeks (Palzer et al., 2018) or even as little as two (Terakata et al., 2012). Effects of mild niacin deficiency on body weight have been reported to occur after 3 weeks on a niacin deficient diet (Boyonoski et al., 2000, Spirtes & Alper, 1961). On the other hand, several previous studies failed to observe a change in body weight due to (mild) niacin deficiency (Natsumi et al., 2020; Rawling et al., 1994). This was additionally confirmed by Shi et al. (2017) as well as Shi et al. (2019), who further found that food-seeking behaviour also was unaltered. These findings are in line with the results of our present study. Despite the steps of the concentration lowering being merely 3 days long, the experimental animals were kept on a niacin-deficient diet for weeks and thus long-term effects of possible niacin deficiency could have been observed nonetheless.

In the present experiment, body weight change was the only indicator of niacin deficiency and thus the only criterion for the minimal required niacin level. To improve the reliability of the minimal niacin requirement found here, molecular markers should additionally be used as indicators for niacin deficiency. Candidates for this include NAD and NADP, which can be measured in the blood, urine, and liver (Mizutani et al., 2023; Rawling et al., 1994; Terakata et al., 2012). Whilst not a part of this paper, mice at the end of experiment 1 were put on a stable niacin concentration for 3 weeks (either 0 mg/kg, 1 mg/kg, or 5 mg/kg). This is for possible future analysis of molecular markers for niacin deficiency to check whether the decided minimum does not in all actuality result in niacin deficiency.

Experiment 2

With the minimal niacin requirement established, the question could be answered in the second experiment whether excessive niacin in combination with sucrose access affects body weight and food intake. From the results, it becomes apparent that dietary niacin did not have an effect on body weight gain, body mass composition, and food/sucrose intake in females. This holds both for diet alone, as well as for the interaction of dietary niacin with the availability of sucrose. For males, dietary niacin does not influence body mass composition and food intake. However, it does seem to be involved with sucrose intake, as well as body weight independent of sucrose access. Namely, the males on the control diet gained significantly more weight than males on the niacin-deficient diet. Further in line with this is the finding that male mice on a niacin-deficient diet consume less sucrose.

When looking at the animals that did not receive sucrose, these findings contradict the results from the first experiment, where the male mice on a niacin deficient level did not have an altered body

weight. Additionally, the current finding that males with the minimal niacin level have a lower body weight compared to those with excessive niacin levels, also contradicts findings of previous studies. Katsumi et al. (1996), found no significantly higher body weight of rats receiving excessive niacin. The study of Mizutani et al. (2024) is in line with this, they also observed no higher body weight in mice ingesting excessive amounts of niacin. This observed difference could be due to the timescale of the observations. The study of Mizutani and colleagues merely lasted 35 days, whilst that of Katsumi and colleagues was even shorter with 17 days. Similarly, the mice from experiment one were kept at their final niacin concentrations (ND 0 ml/L and control 30 mg/kg) for one week. The animals from the second experiment, on the other hand, received their respective diets for 12 weeks. When looking at Figure S3B, it becomes clear that the body weight differences arise in the final stretch of the experiment. Therefore, the discrepancy between the first and the second experiments might indeed be caused by differences in their duration.

Alternatively, the problem of the conflicting findings could be approached from the other way around. As mentioned in the previous section, molecular markers have not been used to identify niacin deficiency. Therefore, it may be the case that the mice on the niacin-free diet have, unbeknownst to us, become niacin deficient instead of having received their minimal niacin requirement. Consequentially, what we would actually be observing here is that niacin deficiency causes the male mice to lose weight. This reasoning does explain why the same pattern is not observed in females, as females have a higher resilience to niacin deficiency. It has been demonstrated that females without access to niacin in the diet, but with access to tryptophan, have no altered whole-body metabolism and liver nicotinamide levels (Van Der Stelt et al., 2021). Yet, since literature suggests that mice cannot become niacin deficient because of their ability to convert tryptophan into niacin, the first line of reasoning is more likely.

When looking at the calorie sources in the diet, females had higher sucrose intake than males, as has previously already been observed by Kumar et al. (2024), Matas-Navarro et al. (2023), and others. Access to the 30% sucrose solution did not have significant effects on either body weight or body composition, despite the total caloric intake being higher in both males and females (Figure 4). A possible explanation for the observed discrepancy between a higher caloric intake and equal body weight could be higher activity levels. However, homecage activity has not been measured for the duration of the experiment so this theory cannot be verified. Another factor that possibly plays a role in the lack of significant differences due to sucrose intake, is stress. The second cohort of mice showed remarkably high baseline stress levels, especially when compared to the first cohort. This is true for both the males and the females. 2 out of 24 females showed stereotypical behaviour. Additionally, 8 out of 47 mice showed signs of overgrooming. Stress has been found to influence body weight and food intake, although previous studies are conflicting as to the direction of the influence. Several studies have found chronic stress to lower both food intake and body weight (Depke et al., 2008; Jeong et al., 2013), whilst others found the opposite effect (Moles et al., 2006) or none (Sterlemann et al., 2008).

The high levels of stress thus could have influenced sucrose drinking behaviour or interfered with the body weight changes.

Regardless of its cause, the inability of sucrose to produce significant body weight changes results in a further inability to make conclusions concerning the main hypothesis of this study. That is, comparing the effect of the minimally required niacin intake with excessive intake as an interaction with sucrose on body weight. Thus, it becomes impossible to conclude whether the absence of a significant difference in either body weight or fat mass is due to niacin not being involved in this factor, or due to the failure of the weight gain stimulating paradigm. Previous studies have found the paradigm to be effective. Similarly to the mice in our study, male mice in the study of Burke et al. (2018) had ad libitum access to a 30% sucrose solution. Their subjects, however, showed a significantly increased body weight after 2 weeks. Similar results have been obtained by Togo et al. (2019), whilst others report no noticeable changes in body weight (McCluskey et al., 2020). Combining the conflicting results from previous studies with the absence of significant weight gain in the current study leads to the conclusion that alternatives to the 30% sucrose protocol should be considered. Alternate diets focussing on weight gain through metabolising carbohydrates include a high-fructose diet or a high-carbohydrate diet. However, these diets do not consistently result in significant weight gain (Ho et al., 2018; Wang et al., 2020; Zhang et al., 2023). Because of this, the scope of obesogenic diets could be expanded to diets with high fat contents, which additionally represents the human situation better, as people consume both fats and sugars. Niacin is, in addition to its role in carbohydrate metabolism, involved with metabolising fatty acids (Djadjo & Bajaj, 2024). It is therefore interesting to investigate the interaction between niacin's functions with the metabolism of fats and carbohydrates, especially considering the finding that niacin actually could contribute to weight loss in the context of a high-fat diet (Samad et al., 2023). An example of such an obesogenic diet is a high-fat high-sucrose diet, which has been found to successfully induce obesity in mice as shown by Burchfield et al. (2018), Victorio et al. (2021), Yang et al. (2012), and more. A different option, that takes the human situation even more into account would be the use of a cafeteria diet, since this considers other aspects of eating such as variety and novelty (Lalanza & Snoeren, 2021). Indeed, a cafeteria diet successfully induces obesity in rodents (Buyukdere et al., 2019); Lang et al., 2019).

Future prospects

As already touched upon in the materials and methods section, the vcSSR rule was applied in this study. The main outcome of interest in this study is the effect of the interaction between dietary niacin and sucrose access on body weight gain. If the p-value of these comparisons is between 0.026 and 0.3, the experiment will be expanded to 12 animals per group. If the value is lower or higher, the null hypothesis will be accepted or rejected. For females, the found p-value is 0.285 and for males it is

0.697. Therefore, in the case of females, it is suggested here to expand the group size to 12 instead of 6. Thereby, experiment 2 should be repeated for the new cohort.

However, as mentioned in the previous section, the 30% sucrose access alone is not sufficient to induce weight gain. Consequently, conclusions about the interaction between niacin and sucrose are less reliable. Therefore, it is suggested here to firstly improve this and other limitations of the present study, before considering expanding the sample size.

If in these future studies it becomes apparent that dietary niacin does not have weight gain stimulating properties, then the surge in obesity cases as observed in recent history can be attributed to different factors. However, if future findings suggest the opposite to be true, interesting angles to be explored could be the interaction of niacin with other nutrients. For example, the interaction of niacin with other B vitamins, since their functions also include metabolic mechanisms and often are fortified to our diet as well (Hanna et al., 2022). More importantly, if excessive niacin levels actively cause weight gain, then fortification laws should be reconsidered to limit obesity prevalence.

Conclusion

In conclusion, dietary niacin has not been found to influence body weight, body composition, or food intake in female mice, both independently and dependently on sucrose access. For males, niacin independently from sucrose access seems to influence body weight, with excessive niacin levels resulting in higher body weight gain. The latter result suggests that in males, excessive dietary niacin intake may play a role during the development of the metabolic disorder obesity. However, the present study has several limitations, including the inability of the 30% sucrose access to induce significant body weight gain, which decreases the reliability of the found results. It is thus advised that future studies are conducted addressing these shortcomings appropriately.

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Supplementary Information

Product #	**D11051801**		**D23082408**	
	20% Casein		*20% Casein*	
Ingredient	**gm**		**gm**	
Casein	200		200	
L-Cystine	3		3	
Corn Starch	375.7		375.7	
Maltodextrin 10	125		125	
Sucrose	107.0777		107.0777	
Cellulose	50		50	
Soybean Oil	25		25	
Lard	75		75	
Mineral Mix S10022G	0		0	
Mineral Mix S10022C	3.5		3.5	
Calcium Carbonate	12.495		12.495	
Calcium Phosphate, Dibasic	0		0	
Potassium Citrate, 1 H20	2.4773		2.4773	
Potassium Phosphate, Monobasic	6.86		6.86	
Sodium Chloride	2.59		2.59	
Vitamin Mix V10037	10		0	
Vitamin Mix V10045	0		10	
Choline Bitrartrate	2.5		2.5	
FD&C Yellow Dye #5	0.05		0	
FD&C Red Dye #40	0		0.05	
FD&C Blue Dye #1	0		0	
Total	**1001.250**		**1001.250**	
	gm	kcal	gm	kcal
Protein	179	716	179	716
Carbohydrate	618	2471	618	2471
Fat	100	900	100	900
Fibre	50	0	50	0
Total		4087		4087
	gm%	kcal%	gm%	kcal%
Protein	18	18	18	18
Carbohydrate	62	60	62	60
Fat	10	22	10	22
Calcium, grams	5.1		5.1	
Phosphorus, grams	3.2		3.2	
Potassium, grams	3.6		3.6	

Table S1. *Contents diet*. D11051801 is the control diet, D23082408 the niacin deficient diet.

Ingredient:	V10037		V10045	
	gm	Amount in 10 gm	gm	
Vitamin A, Acetate (500,000 IU/gm)	0.8	4000 IU	0.8	
Vitamin D3 (100,000 IU/gm)	1	1,000 IU	1	
Vitamin E Acetate (500 IU/gm)	15	75 IU	15	
Phylloquinone	0.075	0.75 mg	0.075	0.75 mg
Biotin, 1.00%	2	0.2 mg	2	
Cyanocobalamin, 0.10%	2.5	25 µg	2.5	25 µg
Folic Acid	0.2	2 mg	0.2	2 mg
Nicotinic Acid	**3**	**30 mg**	**0**	
Calcium Pantothenate	1.6	16 mg	1.6	16 mg
Pyridoxine-HCl	0.7	7 mg	0.7	7 mg
Riboflavin	0.6	6 mg	0.6	6 mg
Thiamin HCl	0.6	6 mg	0.6	6 mg
Sucrose	971.925		974.925	
Total	**1000**		**1000**	

Table S2. *Vitamin mixes used in diets.* V10037 is added to the control diet, V10045 to the niacin deficient diet. Only difference is the nicotinic acid contents, indicated in bold.

Males	Transition	p_{holm}	**Females**	Transition	p_{holm}
	Base - 30	0.009		Base - 30	0.003
	30 - 15 mg/kg	< 0.001		30 - 15 mg/kg	< 0.001
	15 - 7.5 mg/kg	0.290		15 - 7.5 mg/kg	1.000
	7.5 - 4 mg/kg	0.093		7.5 - 4 mg/kg	1.000
	4 - 2 mg/kg	0.204		4 - 2 mg/kg	1.000
	2 - 1 mg/kg	0.204		2 - 1 mg/kg	1.000
	1 - 0 mg/kg	0.191		1 - 0 mg/kg	1.000

Table S3. *p-values stepwise lowering of niacin contents.* Repeated-measures ANOVA has been conducted for males and females separately, with a consequent Holm post-hoc test.

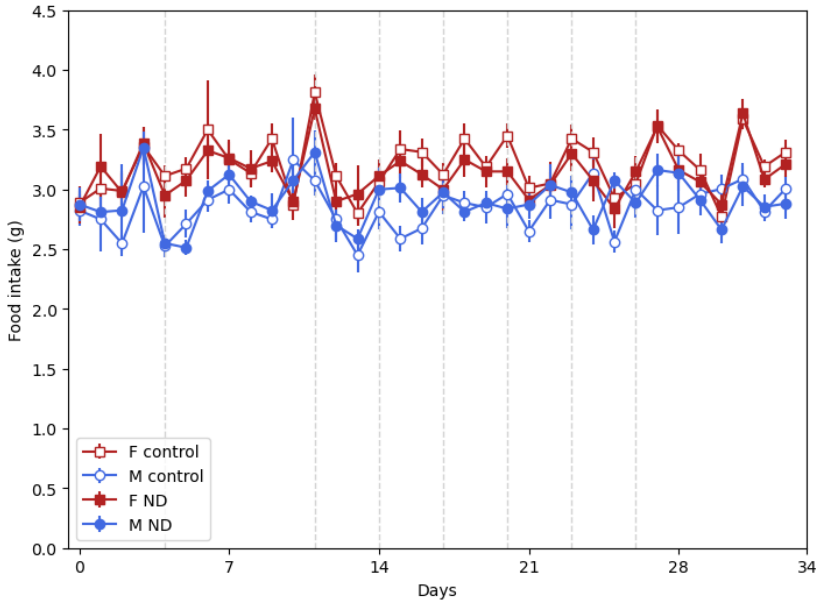

Figure S1. *Food intake during experiment 1.* For both sexes, visual examination of the data reveals no apparent differences between food intake due to supplementation of drinking water with niacin. Further, lowering the niacin concentrations does not seem to be altering food intake. Food intake is shown each day for male (blue) and female (red) mice on either control diet (open circles) or niacin-deficient (ND) diet (filled circles). The first dotted line indicates the switch from food as the niacin source to supplementing. Subsequent dotted lines indicate days on which niacin concentration is halved. Data are mean ± standard error of the mean.

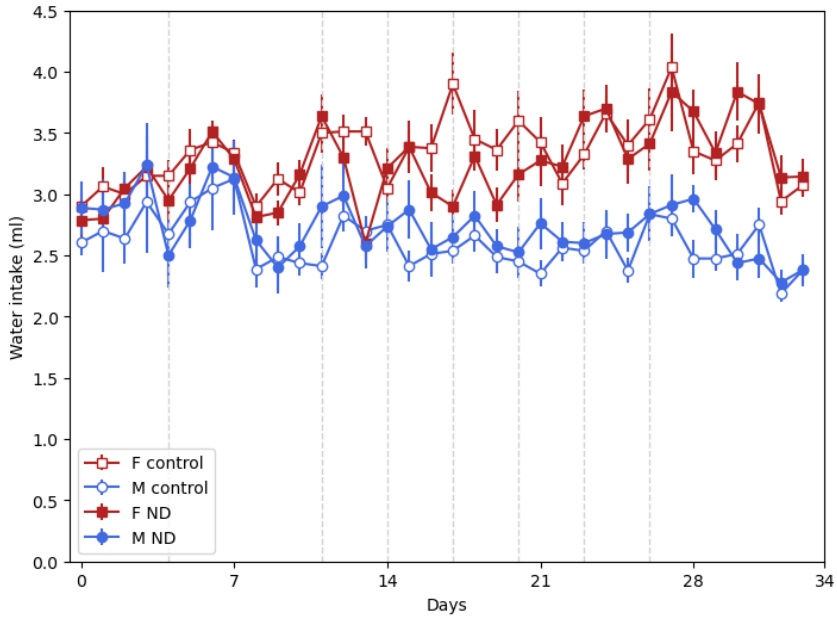

Figure S2. *Water intake during experiment 1.* For both sexes, visual examination of the data reveals no apparent differences between water intake due to supplementation of drinking water with niacin. Further, lowering niacin concentrations does not seem to be altering water drinking behaviour. Water intake is shown each day for male (blue) and female (red) mice on either control diet (open circles) or niacin-deficient (ND) diet (filled circles). The

first dotted line indicates the switch from food as the niacin source to supplementing. Subsequent dotted lines indicate days on which niacin concentration is halved. Data are mean ± standard error of the mean.

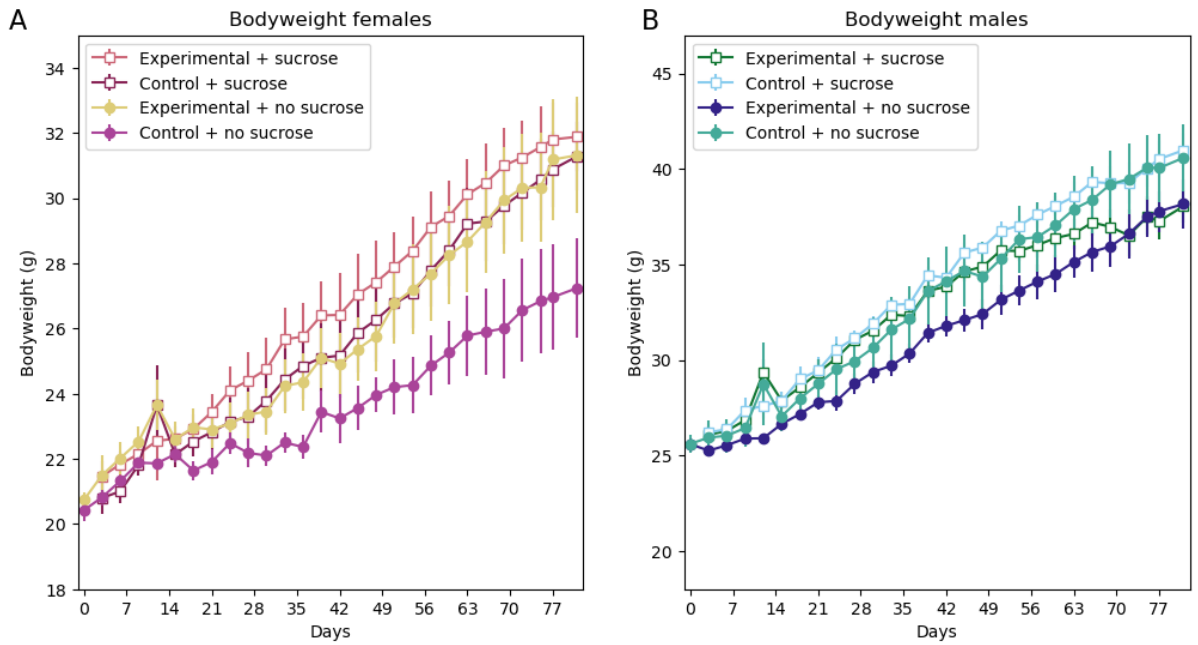

Figure S3. *Overview of body weight changes of experiment 2.* A) Body weight of females. B) Bodyweight of males. For both figures A and B, the error bars display mean ± the standard error of the mean. In the legends, experimental refers to the animals on the niacin deficient diet.

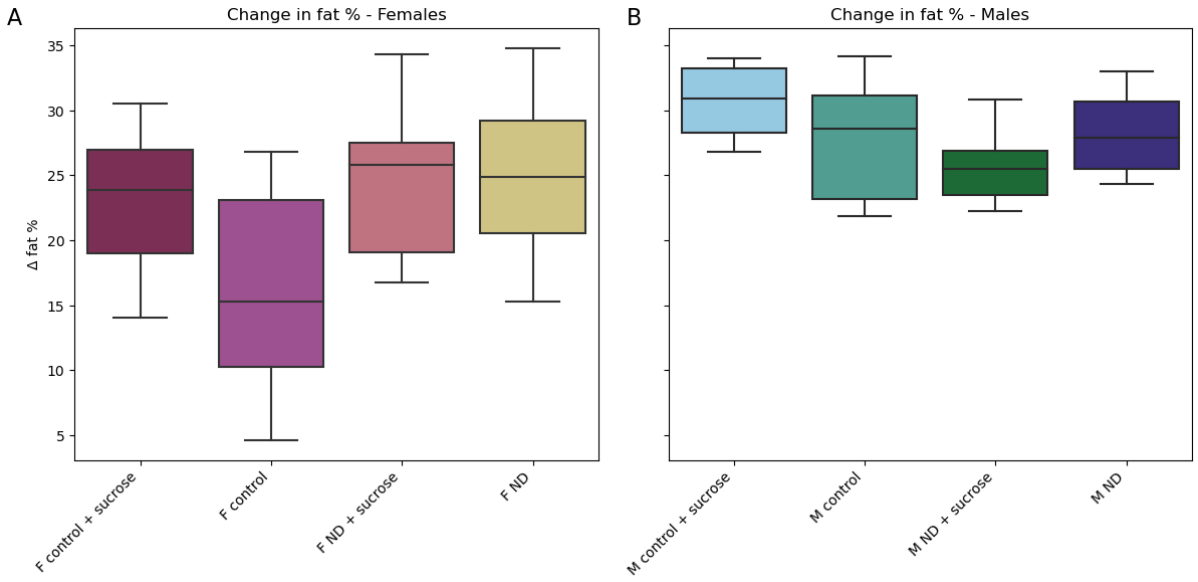

Figure S4. *Changes in the % of lean mass over the course of the experiment.* A) In females, repeated measures ANOVA revealed no effect of diet ($F_{1,19}$ = 3.397, p = 0.081), sucrose ($F_{1,19}$ = 3.904, p = 0.063), or the interaction ($F_{2,19}$ = 3.232, p = 0.088). B) In males, no effect of diet ($F_{1,20}$ = 1.292, p = 0.269), sucrose ($F_{1,20}$ = 0.233, p = 0.635), or the interaction ($F_{2,20}$ = 3.046, p = 0.096) was found.

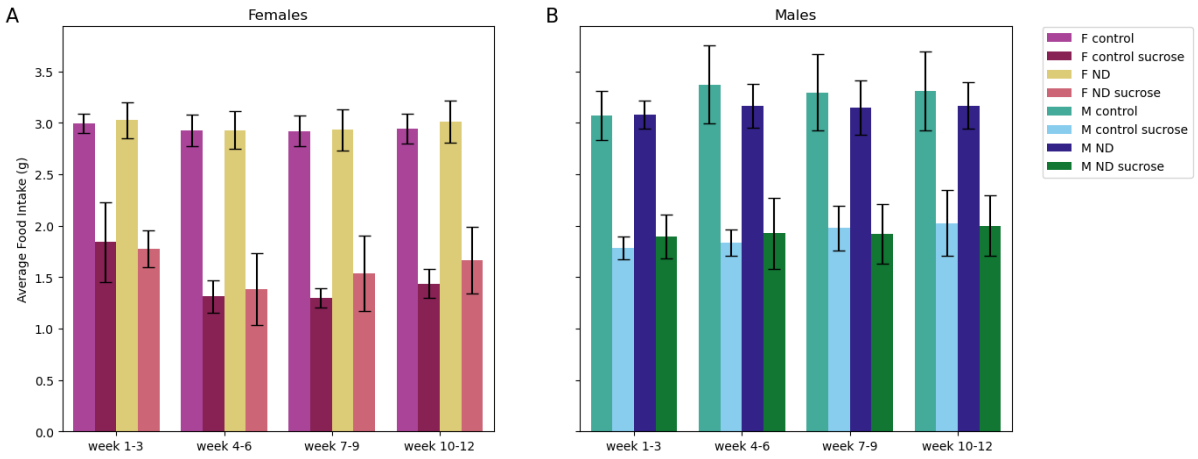

Figure S5. *Average food intake.* Repeated measures ANOVA has been conducted for both sexes separately. A) In females, sucrose access significantly decreases food intake ($F_{1,19}$ = 320.485, p < 0.001). No significant between subject effects of diet or diet * sucrose interactions have been found ($F_{1,19}$ = 0.848, p = 0.369 and $F_{2,19}$ = 0.317, p = 0.580, respectively). B) In males, sucrose access was again found to be a significant between subjects effect with $F_{1,20}$ = 202.587, p < 0.001. No effect of diet and diet * sucrose interactions were present ($F_{1,20}$ = 0.278, p = 0.604 and $F_{2,20}$ = 0.725, p = 0.405, respectively). For both figures A and B, averages worth of 3 weeks have been binned together. Bars display the mean ± standard deviation of the mean.

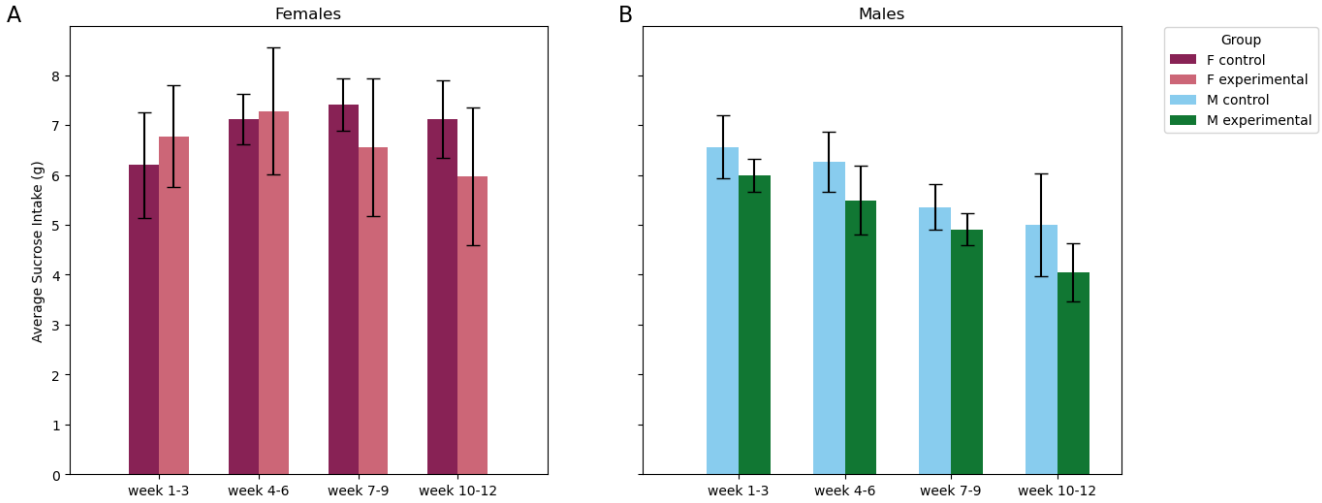

Figure S6. *Average sucrose intake.* A) In females, no effect of diet was found ($F_{1,19}$ = 0.381, p = 0.551). The assumption of homogeneity of variances was violated in the repeated measures ANOVA, transformation of the data did not resolve this issue. B) The male mice with minimal niacin requirements met, consume significantly less 30% sucrose ($F_{1,20}$ = 0.906, p = 0.018). For both figures A and B, averages worth of 3 weeks have been binned together. Bars display the mean ± the standard deviation of the mean.

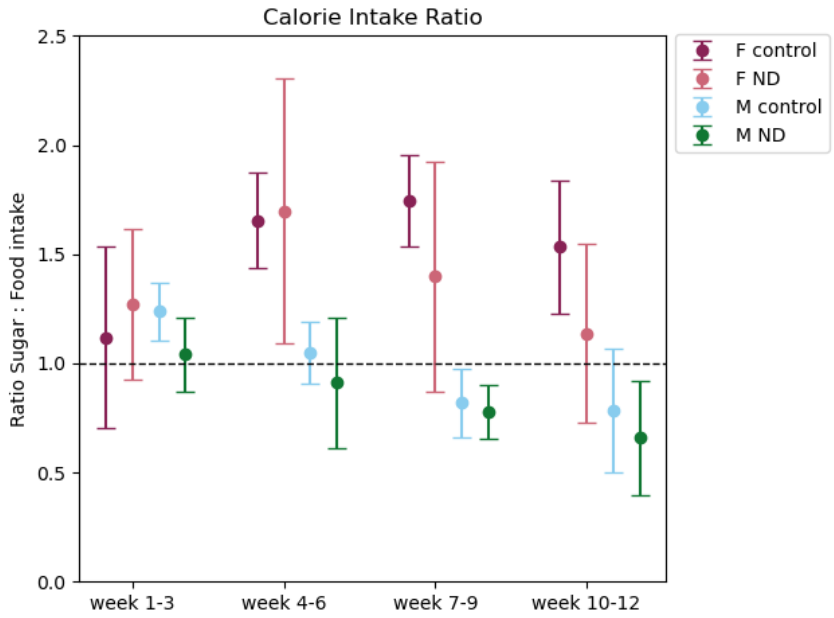

Figure S7. *Sugar : food ratio as calorie source.* Results of all measurement days were averaged and binned for 3 week periods. The initial repeated-measures ANOVA revealed a significant effect of sex on the ratio ($F_{1,39}$ = 25.730, $p < 0.001$), with females gaining more of their calories from sucrose than males. Analysis of the male data reveals no influence of dietary niacin in the sugar : food ratio ($F_{1,20}$ = 2.450, p = 0.149). The same holds for the female data ($F_{1,19}$ = 0.499, p = 0.496). The homogeneity of variances assumption was violated, transformation of data did not resolve this issue.