Effect of Low vs High Eating Frequency on Perceived Appetite, Plasma Appetite Hormones, and Appetite Relationships: A Component of the Frequency of Eating and Satiety Hormones (FRESH) Study

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Abstract

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Purpose

Human appetite hormones are affected by various factors and there is a lack of detailed evidence on the impact of eating frequency on self-reported and serum markers of appetite. If self-reported measures of appetite and serum biomarkers are dysregulated, imbalanced caloric intake could occur, leading to related significant health problems. This study seeks to identify whether three or six meals a day has an impact on the relationships and differences of self-reported measures or serum biomarkers of appetite, along with the concordance of the relationships between the two, while the participants are in the fasted or semi-fasted state. This

topic is a part of a data analysis of the Frequency of Eating and Satiety Hormones (FRESH) randomized cross-over clinical trial.

Methods

The FRESH randomized cross-over clinical trial enrolled 50 participants aged 18-50 who completed three weeks of either high eating frequency (6 eating sessions/day) or low eating frequency (3 eating sessions/day), and then swapped to the other eating frequency after a two-week wash-out period. Participants selected and prepared their own foods that followed a study protocol-directed meal and eating plan throughout the intervention. The protocol-directed eating plan offered individualized daily energy intakes to maintain baseline body weight throughout the study and both macronutrients and micronutrient intakes were consistent across both eating frequency periods. Self-reported measures of appetite were "Hunger," "Full," and "Desire" using a standardized 0-100 visual analog scale and plasma biomarkers of appetite were the hormones ghrelin (pg/mL), leptin (ng/mL), and peptide-YY (pg/mL). Paired t-tests were used to measure differences between continuous self-reported and plasma biomarker variables and Fisher Z transformation tests for differences between correlations. Appetite measurements were collected with blood draws and self-report surveys at the end of each eating frequency period in the morning in a fasted state for the perceived appetite variables, ghrelin, and leptin. Peptide-YY was then collected in the semi-fasted state one half-hour later after a standardized breakfast for those in the low eating frequency period, or a standardized breakfast that was one-half the size for those in the high eating frequency period. Results of the continuous variable analysis were also stratified into low vs high physical activity, split by the median, in order to determine whether physical activity had an effect modification on the data.

Results

Eating frequency had no effect on plasma ghrelin and leptin levels and had no effect on any of the self-reported measures of appetite ($p>0.05$ for all). High eating frequency led to significantly lower (9.7%) plasma peptide-YY ($p<0.01$) compared to the low eating frequency. No differences were discovered in concordance of self-reported and serum biomarkers of appetite across low vs high eating frequency, and all correlations found were of either weak or very weak strength. When the continuous variable differences were stratified by physical activity, an effect modification was found in that only those in the high physical activity stratum had significantly lower peptide-YY in the high eating frequency group compared to the low eating frequency group.

Conclusion

Three versus six periods of daily eating frequency did not play a significant role in overall appetite regulation based on the fasted plasma hormone values and perceived appetite values reported. While peptide-YY was significantly higher in the low eating frequency group, this was expected due to the sensitivity of peptide-YY to meal intakes with the blood draw taking place after eating a larger or smaller meal in the respective eating frequencies. Because all correlations between fasted plasma hormones and perceived appetite were either weak or very weak, currently commonly used perceived appetite scale measures may not accurately reflect biological appetite. However, some specific expected relationships were seen with a closer categorical analysis, demonstrating some coordination between the self-reported and plasma biomarker variables. Further research will be required to identify how eating frequency and appetite relationships could play a role in human health outcomes.

Introduction:

The human hunger and appetite systems are regulated in part by hormones; significant problems may arise should these hormones become dysregulated.[1] Energy balance is managed through both food intake, using hunger and satiety cues, and with energy expenditure, through activity and bodily functions.[2] Long-term caloric energy imbalances brought about by a malfunctioning hormonal system could increase risk for diet-related chronic diseases and conditions such as obesity, cancer, and cardiovascular disease.[1] If perceived appetite does not precisely reflect blood levels of appetite-related hormones through a strong and consistent correlated relationship, energy imbalance issues could arise through food intake that is not aligned with biological cues. Inversely, these system imbalances could also lead to unintended weight loss, muscle wasting, or cachexia.[3] Appetite-related hormones and self-reported perceptions of appetite appear to be heterogeneously affected by a variety of factors including macronutrient proportions in the diet,[4] exercise,[5] psychological factors,[6] and BMI.[6] Additionally, research indicates that certain conditions can also lead to the discordance of some hormonal influences on perceived appetite, such as anorexia nervosa.[7] To the best of this study's researcher's knowledge, no study has been conducted on whether one pattern of eating frequency leads to greater concordance of the relationship between blood biomarkers of appetite and the self-perception of appetite.

Three key appetite-related hormones involved in appetite include ghrelin, peptide-YY (PYY), and leptin. Ghrelin is a hormone that increases the sensations of hunger and increases food intake.[7] Ghrelin is produced in the upper regions of the stomach during negative energy balance and travels through the blood to the hypothalamus to trigger hunger and decrease satiety.[7] It is also involved in gut motility, gastric acid secretion, taste sensation, stress, and

anxiety.[7] PYY is a shorter-lasting hormone that is produced in the gastrointestinal tract that increases for several hours after eating and decreases food intake in response to satiety.[8] Leptin acts on the hypothalamus to decrease appetite and acts as a chemical signal of adipose storage due to the decreased production of leptin as adipose stores decrease.[9] Because of these hormonal actions, it would be expected that a higher perceived appetite in a fasted or semi-fasted state would be correlated with higher ghrelin, lower PYY, and lower leptin.

There have been some experimental studies showing mixed results on the effects of eating frequency on appetite. One review of studies testing the effect of eating frequency on appetite control and food intake generally found decreased maximum peaks in perceived appetite, satiety, ghrelin, and PYY in groups with higher eating frequency (>3 eating occasions per day) compared to those with lower eating frequency (3 eating occasions per day), indicating that those eating more frequently may experience less maximal severe feelings of appetite throughout the day.[10] However, the total sum of the appetite measurements throughout the day, as measured through area under the curve assessments, was still approximately the same as the lower eating frequency group, only spread more out throughout the day without the larger peaks.[10] This review did not include measurements of the strength of the correlations between perceived and blood-based appetite markers.

The purpose of this present study was to analyze the relationships between self-reported and blood-based markers of appetite. This included measuring fasted or semi-fasted plasma concentrations of hormones related to appetite and satiety, perceived appetite status using standardized self-report scales, and measuring the correlation between the two, among healthy adult participants in a randomized crossover intervention testing high eating frequency vs low eating frequency. While there has been research indicating that energy intake or output has the

potential to change the responsiveness of perceived appetite from appetite-related hormonal changes, there has been a lack of research indicating which lifestyle factors could influence these relationships in a eucaloric, energy balanced study setting. By investigating these factors, nutrition related care may be able to be more appropriately planned for patients or individuals with and without specific appetite-related factors that incorporates their perception of appetite responsiveness to hormones. This study will explore whether eating frequency, while keeping energy intake constant, influences measures of self-reported appetite, serum biomarkers of appetite, or the correlations between the two, and will explore effect modification of on the continuous variables by physical activity.

Methods:

Study Design

The Frequency of Eating and Satiety Hormones (FRESH) Study was a randomized cross-over trial and all participants completed both study eating frequency periods in a randomly assigned order. The study was a behavioral intervention where study staff worked with each participant to create an individualized meal plan where participants consumed their own selected foods but ate the same menus in both study periods with constant energy and macronutrient intakes as either three or six meals per day. The participants were randomly assigned to be in either the low eating frequency group (3 meals / day) or high eating frequency group (6 meals / day) for three week periods with a crossover to the opposite eating frequency after a two-week washout period to decrease the possibility of carryover effects. At the end of each three-week eating frequency period, participants completed a fasted blood draw along with several questionnaires on perceived appetite before completing sequential postprandial eating tests, to be used for the broader FRESH trial analyses, by being given a large meal for those finishing the low eating frequency period, or a smaller meal, half of the size, for those finishing the high eating frequency period. For the purposes of this study, only the first measurement for each appetite value was used, which includes the fasted ghrelin, leptin, and perceived appetite values, and the PYY value obtained half an hour later after eating their respective meals.

This current study analyzed three major components of the relationships between variables gathered from the FRESH trial:

1. This study analyzed the effects of low vs high eating frequency on plasma appetite hormone values and self-reported appetite values.

2. This study compared the correlations between plasma concentrations of ghrelin, PYY, and leptin with the self-reported appetite measures of full, desire, and hunger in order to test whether one eating frequency has greater or lesser concordance, or correlation strength, of the relationships between the blood biomarkers and perceived levels of appetite.
3. This study examined whether measures of plasma biomarkers of appetite significantly varied between categorized low (0-33), medium (34-66), or high (67-100) categorizations of self-reported appetite variables within each eating frequency.

Study Participants

The participants were recruited to the study using print, radio, and digital advertisements. These advertisements directed those who were interested to a screening website to determine initial eligibility. Those who were interested and eligible based on the website screening were contacted for an in-person orientation where height, weight, and fasting blood glucose were measured to determine if the participant would meet the study eligibility criteria. The participants were individuals of normal, overweight, or obese BMI, otherwise healthy, both male and female, living in the metropolitan Seattle, WA, between the ages of 18 and 50 years, and varied ethnicities. 60 participants began the study after meeting the eligibility criteria and signing informed consent and ten dropped out for a final sample of 50. At the end of the study, $300 was given to each participant who completed the study.[11] The study protocol and all procedures were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board and all participants signed written informed consent. The study is registered at clinicaltrials.gov as NCT02392897.

Participants were excluded if their BMI was less than 18.5, between 25.0 and 27.9, or greater than 40 kg/m^2. The BMI lower and upper extremes were excluded due to potential metabolic dysfunction that could confound interpretation of appetite measures and BMI=25-27.9kg/m^2 were excluded to allow for potential contrast between normal and overweight or obese participants that may not be observed at the lower end of the overweight distribution. Participants were also excluded if they had a fasting blood glucose over 100 mg/dL during the study eligibility screening, a diagnosed condition that required physician-directed dietary modification such as cardiovascular disease or end stage renal disease, current pregnancy or plans to become pregnant, current breastfeeding, had history of an eating disorder or restrained eating, were on prescription drugs that could interfere with appetite biomarkers or perceptions, used tobacco or marijuana, or had any situations or conditions that would prevent completion of study protocol.[11]

Measurements

After each three week assigned eating frequency period, participants returned for a visit for the researchers to obtain fasted measurement values for the appetite variables and to complete a postprandial meal test that included timed questions on hunger and appetite using a standardized protocol. For the purposes of this study, this includes blood-based biomarkers of appetite including PYY, leptin, and ghrelin, as well as self-reported markers of appetite including fullness, desire to eat, and hunger. These questions were asked as standardized visual analog scale appetite questions established in previous literature[12], with potential answers ranging from 0-100, using the questions “How full do you feel right now?,” “How strong is your desire to eat right now?,” and “How hungry do you feel right now?,” respectively. The anchors ranged from not at all, to extremely, for each asked category. Participants were able to answer on a scale from

0 to 100 for each prompt. For the categorical variable analysis, these self-reported appetite variable answers were divided into subscales of “Low,” (0-33) “Medium,” (34-66) and “High” (67-100); both eating frequencies were then tested where these subscales were analyzed to determine whether serum hormone concentration differences existed across low vs medium vs high for each plasma biomarker variable.

The original FRESH study data collection included blood draws and perceived appetite survey measurements at multiple time points throughout each end of phase visit. For the purposes of this study, the data analyzed only included the first collected measurements of each variable, in either the fasted or semi-fasted state, of serum appetite and self-reported appetite data taken at the beginning of each end of phase procedure appointment; this would include all self-reported appetite values in the fasted state, as well as ghrelin and leptin in the fasted state, and PYY in the semi-fasted state with the vial for analysis being drawn one half-hour later.

After twenty-one days of each eating frequency period, participants came in to be measured and assessed while instructed to have fasted and not eaten the morning of arrival. At about 8:00am that morning after both phases, participants answered the visual analog scale questions for perceived appetite testing and had blood drawn for use in the ghrelin and leptin analyses. It was also at this time when participants were either given a standardized breakfast if they were in the low eating frequency period, or a standardized breakfast that was one-half the size if they were in the high eating frequency period. These meals were provided for postprandial meal tests with further tests used in the broader FRESH study. At 8:30am, after completion of the meal, blood draws were taken from participants to be measured and used for the PYY serum concentration analyses.

Participant demographic characteristics of age, sex, and self-reported race/ethnicity were collected through a survey at the orientation at the beginning of the study. At this time, participants also took a survey on the Pittsburgh Sleep Quality Index including how many average hours of sleep they received with options of hours 0-12 in one hour increments, completed a dual-energy X-ray absorptiometry analysis (DEXA) test to determine body fat percentage, and answered a survey question determining a reported physical activity "score" to be used as a multiplier in an energy requirements equation with four available answers including "Little to no activity or exercise" (1.3), "Light exercise/sports 1-3 days/week" (1.5), "Moderate exercise/sports 3-5 days/week" (1.6) and "Hard exercise/sports 6-7 days/week" (1.9). For further details on the design protocol for data collection, please see the rationale and design paper for the FRESH study.[11]

Statistical Analysis

Participants were compared across treatment categories of high vs low eating frequency for the statistical analyses. All statistics were conducted within R Studio. Differences between high and low eating frequency of continuous self-reported and plasma biomarker variable means were analyzed using two-sided paired t-tests and statistical significance was set at $p<0.05$. Pearson correlations of self-reported appetite variables and plasma appetite hormones were compared using two-sided Fisher's Z transformation tests of the correlations between variables; for this correlation comparison, non-normal distribution data ranges, defined using Shapiro-Wilk tests, were transformed using Tukey's Ladder of Powers transformations to meet test statistical assumptions. Two-sided Wilcox signed-rank non-parametric tests were used to determine whether measures of blood biomarkers significantly varied between categorized low (0-33), medium (34-66), or high (67-100) self-reported appetite variables. To test for effect

modification, the physical activity data was stratified by dividing the data into two equally populated groups, by the median, to analyze whether any differences found between continuous variables across low vs high eating frequency were equivalent across physical activity stratums. Additionally, frequencies of demographic data were compared across arms and proportions of participants who were in low, medium, and high self-reported appetite categories were compared across eating frequency periods using two-sided Pearson chi-squared frequency tests. For all tests, p-values of <0.05 were regarded as statistically significant.

Results:

Demographics

Details of the overall characteristics and those in each arm of randomization are described in Table 1. Baseline characteristics were comparable across arm orders of the trial, with all characteristic frequencies between arms having no significant differences ($p>0.05$ for all).

TABLE 1.
Baseline Characteristics of Frequency of Eating and Satiety Hormones (FRESH) Trial Participants by Overall and Study Arms (n=50)

	Participants, Number		
Characteristic	**Overall (n=50)**	**Arm 1 (n=25) (LF followed by HF)**	**Arm 2 (n=25) (HF followed by LF)**
Sex (Female)	39 (78%)[1]	19 (76%)[1]	20 (80%)[1]
Mean age, SD	32.1, 7.72	30.88, 6.43	33.28, 8.79
Hispanic	6 (12%)[1]	2 (8%)[1]	4 (16%)[1]
Ethnicity[a]:			
Asian	12 (24%)[1]	8 (32%)[1]	4 (16%)[1]
Black or African American	2 (4%)[1]	1 (4%)[1]	1 (4%)[1]
Caucasian	34 (68%)[1]	16 (64%)[1]	18 (72%)[1]
More than one race	3 (6%)[1]	1 (4%)[1]	2 (8%)[1]
Mean Physical Activity Score[b] (Median=1.5)	1.43	1.44	1.43
Number below 50% Physical Activity Median Split	25 (50%)[1]	11 (44%)[1]	14 (56%)[1]
Mean Hours of Sleep, SD	7.4, 0.73	7.4, 0.58	7.4, 0.86
Mean DEXA Body Fat %, SD	31.7, 8.65	31.3, 9.24	32.1, 8.19

Abbreviations: LF = Low eating frequency (3 meals/day), HF = High eating frequency (6 meals/day), DEXA = Dual-energy x-ray absorptiometry, SD = Standard deviation

[a] Ethnicity was gathered through a "pick all that apply" question where participants could select multiple answers.

[b] Physical Activity Score was collected as a multiple choice question ranging from "Little to no activity or exercise" as 1.3 and "Hard exercise/sports 6-7 days/week" as 1.9, used in calculating daily energy expenditure.

[1-3]: Values in rows sharing the same numeric superscript indicate that no significant differences were found between frequencies using Pearson chi-squared frequency tests.

Primary Analysis

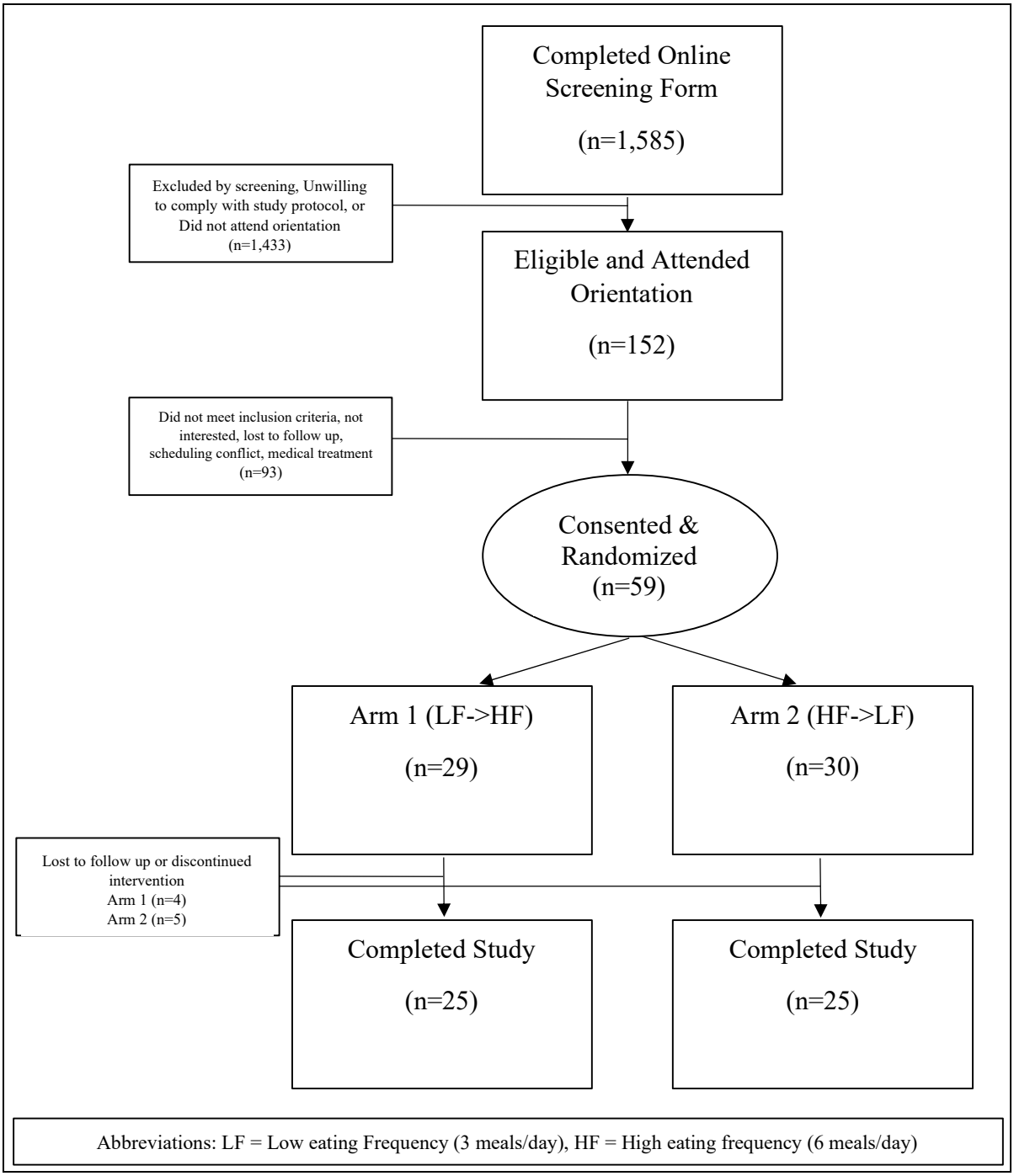


FIGURE 1. Participant flow through screening and trial completion within the FRESH Trial

Table 2. provides the results of the paired t-tests determining the comparison of the low and high eating frequency periods on serum-hormone values and perceived appetite ratings. No significant differences were found for the self-reported appetite variables full, hunger, or desire, or plasma hormone appetite variables leptin or ghrelin between low eating frequency and high eating frequency periods. PYY was 9.7% higher for the low eating frequency assignment (84.82pg/mL) compared to high eating frequency (76.62pg/mL), which was significantly different ($p<0.01$).

TABLE 2.
Comparison of Self-Reported Appetite Scales and Mean Appetite Hormone Values after completing a Low VS High Eating Frequency period, compared with Two-Sided Paired t-tests (n=50)

	LF Mean	HF Mean	Difference between Means CI	p-value
Full[a]	22.70	21.98	(-4.70, 6.14)	0.79
Hunger[a]	61.36	59.02	(-2.85, 7.53)	0.37
Desire[a]	61.14	62.72	(-6.02, 2.86)	0.48
Leptin (ng/mL)	27.28	27.60	(-3.87, 3.24)	0.86
Ghrelin (pg/mL)	1074.78	1083.18	(-86.65, 69.83)	0.83
PYY (pg/mL)	84.82	76.62	**(2.31, 14.09)**	**<0.01**

Abbreviations: LF = Measure at the end of low eating frequency (3 meals/day) for 3 weeks, HF = Measure at the end of high eating frequency (3 meals/day) for 3 weeks, CI = 95% Confidence Interval, PYY = Peptide YY
Variables Full, Hunger, Desire, Leptin, and Ghrelin were collected in the fasted state, and PYY was collected in the semi-fasted state after a large meal for the LF period or a meal half the size for the HF period.
[a] Self-reported appetite measures were asked using visual analog scale questions ranging from 0-100.

Table 3. Demonstrates the Pearson correlation r-values between the self-reported appetite variables as columns and plasma appetite measures as rows, split between high vs low eating frequency. There were no significant differences in correlations of the serum hormone appetite measures of appetite and the self-reported measures of appetite between high vs low eating frequency for all relationships analyzed. The relationships of PYY and hunger, between low EF vs high EF, appear to potentially approach significance with a p-value of .08, with the high EF hunger and PYY Pearson correlation being .32 and the low EF hunger and PYY Pearson correlation being -.03. All correlations were found to be of very weak (.00-.19) or weak (.20-.35) strength.

TABLE 3.
Pearson Correlations between Hormone Appetite Variables and Blood Hormone Appetite Variables, compared with Two-Sided Fisher's Z transformation test (n=50)

	Full			Hunger			Desire		
	High EF	Low EF	p-value	High EF	Low EF	p-value	High EF	Low EF	p-value
Leptin	-.02	-.11	.69	-.11	-.12	.95	-.18	-.15	.91
Ghrelin	-.21	-.16	.81	.33	.02	.12	.20	.05	.45
PYY	.26	.02	.81	.32	-.03	.08	-.03	-.15	.12

Abbreviations: EF = Eating Frequency with High EF indicating 6 meals/day and Low EF indicating 3 meals/day, PYY = Peptide YY.
Self-Reported Variables were measured using a standardized visual analog scale questions ranging from 0-100.
Plasma Appetite Variables were measured with pg/mL (Ghrelin and PYY) and ng/mL (Leptin).

Table 4. shows the overall pooled scatterplots and Pearson correlations (r-values) of the relationships between the plasma appetite hormones and the perceived appetite variables. This was done in order to create a visualization of the trends of the data. Because Table 3. demonstrated no differences between high and low eating frequency, a pooled analysis was considered an appropriate visualization. The data was pooled by taking the average of each participant's results of high and low frequency for each variable. With the exception of pooled hunger, all other variables were found to be non-normally distributed and were Tukey-transformed for finding the r-value listed within the table, although all of the scatterplots are of the untransformed data to provide a visual representation of the collected data. All correlation values found were to be of very weak or weak strength with the highest correlation r-value being 0.24.

TABLE 4.

Scatterplots and Pearson Correlations of the Pooled Data of Plasma Appetite Hormones and Perceived Appetite Variables (n=50)

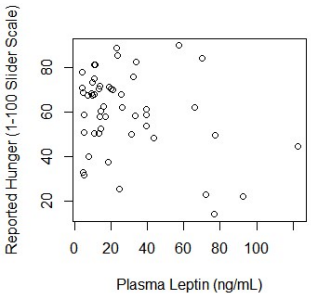
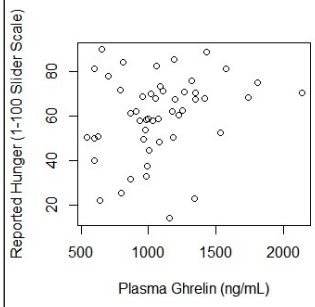
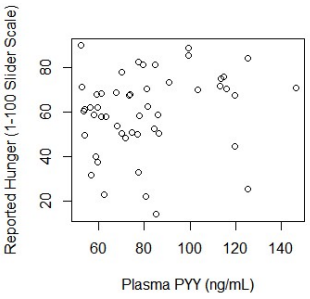
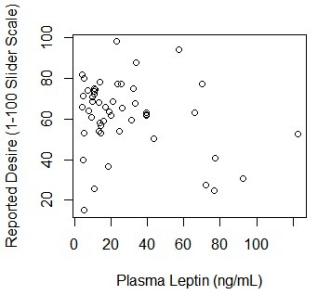
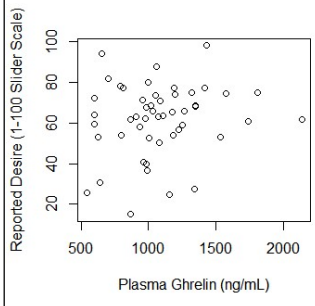
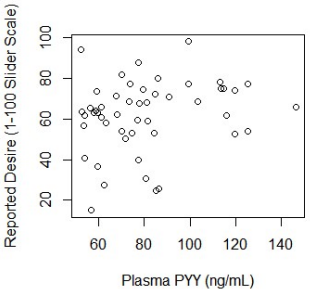
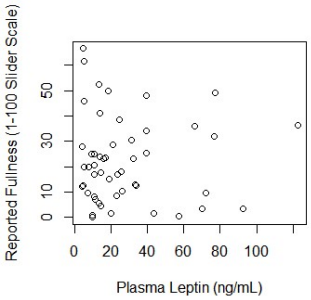
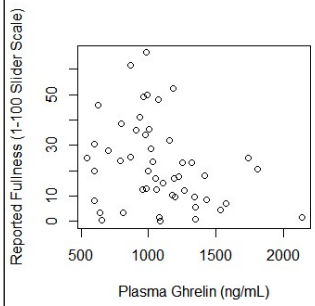
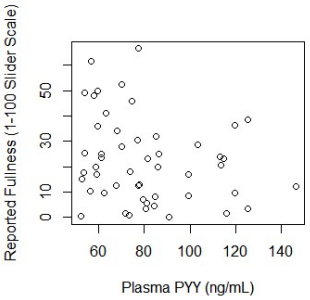
	Leptin (T)	Ghrelin (T)	PYY (T)
Hunger	 r=-0.18	 r=0.23	 r=0.17
Desire (T)	 r=-0.12	 r=0.13	 r=0.17
Full (T)	 r=-0.09	 r=-0.24	 r=-0.21
(T): The scatterplots consist of the untransformed data, but for the purposes of calculating the correlational coefficient, (T) indicates that the data was tukey-transformed to meet normality requirements.			

Table 5. describes the mean plasma hormone values across self-reported appetite variable subscale categories of “Low,” (0-33) “Medium,” (34-66) and “High” (67-100) in both low and high eating frequencies, where comparisons were made between the plasma hormone means across the perceived appetite value categories. While no strong correlations were found between any self-reported appetite and serum hormone pairing in Table 3., after dividing each self-reported appetite scale into subscales of “Low,” “Medium,” and “High” categories, some significant differences in were found between these groupings.

In the low eating frequency period, no significant differences were found across serum hormones between low and medium reported fullness, and no participants reported high fullness. For those with low hunger, there was significantly higher leptin than those with medium or high hunger. No significant differences were found between categorical levels of desire.

In the high eating frequency period, no significant differences were found across low, medium, or high reported fullness, although only one participant reported high fullness. For those with low hunger, there was significantly lower ghrelin than those with high hunger, but this value was not significantly different with those with medium hunger. For those with high reported desire, there was significantly lower leptin than those with medium desire, but this was not significantly different from those with low desire.

TABLE 5.

Mean Serum Hormone Values for Low, Medium, and High Measures of Self-Reported Appetite Variables within the FRESH Trial, compared with Two-sided Wilcox signed-rank non-parametric tests (n=50)

	n	Leptin (ng/mL)	Ghrelin (pg/mL)	PYY (pg/mL)
Low EF				
Low[1] "Full"	37	24.46[a]	1119.76[a]	82.94[a]
Medium[1] "Full"	13	35.29[a]	946.75[a]	90.19[a]
High[1] "Full"	0	N/A	N/A	N/A
Low[1] "Hunger"	5	**81.7[a]**	990.44[a]	76.41[a]
Medium[1] "Hunger"	24	21.60[b]	1095.43[a]	79.6[a]
High[1] "Hunger"	21	20.82[b]	1071.26[a]	92.79[a]
Low[1] "Desire"	3	52.77[a]	1131.50[a]	72.91[a]
Medium[1] "Desire"	30	26.03[a]	1075.47[a]	84.24[a]
High[1] "Desire	17	24.99[a]	1063.54[a]	87.94[a]
High EF				
Low[1] "Full"	36	28.56[a]	1096.14[a]	82.98[a]
Medium[1] "Full"	13	25.53[a]	1015.02[a]	88.89[a]
High[1] "Full"	1	3.80[a]	1082.70[a]	97.97[a]
Low[1] "Hunger"	9	29.67[a]	**852.87[a]**	75.09[a]
Medium[1] "Hunger"	19	34.58[a]	1028.98[ab]	79.90[a]
High[1] "Hunger"	22	20.00[a]	1205.11[b]	93.05[a]
Low[1] "Desire"	6	35.27[ab]	958.35[a]	77.81[a]
Medium[1] "Desire"	21	37.02[a]	1069.22[a]	79.72[a]
High[1] "Desire	23	**16.30[b]**	1110.23[a]	91.31[a]

Abbreviations: EF = Eating Frequency with High EF indicating 6 meals/day and Low EF indicating 3 meals/day, PYY = Peptide YY.
1: Categorical Value Ranges for Self-Reported Appetite Variables includes 0-33 for "Low,", 34-66 for "Medium," and 67-100 for "High."
Means sharing the same superscript letter are not significantly different within each Low/Medium/High categorization per each self-reported variable within the eating frequency using an alpha of =.05.

Additionally, the frequency of low, medium, and high categories for each self-reported hormone were also compared across low vs high eating frequency (e.g. the frequencies of 23/50 had "high" desire in the high eating frequency period and 17/50 had "high" desire in the low eating frequency period, and these frequencies were compared), and no significant differences were found (all $p>0.05$).

Secondary Analysis

Table 6. provides the means of continuous variables for both low and high eating frequency for both those with “Low” and “High” physical activity in order to determine whether physical activity was a variable that had an effect modification on the results of the trial. Separate result models were developed where low and high physical activity were stratified into 25 participants each, by the median of the data, to analyze the results of the continuous variable analysis. As Table 1. demonstrates, the frequency of participants in low and high physical activity categories is similar across arms, with 11/25 participants in arm one and 14/25 participants in arm two being in the low eating frequency category (p=.57). All variable mean differences were analyzed between low vs high eating frequency, separated by low vs high physical activity, which found that the results were different across stratums. The significant PYY difference discovered in the continuous variable analysis, demonstrated in Table 2., only exhibited the same result in the “High” physical activity category, with the difference of the mean PYY between high and low eating frequency was 7.95pg/mL for the low physical activity group (p=.09), and 8.44pg/mL for the high physical activity group (p=.04), whereas only the high physical activity difference was found to be significantly different from zero with a p-value less than .05. Because there were different results across low vs high physical activity, this would indicate that physical activity was an effect modifier on the overall results.

TABLE 6.
Average Hormone Levels and Self-Reported Appetite Variables across Low VS High Eating Frequency for those with Low and High Physical Activity within the FRESH Trial compared with Two-Sided Paired t-tests (n=50)

	LF Mean	HF Mean	Difference between Means CI	p-value
Low[a] Physical Activity				
Full[b]	26.6	27.12	(-9.54, 8.50)	.91
Hunger[b]	57.48	51.52	(-2.86, 14.78)	.18
Desire[b]	57.36	55.40	(-4.51, 8.43)	.54
Leptin (ng/mL)	32.27	32.19	(-7.09, 7.25)	.98
Ghrelin (pg/mL)	1054.09	1027.16	(-66.17, 120.02)	.56
PYY (pg/mL)	77.85	69.90	(-1.19, 17.09)	.09
High[a] Physical Activity				
Full[b]	18.8	16.84	(-4.71, 8.63)	.55
Hunger[b]	65.24	66.52	(-7.06, 4.50)	.65
Desire[b]	64.92	70.04	(-10.55, 1.39)	.10
Leptin (ng/mL)	22.29	23.00	(-2.41, 0.99)	.39
Ghrelin (pg/mL)	1095.46	1139.2	(-175.11, 87.63)	.50
PYY (pg/mL)	91.79	83.35	**(0.32, 16.56)**	**.04**

Abbreviations: LF = Measure at the end of low eating frequency (3 meals/day) for 3 weeks, HF = Measure at the end of high eating frequency (3 meals/day) for 3 weeks, CI = 95% Confidence Interval

[a] "Low" Physical Activity indicates being in the bottom half of the data set, stratified into halves by the median, of reported participant physical activity at the beginning of the study. "High" Physical Activity indicates being in the top half of the data set, stratified by the median, of reported participant physical activity at the beginning of the study.

[b] Self-reported appetite measures were asked using visual analog scale questions ranging from 0-100.

Compliance to Isocaloric / Weight Neutral Intake

One consequential and novel component of this study design was that the participants were instructed to eat a eucaloric diet to maintain their weight during the entire study and to ensure that energy intake did not confound any study results. Because weights were measured at the beginning and end of each period for each participant, the change in weight can be analyzed for compliance. As Table 7. demonstrates, mean weight change for both the low eating frequency period and high eating frequency period was small and includes 0 within each confidence interval, indicating that weight maintenance was satisfactorily achieved with compliance throughout both study period with no significant weight changes. Additionally, the mean weight changes were found to not be different across low vs high eating frequency through a two-sided paired t-test (p=.41).

TABLE 7.
Average Weight Change Over Each Three Week Period of Low and High Eating Frequency within the FRESH Trial (n=50)

	Weight Change (kg) Mean	95% Confidence Interval of Mean
Low Eating Frequency (n=50)	+.57	(-1.53, 2.67)
High Eating Frequency (n=50)	+.75	(-1.31, 2.82)

Discussion:

The Frequency of Eating and Satiety Hormones (FRESH) study measured variables to test whether eating frequency had an impact on various measures of appetite which have been examined through this data analysis. This study had a unique design in that relationships between perceived and biomarkers of appetite were measured, and caloric intakes were managed through directions by the researching staff in order to have a procedure with weight maintenance, all within a randomized crossover trial. While previous known studies have identified that certain factors can play a role in the ability of serum biomarkers to affect perceived appetite,[7] previous literature has not fully explored how eating frequency could affect appetite correlational relationships or the impact of high vs low eating frequency with controlled eucaloric intakes on continuous appetite variables.

For differences between the measured appetite variables, the results of this study indicate that eating frequency period had an effect on the appetite hormone PYY, which was found to be significantly different with the low eating frequency period having 9.7% higher PYY than the high eating frequency period ($p<0.01$). No other significant differences were found between either any of the other hormones or any self-reported measures. PYY is a hormone created in the gastrointestinal tract, that is produced after eating, which increases satiety to decrease food intake,[8] which would explain why PYY in the low eating frequency group was higher after consuming a larger meal than those in the high eating frequency period. Because those within the low eating frequency period were given a larger meal and those with high eating frequency were given a smaller meal, a higher PYY value would be expected for those in the low eating frequency based on the outlined mechanisms of PYY increasing. Ghrelin and leptin are responsive to overall energy balance[7] and adipose storage,[9] respectively, which may explain why

these hormones were not significantly different across eating frequencies in the fasted state. Additionally, even though PYY was expected to be different, ghrelin and leptin are the two hormones most closely associated with energy homeostasis[13] and were both found to be consistent across eating frequencies.

Low vs high eating frequency revealed no significant differences discovered regarding the concordance or discordance of the Pearson correlation relationships between perceived and serum biomarker appetite variables. Only the difference in relationships between PYY and hunger approached significance with a p-value of .08, which warrants further investigation within future studies, and is expected to be related to the intake of different meal sizes. The lack of significant differences in correlations indicates no significant effect of eating frequency on appetite concordance within the trial. Of particular note, nearly all of the correlations discovered were very weak, with a minimum absolute strength of .02 and a maximum strength of .33, which was an unexpected result. This would indicate that, in this study, these standardized, pre-established perceived appetite measurement scales were very poor predictors of biological measures of appetite for the variables used. It may be that these measures are not sensitive or accurate enough in the fasted or semi-fasted state due to the large skew on many variables within the study, as most self-reported appetite variables were skewed to reflect a high perceived appetite given that they had not eaten overnight. It may also simply be that perceived appetite is not experienced in a way that reflects biological measures in general, such that perceived measures could not reliably provide information about hormone values. Previous literature on the concordance between subjective appetite and plasma hormones is limited. Two studies, that measured postprandial changes in subjective appetite and appetite-related hormones ghrelin & PYY found no significant correlation between them.[14-15] This literature is similar to the results in

that there does not appear to be a strong relationship between perceived appetite and plasma appetite hormones. However, one study did find that postprandial PYY area under the curve was positively associated with satiety ratings (r= .47).[16] and another study found a relationship between ghrelin and subjective appetite (r= -.80)[17]. Perceived appetite is psychological and related to a complex control system regarding various hormones and systems, which may explain why relationships to single hormones are not often found to be directly related. While there may be discordance between the perceived appetite and serum appetite variables in this study, whether perceived appetite or plasma hormones could better estimate eating behaviors or caloric intake remains unknown due to this study directing eucaloric intake in order to minimize confounding variables.

After categorizing the data into three categorical variables of "Low," "Medium," and "High," and identifying differences, a more detailed view of the variables was available. While there were no differences in frequencies across low vs high frequency, there are some examples of appropriately expected hormonal effects such as those with low hunger in the low eating frequency period having higher leptin, those with high desire in the high eating frequency period having lower leptin, and those with low hunger in the high eating frequency period having lower ghrelin; these would match up with the mechanistic actions of leptin decreasing appetite[9] and ghrelin increasing hunger.[7] With these categorizations of the data, we can begin to see more relationships that we would expect between perceived and biological measures of appetite that we were unable to with the correlational analysis alone. As an example, although Leptin and Desire in those in the high eating frequency period were very weakly correlated (-.18), we can see that those with high desire had significantly lower leptin and those with medium desire, which is the outcome that would be expected due to leptin decreasing appetite. Because of this,

the perceived appetite variables may have some aspects of noticeable relationships with plasma hormone values, but not of a direct magnitude that this study can detect with Pearson correlations.

Study strengths include that this trial was a randomized crossover design in which each individual was able to be used as their own control. This study has multiple limitations. While the study was designed to measure PYY after a meal due to the hormone's mechanism of action, the results are limited in that the original study measured PYY after eating rather than in the fasted state like the other collected variables, which significantly limits the interpretation of the data due to the difference in meal size at that time period. While having caloric maintenance as a part of the study procedure provided a useful function in isolating the effect of eating frequency alone, one limitation of this study would also be that because the study specifically directed eucaloric intake, it did not discover whether any potential appetite results found could lead to changes in nutrient or caloric intake and weight change. One study that measured both perceived appetite on visual analog scales as well as PYY and ghrelin found that self-reported appetite variables were stronger predictors of energy intake,[15] and more research may be warranted on the topic. Because of this, while the literature was benefitted through this novel aspect where participants had eucaloric intake in order to assess the effect of eating frequency alone, the results of this study do not identify whether eating frequency could affect the risk external health factors or nutrition, only whether appetite was affected, and a future study would be necessary where caloric intake is not controlled in order to assess whether high vs low eating frequency affects actual eating behaviors such as energy intake. Another limitation would be that because all of these measurements for the purpose of study were collected in a fasted or semi-fasted state, it is unknown whether they would remain consistent at other points throughout the day. Study

participants were also from the greater Seattle area who were able and willing to participate within the study and the results may not be representative of the general population. Additionally, because the correlations between perceived and serum appetite measures were all either very weak or weak, the pre-established perceived appetite measures may be inadequate assessment tools. Further research is warranted to address these limitations.

Conclusion:

The effect of eating frequency on appetite is an important piece of data that can be useful in uncovering factors of what behaviors could lead to human health differences. While semi-fasted PYY was significantly higher in the low eating frequency group, this result was expected due to the blood draw taking place after eating a large or small meal in the respective eating frequencies. This study found that fasted ghrelin and leptin plasma hormones, as well as fasted perceived appetite values, do not differ between three versus six eating periods daily, and no differences of the concordance or discordance of correlational relationships between self-reported appetite variables and serum biomarkers were found between low eating frequency and high eating frequency. Because all correlations between fasted plasma hormones and perceived appetite were of either weak or very weak strength, pre-established perceived appetite measures may not accurately reflect biological appetite. However, some specific expected relationships were seen with a closer categorical analysis. Further research is necessary to discover how this data could influence human eating behaviors and health with varied levels of eating frequency.

Footnotes:

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