

Eating Frequency, Disease-Related Biomarkers and Appetite

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Abstract

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Nutritional Sciences

Observational studies have demonstrated an inverse relationship between eating frequency (EF), obesity, and other markers for disease risk. It has been suggested that consumption of several small, frequent meals may influence physiological mechanisms, reducing the risk for disease and lowering appetite. Participants in this randomized crossover study completed two intervention phases lasting three weeks each: one of low eating frequency (“low-EF”; 3 eating occasions/day) and one of high eating frequency (“high-EF”; 8 eating occasions/day). Fasting C-reactive protein, insulin-like growth factor, and leptin were measured at baseline and endpoint of each phase and an optional subjective appetite testing session lasting four hours was offered at the endpoint of each phase. During appetite testing sessions, participants consumed an amount of food equal in total energy and macronutrient

content at either one occasion at 8:00 am (“low-EF” condition) or spread evenly over two smaller eating occasions at 8:00 am and 10:30 am (“high-EF” condition). Ratings of hunger, desire to eat, fullness, thirst, and nausea were made every 30 minutes using paper-and-pencil semi-anchored 100-mm Visual Analog Scales. A composite appetite score was calculated as the mean of hunger, desire to eat, and 100-fullness. The generalized estimating equation modification of linear regression was used to compare fasting plasma biomarkers and mean ratings of subjective appetite. A total of 15 participants completed both study phases (4 males, 11 females). Mean (\pm SD) age was 28.5 ± 8.70 , and mean (\pm SD) BMI was 23.3 ± 3.4 . There was a non-significant, but suggestive trend toward higher plasma hsCRP in the high-EF condition compared to the low-EF condition ($p=0.09$). Mean IGF-I was lower in the high-EF condition as compared to the low-EF condition ($p<0.001$). Regression models showed no association between EF and plasma leptin concentrations ($p=0.83$). Twelve participants completed appetite testing (4 males, 8 females). Mean composite appetite was higher in the high-EF condition for the total testing period baseline through 12:00 pm ($p<0.05$) and for the time period baseline through 10:30 am ($p<0.001$). No significant difference was detected between conditions in mean composite appetite for the final hour of testing ($p=0.12$). Findings suggest that when the energy and macronutrient content of the diet is equal, the consumption of smaller, more frequent meals may not positively impact inflammatory profiles or aid in appetite control. Further studies are needed to confirm the inflammatory impact of more frequent food consumption and to determine the physiological mechanisms driving the relationship between eating frequency and health.

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Dedication

This work is dedicated to Dad, Manda, Zan, Bets, and Mike. I love you!

Introduction

Background

Overwhelming evidence indicates that excess energy intake and poor diet quality are associated with increased risk for obesity, heart disease, diabetes, and cancer. Currently, these diet-related diseases are among the leading causes of death for adults in the United States (1). Specific guidelines indicating the number of calories, foods, and particular nutrients that one should consume to reduce their risk for disease are widely available (2). Although popular diet literature offers a wide array of recommendations concerning meal and snack frequency, there is currently no research-based guideline indicating the frequency of eating occasions, meal pattern, or number of times per day an individual should consume food for optimal health or disease prevention.

Many observational studies have demonstrated an inverse relationship between eating frequency (EF), obesity, and other markers for disease risk (3-7). It has thus been hypothesized that the consumption of several small, frequent meals may influence various physiological mechanisms, reducing risk for disease. Additionally, it is often assumed that higher meal frequency leads to higher satiety, lower overall caloric intake, and lower body weight. The health impact of high vs. low eating frequency in the absence of caloric manipulation has been examined in interventional studies as well. Researchers have measured a number of outcomes that indicate risk for heart disease (8-19), risk for diabetes (8, 9, 11, 16, 20-22), circulating hormones (11, 19, 22), and appetite (19, 23-27). Findings from these investigations are of particular importance because they can yield clues to the physiological processes impacted by alterations in eating frequency, and inform future efforts for research on dietary methods of preventing heart disease, diabetes and cancer.

Manipulation of eating frequency may be an important tool for maintaining overall health and disease prevention. As rates of nutrition-related diseases such as diabetes, cardiovascular disease, and cancer rise worldwide, it becomes increasingly important that intake guidelines incorporate recommendations for eating frequency based on high-quality scientific evidence. This study was conducted to examine the effects of high vs. low eating frequency on previously unstudied markers of risk for disease and subjective appetite in normal, overweight and obese males and females.

Specific Aims

1. To test whether a low-EF (3/day) vs. high-EF (8/day) pattern for a period of 3 weeks decreases plasma hsCRP, IGF-I, and leptin in overweight and obese individuals
2. To test whether consumption of food as one eating occasion (low-EF) would result in higher or lower subjective appetite ratings than the same amount of food as two equal servings (high-EF) over the same time period.

Research Design

The overall study design is provided in **Figure 1.1**. We used a within-subjects design (cross-over) and included normal, overweight and obese males and females. Each participant completed two study phases. During Phase 1 (3 weeks), participants were randomized to follow either an eating pattern with high eating frequency (high-EF"; 8 eating occasions per day) or an eating pattern with low eating frequency ("low-EF"; 3 eating occasions per day). During Phase 2 (3 weeks), participants having completed the high-EF condition will switch to the low-EF condition, and so forth. Energy consumed was based on each individual's habitual food intake, and thus designed for weight maintenance, not loss.

On day 21 of each phase, an optional half-day appetite testing session was completed. Starting at 8:00 am and every 30 minutes thereafter until 12:00 pm, subjective appetite was rated using 100-mm paper and pencil Visual Analog Scales for hunger, desire to eat, fullness, thirst, and nausea. At 8:00 am and 10:30 am (on the high-EF pattern) and at 8:00 am (on the low-EF pattern), participants consumed foods similar to those usually eaten in the morning during intervention phases.

This dissertation consists of manuscripts on the effects of high vs. low eating frequency on C-reactive protein (Chapter 1), Insulin-like growth factor-I and leptin (Chapter 2) and subjective appetite (Chapter 3) generated from this research project. Each chapter provides a detailed topic-specific background, methods, results, and discussion section.

Figure 1. Study Procedures. Participants attended one orientation session and four clinic visits at the FHCRC Prevention Center and all other study procedures were carried out at home. Order of conditions was randomly assigned.

STUDY PROCEDURES

Orientation Session: FHCRC Prevention Center

1. Verify eligibility criteria
2. Informed consent
3. 7-Day Food Record instructions

7-Day Food Record: At-Home

1. Record type and quantity of all food and drink except water for 7 consecutive days
2. Return food record to study staff via hand delivery or U.S. Post

Meal Plan Checklist Creation: Study RD

1. 7-Day Food Record analyzed for energy and macronutrient content
2. Isoenergetic meal plans created for Low-EF "Meals" and high-EF "Grazing" conditions

Randomization

- "Meals" Low-EF (3/day)
- "Grazing" High-EF (8/day)

PHASE I

Day 1: FHCRC Prevention Center
Blood draw IGF-I, CRP, leptin
Body weight, waist/hip, blood pressure
DEXA

Day 21: FHCRC Prevention Center
Blood draw IGF-I, CRP, leptin
Body weight, waist/hip, blood pressure
APPETITE TESTING SESSION

2 WEEK WASHOUT CROSSOVER

PHASE 2

Day 1: FHCRC Prevention Center
Blood draw IGF-I, CRP, leptin
Body weight, waist/hip, blood pressure

Day 21: FHCRC Prevention Center
Blood draw IGF-I, CRP, leptin
Body weight, waist/hip, blood pressure
APPETITE TESTING SESSION

Chapter 1: The effects of high vs. low eating frequency on plasma C-reactive protein

Introduction

The inflammatory process has recently been identified as a key mechanistic link between dietary behavior and chronic disease (28). Not only the composition of one's diet and the total energy it provides, but also the frequency with which one consumes food can be associated with known markers of inflammation and risk for disease (29). The inflammatory biomarker C-reactive protein (CRP) has frequently been measured in dietary studies as a predictor of health outcome (30-33). This acute-phase protein is synthesized primarily in the liver in response to activation by the cytokine interleukin-6 (IL-6) which is produced by adipose tissue, lymphocytes, and macrophages during the immune response. High levels of circulating CRP are directly related to a sedentary lifestyle and obesity (20, 34, 35), are indicative of systemic inflammation, and are associated with development of heart disease (30, 36), diabetes (37-39), and cancer (40-42), supporting the idea that dietary behavior can impact disease risk via inflammatory pathways.

The association of eating frequency with CRP may depend largely on fluctuations of blood glucose and triglycerides occurring in the post-prandial state. Post-prandial hyperglycemia and hypertriglyceridemia can contribute to the development of endothelial dysfunction, inflammation, and ultimately increased disease risk (28). By moderating caloric intake and glycemic excursions at each meal, a high-eating frequency (EF) diet may lead to decreases in post-prandial insulin secretion, and lower production of low-density lipoprotein (LDL) and inflammatory proteins including CRP. The association of eating frequency and inflammatory biomarkers has not been adequately explored (43).

We conducted a randomized, controlled crossover trial examining the influence of eating frequency on health related biomarkers called the Meals and Grazing Study. The purpose of the current analysis was to explore associations between eating frequency and a biomarker indicative of systemic inflammation. We hypothesized that increased eating occasions would be associated with decreased CRP. To test this hypothesis, we compared whether eating eight times per day (high-EF condition) vs. eating three times per day (low-EF condition) for a period of three weeks would decrease plasma hsCRP among a sample of healthy individuals.

Materials and methods

Participants

Participants were recruited for the Meals and Grazing Study using posters and online advertisements at the Fred Hutchinson Cancer Research Center (FHCRC) and the University of Washington campus in Seattle, WA. Participants were healthy 18-50 year-old males and females with a body mass index (BMI) greater than or equal to 18 kg/m² (normal to obese). Exclusion criteria included diabetes, smoking, following a diet to gain or lose weight, athletes in training, abnormal self-reported cholesterol or blood pressure, taking prescribed medication other than oral contraceptives, and pregnancy or nursing (females). All experimental protocols were approved by the Institutional Review Office at the Fred Hutchinson Cancer Research Center. All participants provided informed consent and were compensated for their time.

Study Design

The overall study design for the Meals and Grazing Study is shown in **Figure 1**. Interested individuals were invited via email or telephone for an initial session at FHCRC in which eligibility criteria were verified and study procedures were explained in detail. Participants were free-living and consumed their own food throughout the study using an individually tailored meal plan. Eligible participants who enrolled in the study were provided detailed written and verbal instructions on keeping a seven-day food record. Participants then recorded details (type and quantity) for all food and drink except water consumed for seven consecutive days. The completed food records were returned to study staff. Upon receipt, the study dietitian analyzed the seven-day food record for energy and macronutrient content using The Food Processor software (ESHA Research, Salem OR.) Keeping food items, energy, and macronutrient content of the diet constant, a low-EF “Meals” meal plan (providing all energy as three evenly spaced eating occasions per day) and a high-EF “Grazing” meal plan (providing all energy as eight evenly spaced eating occasions per day) were individually designed for each participant. Using the food record as a guide, weight-maintaining meal plans were designed to rotate every seven days and participants were instructed to eat only the foods on their individual eating plan at the specified hours. All meal and snack consumption (including time of intake) were reported daily using an electronic Meal Plan Checklist. Thirty-four participants attended the initial session, of which 32 were eligible to participate and enrolled. Of those, 15 completed the Meals and Grazing Study. Reasons for drop-out included loss to follow-up after the initial session (8), received medical advice not to participate after initial session (1), decided not to participate after completing seven-day food record (1), decided not to participate-reason not indicated (1), loss to follow-up after the first clinic visit (3), and personal schedule conflicts with meal timing (3).

This study used a randomized crossover design so that each participant served as his or her own control.

A computerized random number generator was used to assign order of conditions. Participants in the Meals and Grazing Study completed two 21-day study phases in random order (Phase 1 and Phase 2 in **Figure 1**), with a 14-day washout period during which they were instructed to consume their habitual diet (44). Clinic visits and blood draws were completed at the beginning and end of each study phase. In both phases, the foods, energy, and macronutrient content of the participant's diet were kept constant and were matched with the participant's normal diet as reported in the seven-day food record. In both conditions, the first eating occasion of the day was usually timed at 8:00 am. In the low-EF "Meals" condition, foods were divided into three approximately equal eating occasions per day. Eating occasions were spaced apart by 5.6 (SD 0.52) hours and spanning 11.0 (SD 1.0) hours on average. In the high-EF "Grazing" condition, foods were divided into eight approximately equal eating occasions per day, spaced apart by 1.77 (SD 0.25) hours and spanning 12.6 (SD 1.89) hours on average. Each day, participants received an automatically-generated email from the study, linking to an online Meal Plan Checklist that indicated the specific foods and serving sizes to consume and the times of each daily eating occasion (see **Appendix 1**). Participants were provided with verbal and written guidance on meal and food substitutions prior to beginning the study. For example, an appropriate substitution for a medium apple would be another medium fruit such as a pear or an orange. Meal Plan Checklists, specifying deviations including substituted foods, different portions, or missed/extra meals were submitted online daily to study staff. Participants were encouraged to contact the study coordinator if they were not able to complete the assigned eating occasions or consume the foods on their Meal Plan Checklists.

Participants attended in-person clinic visits at 8:00 am on day one and day 21 of both phases for a total of four visits. Clinic visits were scheduled on the same day of the week whenever possible. All other study activities were completed at home. Participants were asked to consume nothing other than

noncarbonated water for 12 hours prior to their appointment, and to refrain from drinking alcohol or eating or exercising outside of their normal routine. In addition, they were asked not to use any non-steroidal anti-inflammatory drugs (NSAIDs; such as ibuprofen) for 72 hours prior to their appointment. Any deviations from protocol, including use of NSAIDs and alcohol ingestion, were reported to study staff and recorded. Study sessions were rescheduled for a later date when possible to ensure protocol compliance. During all four clinic visits, body weight, height, waist and hip circumference, pulse, diastolic and systolic blood pressure were measured and venous blood samples were collected using standardized procedures by trained staff. During the first clinic visit, Dual energy X-ray absorptiometry (DEXA) using a GE Lunar DPX Pro (GE Healthcare Lunar, Madison, WI) was used to measure body fat percentage.

Plasma CRP concentrations were measured using the High-Sensitive CRP reagent (Kamiya Biochemical Company, Seattle, WA) on a Roche Cobas Mira chemistry analyzer. The limit of quantification for this assay in our lab was 0.08 mg/l. Samples were run in duplicate, and the median duplicate intra-assay coefficient of variation (CV) was 3.4% for hsCRP. The inter-assay CV was 8.0%. The assays were performed on never-thawed samples. All samples from the same individual were run in the same batch.

Statistical Analysis

The goal of statistical analysis was to measure within subject differences in plasma hsCRP between the high-EF and low-EF conditions. Logarithmic transformations were used to improve the normality of distributions. The generalized estimated equations (GEE) modification of linear regression was used to estimate mean within-subjects differences in plasma hsCRP after three weeks on the low-EF condition

vs. three weeks on the high-EF condition while adjusting for baseline plasma hsCRP concentrations. Models were adjusted for order of conditions, gender, and body fat percentage only if they altered parameter estimates by more than 10%. This did not occur in any of the models tested. We also used paired t-tests to compare endpoint plasma hsCRP between conditions and to compare the change from baseline to endpoint plasma hsCRP between conditions. Weight change, waist to hip ratio, blood pressure, and compliance to study diets were also analyzed for between-conditions differences using generalized estimating equations. Condition- and timepoint-specific mean diastolic blood pressure was imputed for one male participant whose diastolic blood pressure was recorded erroneously at baseline of the low-EF condition. A value of 0.04 mg/dL (half of the lower limit of detection) was imputed for one participant whose plasma hsCRP was too low to be detected by our assay at the endpoint of the low-EF condition. Statistical analyses were performed using Stata version 12.0 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.)

Results

Participant characteristics are shown in **Table 1**. A total of 15 participants completed both study phases (4 males, 11 females). Mean (\pm SD) age was 28.5 ± 8.70 (25.0 ± 4.7 for males; 29.8 ± 9.6 for females.) Mean (\pm SD) BMI was 23.3 ± 3.4 (22.4 ± 1.2 for males; 23.6 ± 3.9 for females.) Mean body fat percentage was 31.8 ± 6.5 (25.5 ± 2.8 for males; 34.0 ± 5.9 for females.)

Participant diet composition from the seven-day food record is shown in **Table 2**. Per day, participants consumed on average 2262 ± 436 kcals, with 48.0% energy from carbohydrates, 16.8% energy from protein, and 33.7% energy from fat. No significant dietary differences were observed between groups of

normal and overweight/obese participants (normal : BMI 18-24.9 kg/m², overweight/obese: ≥ 25kg/m²) in terms of daily intake. Compliance to the study diets was calculated by dividing the total number of completed eating occasions by the total number of assigned eating occasions in each phase for each individual. For instance, in the low-EF phase, a participant who consumed 58 of the 63 assigned eating occasions would have 92% compliance. Excellent rates of compliance were achieved in both low-EF and high-EF conditions (96% and 100%, respectively). Using a paired t-test, we found a slight tendency toward higher compliance in the high-EF condition (p=0.07) (data not shown).

Participant body weight, BMI, waist to hip ratio, and systolic and diastolic blood pressure are shown in **Table 3**. Participant body weight increased by 0.14% and decreased by 0.44% in the high-EF condition. Body Mass Index (BMI; kg/m²) decreased by 2.7% in the low-EF condition and decreased by 0.83% in the high-EF condition. The waist to hip ratio decreased by 7.3% in the low-EF condition and increased by 2.56% in the high-EF condition. Diastolic blood pressure decreased by 3.5% in the low-EF condition and increased by 1.7% in the high-EF condition. Systolic blood pressure decreased by 7.46% in the low-EF condition and increased by 3.37% in the high-EF condition. Using generalized estimating equations with adjustment for baseline values, we found no significant differences in mean body weight, BMI, waist to hip ratio, or diastolic blood pressure between the low-EF and high-EF conditions. Systolic blood pressure was significantly lower in the low-EF condition than the high-EF condition (p<0.001).

Plasma hsCRP values by study condition are shown in **Table 4**. In the linear regression analysis, we found a non-significant, but suggestive trend toward higher plasma hsCRP in the high-EF condition compared to the low-EF condition (p=0.09). Further, the direction of change varied by condition. On average, plasma hsCRP decreased by 41% in the low-EF condition and increased by 6.5% in the high-EF

condition.

Discussion

This randomized cross-over intervention assessed the impact of eating frequency on plasma C-reactive protein, a sensitive inflammatory biomarker. Results from our linear regression analysis suggested higher mean hsCRP (adjusted for baseline values) when eating eight times per day (high-EF condition) vs. three times per day (low-EF condition), although differences were not significant.

Recent clinical studies have found associations between eating frequency and disease-related biomarkers. Higher eating frequency has been linked with lower total cholesterol (8, 9, 11-13) and lower low-density lipoprotein (8, 9, 17, 19), although these results were not always consistent (10, 15, 16). However, other studies have demonstrated a relationship between higher eating frequency and lower high-density lipoprotein (15-17, 19). Our analyses detected a trend toward higher plasma hsCRP in the high-EF condition, contrary to the majority of previously conducted clinical trials and supporting a direct relationship between eating frequency and an inflammatory biomarker predictive of later disease.

Based on results from observational and clinical investigations, we hypothesized that higher eating frequency would be associated with lower plasma hsCRP in our sample. Our rationale was based on the presumed lower peaks in postprandial glucose, insulin, lipids and associated molecules that might influence CRP following a high EF eating pattern. However, it could be that because a high-EF pattern increases the total amount of time spent in the post-prandial state, the concentrations of pro-inflammatory metabolites and cytokines become elevated. It is possible that the positive trend we

observed between eating frequency and plasma hsCRP reflects development of a pro-inflammatory milieu resulting from more frequent food ingestion.

Although we detected a significant difference in change in systolic blood pressure such that the low-EF condition led to a 7.46% decrease and the high-EF condition led to a 3.37% increase, the clinical relevance of this change is unknown. Further research is needed to determine whether these eating-frequency related changes in systolic blood pressure are clinically relevant or whether our finding is due to chance.

The current study had several strengths. The use of a randomized crossover design allowed for comparison of plasma hsCRP between conditions of low and high EF while limiting sources of random variation from between-subjects differences. The energy and macronutrient content of diets consumed during both conditions were identical and compliance in both conditions was high, ensuring that the direct trend between eating frequency and plasma hsCRP was not confounded by differences in dietary composition or total energy consumed. Further, diets were weight-maintaining and no differences were detected in anthropometric measurements between conditions, eliminating the possibility that results were reflective of changes in body weight or adiposity in either condition.

Certain limitations were present in this study. The provision of all study foods to participants has frequently been used in dietary interventions to increase compliance. In this investigation, participants consumed their own foods in portions designated by the study dietitian. It is possible that intake during both conditions would have been more precisely equal if all foods had been pre-portioned and provided

to participants at the facility. Our crossover design kept diet composition the same across conditions to allow consideration of the isolated effects of eating frequency. In a natural setting, it is likely that any impact of eating frequency will depend on diet quality. Our study did not address this issue.

Additionally, although CRP is frequently used as a reliable predictor of later disease, this acute-phase protein fluctuates rapidly during the immune response, and high levels can result from minor infections. Participants in the study were asked to reschedule study appointments for any illness. It is still possible that changes in plasma hsCRP in this study could be attributed to immune function, rather than changes in eating frequency. Since the order of conditions was randomly assigned, and we conducted within-subjects analyses, systematically high or low CRP in either condition is not likely.

The majority of participants in this study were of normal BMI, and all were healthy with initial CRP within normal limits. Including overweight and obese individuals in our study population, or those with chronic disease and likely to have high initial plasma hsCRP, may have resulted in more substantial effect in our outcome. Our sample was small (n=15), but comparable in size to other similar investigations (9, 10, 12, 18-20, 29, 45). A larger sample size might have also increased our ability to detect a significant difference between conditions.

Conclusion

With all other dietary factors standardized, the consumption of a high-EF vs. a low-EF diet may increase plasma hsCRP in healthy individuals. Although between-conditions differences were not significant, these results are suggestive of a direct relationship between eating frequency and systemic inflammation. Further studies should investigate any possible interactions between eating frequency

and diet composition and the impact of subject characteristics such as BMI.

Table 1. Participant characteristics by gender (n=15).^{1,2}

	Males (n=4)	Females (n=11)	Total(n=15)
Age (y)	25.0 (4.7)	28.2 (8.4)	28.5 (8.7)
Body Mass Index (kg/m ²)	22.4 (1.2)	24.3 (4.2)	23.3 (3.4)
Total body fat %	25.5 (2.8)	35.2 (6.2)	31.8 (6.5)
Waist/hip ratio	0.84 (0.05)	0.80 (0.06)	0.8 (0.07)
Systolic blood pressure (mmHg)	118.5 (9.1)	112.5 (8.5)	112.1 (10.1)
Diastolic blood pressure (mmHg)	76.3(3.2)	72.5 (4.2)	72.2 (5.1)

¹ Anthropometric measures collected during participant's first study session.

² Values are means (Standard Deviation; SD).

Table 2. Participant diet composition by Body Mass Index (kg/m²) (n=15).^{1,2,3}

	Normal Weight (BMI 18-24.9) (n=13)	Overweight (BMI ≥ 25) (n=2)	Total (n=15)
Total daily energy intake	2219.0 (425)	2538 (560)	2262 (436)
Carbohydrate (g)	274.9 (50.1)	269.2 (75.2)	274.1 (50.6)
Carbohydrate (% energy)	49.0 (4.7)	41.0 (1.4)	47.9 (5.2)
Protein (g)	95.9 (26.8)	102.3 (30.1)	96.8 (26.2)
Protein (% energy)	17 (3.54)	15.5 (0.7)	16.8 (3.3)
Fat (g)	83.0 (26.5)	113.8 (15.0)	87.1 (27.1)
Fat (% energy)	0.33 (0.06)	40 (0.04)	33.7 (5.9)
Fiber (g)	25.46 (6.9)	35.4 (24.1)	26.8 (9.8)

¹Dietary data obtained from 7-day food record. Body Mass Index calculated at participant's first clinic visit.

²Values are means (Standard Deviation; SD).

³ No significant differences were observed between the two categories BMI 18-24.9 and BMI ≥25 (two-sample t-test with unequal variance; p<0.05).

Table 3. Participant anthropometric measurements and blood pressure by study condition (n=15).¹

		Weight (kg)	BMI (kg/m ²)	Waist/hip ratio	Systolic BP (mmHg)	Diastolic BP (mmHg)
Low-EF	Baseline	68.2(13.8)	23.3(3.2)	0.82(0.06)	114.3(9.5)	73.2(6.6)
	Endpoint	68.3(14.4)	23.3(3.5)	0.79(0.07)	105.3(7.6)	70.9(6.0)*
High-EF	Baseline	68.5(14.3)	23.4(3.5)	0.79(0.07)	107.5(7.5)	69.9(4.4)
	Endpoint	68.2(14.1)	23.3(3.4)	0.79(0.06)	110.8(8.0)	71.1(4.6)*

¹Values are means (Standard Deviation; SD).

*Generalized estimating equation (GEE) modification of the linear regression compared weight, BMI (Body Mass Index; kg/m²), waist to hip ratio, systolic blood pressure and diastolic blood pressure in low-EF and high-EF conditions, adjusted for baseline values. Systolic blood pressure was significantly lower in the low-EF condition (p<0.001); no other significant differences were detected.

Table 4. Effect of low-EF vs. high-EF experimental conditions on plasma hsCRP (mg/L), by study condition (n=15)^{1,2,3}

	Baseline	Endpoint
Low-EF	0.27 (0.10)	0.16 (0.06)*
High-EF	0.27 (0.07)	0.29 (0.14)*

¹Values are geometric means (Standard Error of the Mean; SEM).

²Each participant completed two dietary interventions lasting approximately three weeks each: low-EF (three eating occasions per day); high-EF (eight eating occasions per day).

³Plasma hsCRP measured using High-Sensitive CRP reagent on never-thawed samples.

*Generalized estimating equation (GEE) modification of the linear regression compared hsCRP in low-EF and high-EF conditions, adjusted for baseline plasma hsCRP (p=0.09).

Chapter 2: Effects of high vs. low eating frequency on plasma insulin-like growth factor-I and leptin

Introduction

Research has demonstrated a clear link between excess caloric intake, obesity, and development of heart disease, cancer, and diabetes (46-52). The physiological mechanisms governing the relationship between dietary behavior and health depend on the energy and nutrient content of the diet, and may also be impacted by the frequency of food consumption. It is often said that daily energy intake should be spread evenly throughout the day in the form of small, frequent meals rather than fewer larger meals, as this pattern of consumption may help to limit the magnitude of post-prandial spikes in hormones and pro-inflammatory cytokines known to raise disease risk (8, 20, 29, 44, 53).

Several studies have attempted to elucidate the physiological consequences of increased eating frequency (EF). Jenkins and colleagues found that mean 12-hour insulin concentrations were lowered in a small sample of males ($n=7$) who ate 17 times per day vs. three times per day (9). However, that study and others have found no significant effect of eating frequency on fasting serum glucose or insulin concentration (9, 10, 16, 19, 22), or glucose and insulin response curves (8, 21). Other studies have found associations between high EF and increased fasting blood glucose and decreased glucose tolerance (11) and increased serum insulin (22). The relationship between eating frequency and diabetes risk markers remains unresolved (29). The influence of eating frequency on markers for heart disease risk also is unclear. The majority of studies measuring blood lipids have found an inverse association between eating frequency and low-density lipoprotein (LDL), but total cholesterol, HDL and total triglycerides were either non-significantly altered or were positively associated with eating

frequency (29).

The physiologic processes driving any effects of eating frequency on health may be better clarified by measurement of circulating hormones involved in pathways of metabolism, appetite regulation, and inflammation. Relatively few studies have measured the impact of eating frequency on hormonal biomarkers other than insulin. Insulin-like growth factor-I (IGF-I) is a peptide hormone synthesized in the liver which is similar in structure and released into the circulation at a rate similar to that of insulin (54). Production of IGF-I is stimulated by pituitary production of Growth Hormone (GH), which is enhanced by hypoglycemia and may also be increased by leptin (55). Circulating levels of IGF-I fall dramatically within a short period of time in fasting conditions, making it a sensitive indicator of nutritional status (54). IGF-I is a mitogen, prevents apoptosis, and increases cell proliferation and differentiation and is expressed in neoplastic tissue (56) and has been implicated in the development of cancer and tumor growth. It is possible that by moderating post-prandial fluctuations in blood glucose and insulin, the consumption of a high-EF diet may reduce production of IGF-I.

Another hormone which may help to mediate the effects of eating frequency on health is leptin. Leptin is produced mainly by adipocytes in direct proportion to the amount of body fat present. Leptin is involved in long-term regulation of intake, energy metabolism, and is important in the immune response (57). A positive correlation between fasting leptin and insulin concentrations has been demonstrated; however, it is unclear from human in vivo studies whether increased insulin directly leads to increased leptin (58). In vitro studies have shown that exposure of human adipocytes to insulin leads to increased leptin mRNA expression (58). Two previous investigations on eating frequency have measured leptin concentrations in the absence of caloric manipulation. Participants in one weight-maintenance trial who

consumed all calories within a four-hour window each day (one meal/day) vs. consuming all calories over three eating occasions per day showed no difference in fasting morning plasma leptin concentrations after a period of eight weeks (11). In another trial, participants who regularly consumed four meals/day and switched to eating three meals/day had significantly increased leptin concentrations after 28 days. Participants who regularly consumed three meals/day and switched to eating four meals/day showed no difference in leptin concentrations (22).

The purpose of the present analysis was to examine the influence of eating frequency on IGF-I and leptin, two hormonal indicators of nutritional status that have the potential to influence disease progression via metabolic and inflammatory pathways. Specifically, we conducted a randomized, crossover study to address whether a high-EF diet (eight eating occasions/day) vs. a low-EF diet (three eating occasions/day) for a period of three weeks would decrease plasma IGF-I and leptin concentrations.

Materials and methods

Participants

Participants were recruited for the Meals and Grazing Study using posters and online advertisements at the Fred Hutchinson Cancer Research Center (FHCRC) and the University of Washington campus in Seattle, WA. Participants were healthy 18-50 year-old males and females with a body mass index (BMI) greater than or equal to 18 kg/m² (normal to obese). Exclusion criteria included diabetes, smoking, following a diet to gain or lose weight, athletes in training, non-normal blood cholesterol or blood pressure, taking prescribed medication other than oral contraceptives, and pregnancy or nursing

(females). All experimental protocols were approved by the Institutional Review Office at the Fred Hutchinson Cancer Research Center (FHCRC). All participants provided informed consent and were compensated for their time.

Study Design

The overall study design for the Meals and Grazing Study is shown in **Figure 1**. Interested individuals were invited via email or telephone for an initial session at FHCRC in which eligibility criteria were verified and study procedures were explained in detail. Participants were free-living and consumed their own food throughout the study using an individually tailored meal plan, which was designed using the following procedures. Eligible participants who enrolled in the study were provided detailed written and verbal instructions on keeping a seven-day food record. Participants then recorded details (type and quantity) for all food and drink except water consumed for 7 consecutive days and returned food records to study staff via hand delivery or U.S. Post. Upon receipt, the study dietitian analyzed the seven-day food record for energy and macronutrient content using The Food Processor software (ESHA Research, Salem OR.) Keeping food items, energy, and macronutrient content of the diet constant, a low-EF “Meals” meal plan (providing all energy as three evenly spaced eating occasions per day) and a high-EF “Grazing” meal plan (providing all energy as eight evenly spaced eating occasions per day) were individually designed for each participant. Weight-maintaining meal plans were designed to rotate every seven days and participants were instructed to eat only the foods on their individual eating plan at the specified hours. All meal and snack consumption (including time of intake) were reported daily using an electronic meal plan checklist. Thirty-four participants attended the initial session, of which 32 were eligible to participate and enrolled. Of those, 15 completed the Meals and Grazing Study. Reasons for drop-out included loss to follow-up after the initial session (8), received medical advice not to

participate after initial session (1), decided not to participate after completing seven-day food record (1), reason not indicated (1), loss to follow-up after the first clinic visit (3), and personal schedule conflicts with meal timing (3).

This study used a randomized crossover design so that each participant served as his or her own control. A computerized random number generator was used to assign order of conditions. Participants in the Meals and Grazing Study completed two 21-day study phases in random order (Phase 1 and Phase 2 in **Figure 1**), with a 14-day washout period during which they were instructed to consume their habitual diet (44). Clinic visits and blood draws were completed at the beginning and end of each study phase. In both phases, the foods, energy, and macronutrient content of the participant's diet were kept constant and were matched with the participant's normal diet as reported in the seven-day food record. In both conditions, the first eating occasion of the day was usually timed at 8:00 am. In the low-EF "Meals" condition, foods were divided into three approximately equal eating occasions per day, spaced apart by 5.6 (SD 0.52) hours and spanning 11.0 (SD 1.0) hours on average. In the high-EF "Grazing" condition, foods were divided into eight approximately equal eating occasions per day, spaced apart by 1.77 (SD 0.25) hours and spanning 12.6 (SD 1.89) hours on average. Each day, participants received an automatically-generated email from the study, linking to an online Meal Plan Checklist that indicated the specific foods and serving sizes to consume and the times of each daily eating occasion (see **Appendix 1**). Participants were provided with verbal and written guidance on meal and food substitutions prior to beginning the study. For example, an appropriate substitution for a medium apple would be another medium fruit such as a pear or an orange. Meal Plan Checklists, specifying deviations including substituted foods, different portions, or missed/extra meals were submitted online daily to study staff. Participants were encouraged to contact the study coordinator if they were not able to complete the

assigned eating occasions or consume the foods on their Meal Plan Checklists.

Participants attended in-person clinic visits at 8:00 am on day one and day 21 of both phases for a total of four visits. Clinic visits were scheduled on the same day of the week whenever possible. All other study activities were completed at home. Participants were asked to consume nothing other than noncarbonated water for 12 hours prior to their appointment, and to refrain from drinking alcohol or eating or exercising outside of their normal routine. In addition, they were asked not to use any non-steroidal anti-inflammatory drugs (NSAIDs; such as ibuprofen) for 72 hours prior to their appointment. Any deviations from protocol, including use of NSAIDs and alcohol ingestion, were reported to study staff and recorded. Study sessions were rescheduled for a later date when possible to ensure protocol compliance. During all four clinic visits, body weight, height, waist and hip circumference, pulse, diastolic and systolic blood pressure were measured and venous blood samples were collected using standardized procedures by trained staff. During the first clinic visit, Dual energy X-ray absorptiometry (DEXA) using a GE Lunar DPX Pro (GE Healthcare Lunar, Madison, WI) was used to measure body fat percentage.

Leptin concentrations were measured using the Human Leptin ELISA (Millipore, Inc., Billerica, MA). The lowest limit of detection for the leptin assay was 0.125ng/ml. IGF-1 levels were measured using the Human IGF-I Quantikine ELISA Kit (R&D Systems, Minneapolis, MN). The lowest limit of detection for the IGF-1 assay was 0.094 ng/ml. Samples were run in duplicate, and the median duplicate intra-assay coefficients of variation (CVs) were: 5.7% for leptin; and 1.2% for IGF. Inter-assay CVs were 1.5% and 4.0% for leptin and IGF-1, respectively. The assays were performed on never-thawed samples. All samples from the same individual were run in the same batch.

Sample size, data analyses, and statistical tests

The primary goal of statistical analysis was to compare mean IGF-I and leptin concentrations in the high-EF vs. low-EF conditions, adjusted for baseline values. Logarithmic transformations were used to improve the normality of distributions. The generalized estimated equations (GEE) modification of linear regression was used to account for the correlation within individuals over time. Regression analyses were adjusted for order of conditions, gender, and body fat percentage only if they altered the effect of condition by more than 10%. This did not occur in any of the models tested and so a more parsimonious model was used. Between-conditions differences in weight change, waist to hip ratio, blood pressure, and compliance to study diets were compared using the generalized estimating equations modification of linear regression. Condition- and timepoint-specific mean diastolic blood pressure was imputed for one male participant whose diastolic blood pressure was recorded erroneously at baseline of the low-EF condition. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using Stata version 12.0 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.)

Results

Participant characteristics are shown in **Table 1**. A total of 15 participants completed both study phases (4 males, 11 females). Mean (\pm SD) age was 28.5 ± 8.70 (25.0 ± 4.7 for males; 29.8 ± 9.6 for females.) Mean (\pm SD) BMI was 23.3 ± 3.4 (22.4 ± 1.2 for males; 23.6 ± 3.9 for females.) Mean body fat percentage was 31.8 ± 6.5 (25.5 ± 2.8 for males; 34.0 ± 5.9 for females.)

Participant diet composition from the seven-day food record is shown in **Table 2**. Per day, participants consumed on average 2262 ± 436 kcals, with 48.0% energy from carbohydrates, 16.8% energy from protein, and 33.7% energy from fat. No significant dietary differences were observed between groups of normal and overweight/obese participants (normal : BMI 18-24.9 kg/m², overweight/obese: ≥ 25 kg/m² in terms of daily intake. Compliance to the study diets was calculated by dividing the total number of completed eating occasions by the total number of assigned eating occasions in each phase for each individual. For instance, in the low-EF phase, a participant who consumed 58 of the 63 assigned eating occasions would have 92% compliance. Excellent rates of compliance were achieved in both low-EF and high-EF conditions (96% and 100%, respectively) and there was no significant difference detected between the conditions ($p=0.07$; data not shown). Using a paired t-test, we found a slight tendency toward higher compliance in the high-EF condition ($p=0.07$); data not shown-

Participant body weight, BMI, waist to hip ratio, and systolic and diastolic blood pressure are shown in **Table 3**. Participant body weight increased by 0.14% and decreased by 0.44% in the high-EF condition. Body Mass Index (BMI; kg/m²) decreased by 2.7% in the low-EF condition and decreased by 0.83% in the high-EF condition. The waist to hip ratio decreased by 7.3% in the low-EF condition and increased by 2.6% in the high-EF condition. Diastolic blood pressure decreased by 3.5% in the low-EF condition and increased by 1.7% in the high-EF condition. Systolic blood pressure decreased by 7.46% in the low-EF condition and increased by 3.37% in the high-EF condition. .Using generalized estimating equations with adjustment for baseline values, we found no significant differences in mean body weight, BMI, waist to hip ratio, or diastolic blood pressure between the low-EF and high-EF conditions. Systolic blood pressure was significantly lower in the low-EF condition than the high-EF condition ($p<0.001$).

Plasma IGF-I and leptin values are shown in **Table 4**. We found lower mean IGF-I in the high-EF condition compared to the low-EF condition ($p<0.001$). This relationship was not modified by order of conditions, gender, or body fat percentage. Between baseline and endpoint, average plasma IGF-I increased by 6.8% in the low-EF condition and decreased by 11.6% in the high-EF condition.

Regression models showed no association between EF and plasma leptin concentrations ($p=0.83$; **Table 4**). Plasma leptin decreased in the low-EF condition by 14.2% and decreased in the high-EF condition by 11.5%.

Discussion

In this randomized controlled crossover study we examined the impact of eating frequency on plasma concentrations of IGF-I and leptin. Over two three-week phases, participants consumed equal energy and macronutrients and intake was divided into either three eating occasions/day (low-EF) or eight eating occasions/day (high-EF). In regression models adjusted for baseline biomarkers, we found significantly lower mean IGF-I in the high-EF condition ($p<0.001$). There was no significant difference between low-EF and high-EF conditions in mean plasma leptin concentrations adjusted for baseline values ($p=0.83$).

In our study, mean IGF-I was lower after completion of the high-EF condition than the low-EF condition after adjustment for baseline values ($p<0.001$). It is possible that by decreasing the magnitude of post-prandial peaks in blood glucose, consumption of a high-EF diet in our study decreased production of

insulin. The resulting downregulation of insulin receptors may have led to decreased production or bioavailability of IGF-I. Another possible explanation for this difference is that diurnal insulin concentrations may have been lower in the high-EF condition due to reduced nighttime intake, leading to decreased hepatic production of IGF-I. Our finding that IGF-I can be decreased by manipulating eating frequency is consistent with the idea that eating, smaller meals more frequently may positively impact health and risk for disease.

In a recent cross-over investigation, no difference in fasting morning plasma leptin concentrations was observed when participants consumed all daily calories within a four-hour window each day (one meal/day) vs. spread over three eating occasions/day for a period of eight weeks (11). Although body weight was intentionally maintained within 2 kg over both intervention periods, body weight and body fat percentage were significantly decreased ($p < 0.01$ and $p < 0.001$, respectively) in the one meal/day condition, making it hard to interpret the leptin data in this study as they relate to EF. In another trial, a significant increase in leptin concentrations was observed in participants who regularly consumed food at four occasions/day and switched to eating three meals/day (*ad libitum* food consumption) for a period of 28 days (22). This omission of one eating occasion per day was associated with a significant decrease in the total daily energy consumed ($p < 0.05$), and a significant increase in fat mass ($p < 0.05$), but no significant change in body weight. In the current study, Meal Plan Checklists were designed to ensure equal caloric and macronutrient intake and we did not detect a significant difference in plasma leptin concentration or body weight between conditions. It is possible that body fat percentage was affected by eating frequency in our trial, but we detected no differences between conditions in body weight or plasma leptin concentrations to suggest that this was the case. In future studies, measurement of ad-libitum intake at each eating occasion and measurement of body composition may

help to clarify whether any observed effects of eating frequency on leptin concentrations are driven by differences in dietary intake and body composition.

We detected 7.46% decrease in systolic blood pressure in the low-EF condition and a 3.37% increase in systolic blood pressure in the high-EF condition. Between conditions, the difference in systolic blood pressure was significant ($p < 0.001$). In contrast, Stote and colleagues found that participants had significantly higher blood pressure in the lower-EF condition ($p < 0.05$) (19). Further research is needed to determine whether these changes are related to differences between conditions in eating frequency or can be attributed to chance.

Strengths and limitations

The current study had several strengths. This is the first study known to authors that measured eating frequency and IGF-I, a hormone that promotes tumor development via mitogenic and anti-apoptotic actions. Our study used a randomized crossover design to eliminate random sources of variation from comparisons of plasma IGF-I and leptin between conditions. Participants consumed diets equal in energy and macronutrient content during both intervention phases, and no differences were detected in anthropometric measurements between conditions. Excellent compliance to both study diets (low-EF and high-EF) was reported. Therefore, we are confident that the trends observed can be solely attributed to differences in eating frequency between conditions.

Limitations also existed. Participants purchased, prepared, and consumed their own foods in portions designated by the study dietitian during both intervention phases. Despite excellent rates of compliance

reported by participants, it is possible that intake was not as precisely controlled as intended. Because participants were assigned isoenergetic diets equal in macronutrient content across study interventions, we were not able to assess the role of diet quality. Further, we were not able to determine whether participants would naturally consume different total amounts of food when the number of eating occasions was increased or decreased. Study participants were mostly women of normal BMI. A more diverse sample including more overweight and obese individuals and a greater percentage of males may have resulted in different findings. Further, a larger sample size may have been necessary to detect significant differences between conditions in our outcome measures; however, our sample was comparable in size to other similar investigations (9, 10, 12, 18-20, 29, 45).

Conclusion

Eating frequency is an important yet unexplored dimension of dietary behavior that could have a major impact on health outcomes. Results from this investigation indicate that increasing eating frequency may reduce levels of circulating IGF-I, a sensitive hormonal indicator of nutritional status that promotes cellular proliferation and tumor development. The impact of eating frequency on leptin may depend on changes in body weight or body fat content driven by energy or macronutrient intake. These changes may be observed when the dietary intake is allowed to fluctuate naturally within the framework of pre-determined eating occasions at a low or high frequency. Future studies should employ a large, diverse sample and provide study meals to participants. The complex hormonal regulation of energy metabolism, inflammation, and disease is not yet fully understood, and future studies should continue to measure hormonal markers to assess the effects of eating frequency on risk for disease.

Table 1. Participant characteristics by gender (n=15).^{1,2}

	Males (n=4)	Females (n=11)	Total(n=15)
Age (y)	25.0 (4.7)	28.2 (8.4)	28.5 (8.7)
Body Mass Index (kg/m ²)	22.4 (1.2)	24.3 (4.2)	23.3 (3.4)
Total body fat %	25.5 (2.8)	35.2 (6.2)	31.8 (6.5)
Waist/hip ratio	0.84 (0.05)	0.80 (0.06)	0.8 (0.07)
Systolic blood pressure (mmHg)	118.5 (9.1)	112.5 (8.5)	112.1 (10.1)
Diastolic blood pressure (mmHg)	76.3(3.2)	72.5 (4.2)	72.2 (5.1)

¹ Anthropometric measures collected during participant's first study session.

² Values are means (Standard Deviation; SD).

Table 2. Participant diet composition by Body Mass Index (kg/m²) (n=15). ^{1,2,3}

	Normal Weight (BMI 18-24.9) (n=13)	Overweight (BMI ≥ 25) (n=2)	Total (n=15)
Total daily energy intake	2219.0 (425)	2538 (560)	2262 (436)
Carbohydrate (g)	274.9 (50.1)	269.2 (75.2)	274.1 (50.6)
Carbohydrate (% energy)	49.0 (4.7)	41.0 (1.4)	47.9 (5.2)
Protein (g)	95.9 (26.8)	102.3 (30.1)	96.8 (26.2)
Protein (% energy)	17 (3.54)	15.5 (0.7)	16.8 (3.3)
Fat (g)	83.0 (26.5)	113.8 (15.0)	87.1 (27.1)
Fat (% energy)	0.33 (0.06)	40 (0.04)	33.7 (5.9)
Fiber (g)	25.46 (6.9)	35.4 (24.1)	26.8 (9.8)

¹Dietary data obtained from 7-day food record. Body Mass Index calculated at participant's first clinic visit.

²Values are means (Standard Deviation; SD).

³ No significant differences were observed between the two categories BMI 18-24.9 and BMI ≥25 (two-sample t-test with unequal variance; p<0.05).

Table 3. Participant anthropometric measurements and blood pressure by study condition (n=15).¹

		Weight (kg)	BMI (kg/m ²)	Waist/hip ratio	Systolic BP (mmHg)	Diastolic BP (mmHg)
Low-EF	Baseline	68.2(13.8)	23.3(3.2)	0.82(0.06)	114.3(9.5)	73.2(6.6)
	Endpoint	68.3(14.4)	23.3(3.5)	0.79(0.07)	105.3(7.6)	70.9(6.0)*
High-EF	Baseline	68.5(14.3)	23.4(3.5)	0.79(0.07)	107.5(7.5)	69.9(4.4)
	Endpoint	68.2(14.1)	23.3(3.4)	0.79(0.06)	110.8(8.0)	71.1(4.6)*

¹Values are means (Standard Deviation; SD).

*Generalized estimating equation (GEE) modification of the linear regression compared weight, BMI (Body Mass Index; kg/m²), waist to hip ratio, systolic blood pressure and diastolic blood pressure in low-EF and high-EF conditions, adjusted for baseline values. Systolic blood pressure was significantly lower in the low-EF condition (p<0.001); no other significant differences were detected.

Table 4. Effect of low-EF vs. high-EF experimental conditions on plasma IGF-I (ng/mL) and leptin (ng/mL), by study condition (n=15)^{1,2,3,4}

		Baseline	Endpoint
IGF-I	Low-EF	123.4 (7.1)	131.8 (7.4)*
	High-EF	133.6 (8.7)	118.2 (6.7)*
leptin	Low-EF	7.0 (1.3)	6.0 (1.2)
	High-EF	7.0(1.0)	6.2 (1.2)

¹Values are geometric means (Standard Error of the Mean; SEM).

²Each participant completed two dietary interventions lasting approximately three weeks each: low-EF (three eating occasions per day); high-EF (eight eating occasions per day).

³Plasma IGF-I measured using the Human IGF-I Quantikine ELISA Kit.

⁴Plasma leptin measured using the Human Leptin ELISA on never-thawed samples.

*Generalized estimating equation (GEE) modification of the linear regression found a significant difference in plasma IGF-I concentrations between low-EF and high-EF conditions, adjusted for baseline values (p=0.000).

Chapter 3: Effects of high vs. low eating frequency on subjective appetite

Introduction

Cardiovascular disease, type 2 diabetes and cancer are among the leading causes of death in the United States and worldwide (1). The burden of these diseases can be partly attributed to overweight and obesity due to excess energy intake and poor diet quality (59, 60). One strategy to improve control of food intake and body weight could involve better regulation of appetite.

The consumption of several small, frequent meals may contribute to feelings of greater fullness and satiety than less frequent, larger meals. Limited data exists on the impact of altered eating frequency on appetite, especially in the context of a diet that is not manipulated in terms of energy or macronutrient content (27). Increased eating frequency has been associated with reduced subjective appetite ratings in two crossover studies (19, 24), and with no significant difference in appetite ratings in another study (25). The role of eating frequency in human appetite regulation remains unclear.

The goal of the present study was to investigate the relationship between eating frequency (EF) and subjective appetite in the absence of alterations to the caloric or macronutrient content of the diet. Specifically, in a randomized cross-over trial, we assessed whether the consumption of food consumed as one eating occasion would result in higher or lower subjective appetite ratings than the same amount of food consumed as two equal servings over the same time period in two separate appetite testing sessions. We hypothesized that increased eating frequency would be associated with decreased appetite in normal, overweight, and obese men and women.

Materials and methods

Participants

This study was a subset of the Meals and Grazing Study, a randomized controlled clinical trial investigating the impact of eating frequency on health-related outcomes. A total of 32 eligible participants were recruited. Of those, 15 completed the Meals and Grazing Study and 12 completed the optional appetite testing sessions for this analysis. Recruitment was completed using posters and online advertisements at the Fred Hutchinson Cancer Research Center (FHCRC) and the University of Washington campus in Seattle, WA. Participants were healthy 18-50 year-old males and females with a body mass index (BMI) greater than or equal to 18 kg/m² (normal to obese). Exclusion criteria included diabetes, smoking, following a diet to gain or lose weight, athletes in training, non-normal blood cholesterol or blood pressure, taking prescribed medication other than oral contraceptives, and pregnancy or nursing (females). There were no additional eligibility criteria and all participants were offered the option to participate in appetite testing sessions. All experimental protocols were approved by the Institutional Review Office at the Fred Hutchinson Cancer Research Center and all participants provided written informed consent. Participants were provided with a small token of \$100.00 USD for completion of the parent study plus the optional appetite testing sessions to compensate for their time and travel expenses.

Study Design

The overall study design for the parent Meals and Grazing Study is shown in **Figure 1**. Interested individuals were invited via email or telephone for an initial orientation session at FHCRC in which eligibility criteria were verified and study procedures were explained in detail. Participants were free-

living and consumed their own food throughout the study using an individually tailored meal plan, which was designed using the following procedures. Eligible participants who enrolled in the study were provided detailed written and verbal instructions on keeping a seven-day food record. Participants then recorded details (type and quantity) for all food and drink except water consumed for seven consecutive days and returned food records to study staff via hand delivery or U.S. Post. Upon receipt, the study dietitian analyzed the seven-day food record for energy and macronutrient content using The Food Processor software (ESHA Research, Salem OR.) Keeping food items, energy, and macronutrient content of the diet constant, a low-EF “Meals” meal plan (providing all energy as three evenly spaced eating occasions per day) and a high-EF “Grazing” meal plan (providing all energy as eight evenly spaced eating occasions per day) were individually designed for each participant. Initial and final eating occasions for each participant were set to match approximately their usual schedule and there was equal time allotted between each daily eating occasion. Weight-maintaining meal plans were designed to rotate every seven days and participants were instructed to eat only the foods on their individual eating plan at the specified hours. All meal and snack consumption (including time of intake) were reported daily using an electronic meal plan checklist.

This study was a crossover trial, with each participant serving as his or her own control. Participants in the Meals and Grazing Study completed two 21-day study phases in random order (Phase I and Phase 2 in **Figure 1**), with a 14-day washout period during which they were instructed to consume their habitual diet (44). Participants attended four clinic visits at the FHCRC Prevention Center at 8:00 am on day one and day 21 of both phases. All other study activities were completed at home. Appointments were scheduled on the same day of the week whenever possible. Participants were asked to consume nothing other than noncarbonated water for 12 hours prior to their clinic appointments and to refrain

from drinking alcohol or eating or exercising outside of their normal routine. In addition, participants did not use any non-steroidal anti-inflammatory drugs (such as ibuprofen) for 72 hours prior to their clinic appointments. During all four clinic visits, body weight, height, waist, hip, pulse, systolic and diastolic blood pressure were measured by trained staff using a highly standardized protocol and blood samples were taken. During the first appointment, Dual energy X-ray absorptiometry (DEXA) GE Lunar DPX Pro (GE Healthcare Lunar, Madison, WI) was used to measure body fat percentage.

Appetite testing sessions

A subset of 12 participants (4 males, 8 females) from the Meals and Grazing Study agreed to participate in optional appetite testing sessions conducted from approximately 8:00 am through 12:00 pm on the last day of each phase (**Figure 2**). Upon arrival at the clinic, participants were seated in private clinic rooms where they would remain for the duration of the test. At baseline and every 30 minutes through 12:00 pm, participants rated subjective feelings of appetite using 100-mm Visual Analogue Scales (**Figure 3**). Immediately after the baseline assessment, participants in both conditions were provided a serving of food which they consumed in entirety within 30 minutes. In the low-EF condition, there was one eating occasion (directly after baseline measurements) and in the high-EF condition, there were two eating occasions (directly after baseline and at 10:30 am). Throughout the testing session, participants remained in clinic rooms and were allowed to engage in quiet activities such as reading, listening to music with headphones, or using a personal computer, and to leave briefly to use the restroom.

We measured the impact of eating frequency on subjective appetite at the end of each three-week phase. Order of conditions followed the crossover design used for the Meals and Grazing Study, so that

each participant in the current analysis completed a low-EF appetite testing session and a high-EF appetite testing session. During appetite testing sessions, participants consumed either one large portion of food at one occasion (low-EF) or two smaller portions of food at two occasions (high-EF) and rated subjective appetite for the duration of the testing session. Foods provided during the appetite testing session were closely matched to the foods normally eaten by the participant at that time and weekday and selected from the FHCRC cafeteria. In the low-EF condition, participants consumed roughly 33% of their usual daily caloric intake in one serving. In the high-EF condition, 33% of the participant's estimated daily caloric intake was consumed in two equally divided servings. For example, if a participant usually ate 1 cup of oatmeal with $\frac{1}{2}$ cup blueberries and 2 hard-boiled eggs for breakfast, they would receive the same foods during their appetite testing session. In the low-EF condition, they would consume all foods at one occasion and in the high-EF condition, they would consume $\frac{1}{2}$ cup oatmeal, $\frac{1}{4}$ cup blueberries, and 1 hard-boiled egg at each of two separate occasions, so total energy intake between the two conditions would be equal. Participants were to consume all of the food and drink provided during the appetite testing session and were not allowed outside food or drink.

Visual Analog Scales

Participants rated their hunger, desire to eat, fullness, thirst, and nausea on a semi-anchored 100-mm Visual Analogue Scale (VAS) every 30 minutes throughout the study. VAS were presented as a booklet with one scale per page with questions such as "How hungry do you feel right now?" and were labeled with anchors such as "not at all" at 0 mm and "extremely" at 100 mm. Participants used a pen to place a single mark on the horizontal bar at the point which best described their feeling at the time of each rating. Ratings were manually scored using a ruler measured to the nearest mm. A composite appetite score was calculated as the mean of hunger, desire to eat and (100-fullness), following previous investigations (61-63).

Statistical Analysis

The primary analytic goal was to assess differences in subjective appetite ratings between the low-EF condition and the high-EF condition. We conducted additional analyses restricted to timepoints from baseline through 10:30 am (approx. two hours), and restricted to timepoints from 11:00 am through 12:00 pm (one hour) to examine whether subjective appetite scores differed at the point in time where the total energy and total food consumed would have been equivalent across the two conditions. The generalized estimated equations (GEE) modification of linear regression was used to estimate mean within-subjects differences in subjective ratings of appetite between conditions. Differences in Area Under the Curve (AUC; calculated using the trapezoidal rule) between the two conditions were examined using paired t-tests. We tested differences over the entire testing period from baseline through 12:00 pm (approx. 4 hours), from baseline through 10:30 am (approx. two hours) and from 11:00 am through 12:00 pm (1 hour.) Total energy (kcal), percent carbohydrate, protein and fat, and fiber (g) consumed during testing sessions, and weight, waist to hip ratio and blood pressure were also analyzed for between-conditions differences using the generalized estimating equations modification of linear regression. Condition- and timepoint-specific mean diastolic blood pressure was imputed for one male participant whose diastolic blood pressure was recorded erroneously at baseline of the low-EF condition. Statistical analyses were performed using Stata version 12.0 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.) All tests were two-sided and statistical significance was set at $p < 0.05$.

Results

Thirty-four participants attended the initial session, of which 32 were eligible to participate and enrolled. Of those, 15 completed the parent Meals and Grazing Study. A total of 12 participants

enrolled in and completed appetite testing (4 males, 8 females). Mean (\pm SD) age for participants who completed appetite testing was 27.08 ± 7.30 (25.0 ± 4.7 for males; 28.13 ± 8.41 for females.) Mean (\pm SD) BMI was 23.67 ± 3.52 (22.4 ± 1.2 for males; 24.3 ± 4.18 for females.) Mean body fat percentage was 31.93 ± 7.00 (25.5 ± 2.8 for males; 35.15 ± 6.17 for females) (**Table 1**). Habitual dietary intake, as measured by the seven-day food record, is shown in **Table 2**. There was no difference in the energy or macronutrient composition of the diets assigned to participants during the 3-week low-EF and high-EF intervention phases (data not shown).

Energy and nutrient intake during appetite testing sessions

Regression models showed no significant differences between conditions in total energy, percent carbohydrate, percent protein, percent fat and grams of fiber provided during appetite testing sessions. Summary characteristics of the foods consumed during appetite testing sessions are provided in **Table 3**.

Anthropometric measurements and vital signs

Mean anthropometric measurements and vital signs by study condition are shown in **Table 4**. Regression models revealed no significant difference in weight, waist to hip ratio, systolic or diastolic blood pressure between conditions ($p=0.84$ and $p=0.99$, $p=0.09$, and $p=0.81$, respectively).

Subjective Appetite Ratings

Hunger

Hunger ratings were highest at baseline in both the low-EF and high-EF conditions (**Figure 4a**). In the low-EF and high-EF conditions, hunger ratings decreased after the first eating occasion, and in the high-EF condition, ratings continued to increase through the final rating at 12:00 pm. High mid-morning hunger in the high-EF condition was suppressed following the second eating occasion, after which

ratings also increased until 12:00 pm. Analysis using GEE showed that mean hunger was significantly lower over the entire testing period in the low-EF condition ($p < 0.001$). In the analysis restricted to baseline through 10:30 am, hunger was significantly lower in the low-EF condition ($p < 0.001$). The 11:00 am through 12:00 pm testing periods were not significantly differently different between the two conditions ($p = 0.23$.) In paired t-tests, area under the hunger curve for 8:00 am through 10:30 am was greater in the high-EF condition compared to the low-EF condition ($p < 0.05$), but did not remain significantly different for the entire testing period ($p = 0.26$), nor for the final hour ($p = 0.69$).

Desire to Eat

In the low-EF condition, desire to eat was suppressed by the first eating occasion with a nadir at 10:00 am, and then increased steadily until completion of the test at 12:00 pm. In the high-EF condition, desire to eat was suppressed to a lesser degree in response to the first eating occasion. Ratings then increased more dramatically until the second eating occasion at 10:30 am, after which they decreased to levels lesser than those seen in the low-EF condition (**Figure 4b**). In regression analyses, mean desire to eat was significantly lower in the low-EF condition compared to the high-EF condition over the entire testing period and in the analysis restricted to baseline through 10:30 am ($p < .001$ for both). No significant differences were observed in regression analyses comparing conditions of low-EF and high-EF in desire to eat from 11:00 through 12:00 ($p = 0.59$.) Area under the curve for desire to eat was greater in the high-EF condition for the entire testing session ($p = 0.02$) and for the restricted time 8:00 am through 10:30 am ($p = 0.07$). No significant difference was observed in area under the curve for desire to eat between the low-EF and high-EF conditions during times 11:00 through 12:00 ($p = 0.65$).

Fullness

Fullness was initially low and increased after the first eating occasion in both conditions, reaching a peak at 10:00 AM. Fullness then decreased in the low-EF condition through 12:00 pm; in the high-EF

condition, fullness increased again after the second eating occasion at 10:30 am (**Figure 4c**). In regression analyses, comparison of mean fullness ratings over the total testing period and for the restricted times baseline through 10:30 am showed lower fullness ratings in the high-EF condition than the low-EF condition ($p=0.05$ and $p<0.05$, respectively). Mean fullness ratings were significantly higher in the high-EF condition in regression analyses comparing times 11:00 am through 12:00 pm ($p<0.05$). Paired t-tests showed no significant difference between conditions in the AUC for fullness over the total testing period ($p=0.13$). Area under the curve for fullness was significantly greater in the high-EF condition when the paired t-test was comparing ratings restricted to times baseline through 10:30 am ($p=0.04$). A paired t-test found no difference in AUC for fullness for the restricted time period 11:00 am through 12:00 pm ($p=0.65$).

Composite Appetite Score

Temporal profiles for the composite appetite score are shown in **Figure 4d**. Mean composite appetite was higher in the high-EF condition for the total testing period ($p<0.05$) and for the time period baseline through 10:30 am ($p<0.001$) in regression analyses. No significant difference was detected between conditions in mean composite appetite for the final hour of testing ($p=0.12$). In paired t-tests, the AUC for composite appetite over the entire testing period was greater in the high-EF condition ($p<0.05$) and for the time period 8:00 am through 10:30 am ($p<0.05$). There was no significant difference between conditions for the time period 11:00 am through 12:00 pm ($p=0.55$).

Thirst

Thirst ratings decreased after the first eating occasion in both conditions, increased slightly mid-morning and remained constant throughout the testing period in both conditions (**Figure 4e**). Regression analyses showed that mean thirst was higher in the high-EF condition throughout the entire testing period, for times baseline through 10:30 am, and for times 11:00 am through 12:00 pm ($p<0.05$ for all.)

In paired t-tests, area under the thirst curve was greater in the high-EF conditions for the total post-baseline study period and final hour of the test ($p < 0.05$) but there was no significant difference detected between the two conditions for the times 8:30 am through 10:30 am ($p = .09$.)

Nausea

Constant low levels of nausea were observed throughout the study period (**Figure 4f**). In regression analyses, mean nausea ratings were higher in the high-EF condition for the total time period ($p < 0.001$) and in both restricted periods baseline through 10:30 am ($p < 0.001$), and 11:00 am through 12:00 pm ($p < 0.05$). There were no statistically significant differences in AUC for nausea over the total time period or for restricted times baseline through 10:30 am or 11:00 am through 12:00 pm ($p = .12$, $p = 0.16$, and $p = 0.06$ respectively).

Discussion

In this randomized crossover trial testing the impact of eating frequency on subjective appetite, we found that participants experienced significantly stronger hunger and desire to eat, and tended toward feeling less full when they consumed equal amounts of food as 2 eating occasions versus 1 over a 4-hour period. In analyses restricted to baseline through 10:30 am, participants in the high-EF condition had completed only one post-baseline eating occasion that provided on average 273 kcals, and reported higher hunger and desire to eat and lower fullness than they did in the low-EF condition after having one eating occasion providing on average 547 kcals. When analyses were restricted to the final hour of testing, participants had consumed equivalent amounts of food in both conditions. Ratings of hunger and desire to eat were indistinguishable between conditions, but fullness was significantly higher in the high-EF condition. Taken together, these findings suggest that the consumption of fewer, smaller meals may not decrease appetite. However, the observations collected during the final hour of appetite testing (11:00 am through 12:00 pm) suggest that when total food and macronutrient consumption is

equal, eating frequency may not impact satiety. Further research may be necessary to definitively test whether total calories consumed or frequency of consumption has the greatest influence on satiety.

In our study, the higher satiating effect of equal total food consumption in the low-EF condition suggests that consumption of fewer, larger meals may suppress appetite to a greater degree over a given period of time. As observed in analyses restricted to the times baseline through 10:30 am, and over the total testing period, higher satiety may be achieved by consumption of higher volume, higher energy eating occasions. These findings may be due to differences between conditions in rate of stomach emptying based on volume, energy, or nutrients consumed, different rates of nutrient metabolism, or may reflect an inadequacy in the length of time of our appetite testing session. Further studies conducted over the course of an entire day may help to elucidate other mechanisms by which equal total energy consumption may lead to similar appetite between conditions of high and low EF.

The notion that increased eating frequency can help suppress appetite and curb intake is supported by very limited experimental evidence. In one recent crossover study by Stote et al., participants consumed total daily calories as three meals per day or as one large evening meal for two 8-week phases. Subjective appetite ratings were collected once per day prior to the evening meal in both conditions. Participants reported significantly higher hunger when consuming one meal per day compared to three meals per day (19). In that study, it is possible that the higher hunger ratings observed in the lower-EF condition were a reflection of the lower overall total energy and volume consumption at the time appetite was measured. In a short-term crossover study with obese males, Speechly et al. provided participants with isoenergetic preloads served at one post-baseline eating occasion or as five hourly eating occasions prior to an *ad-libitum* test lunch (24). No significant

differences in overall subjective ratings of hunger, prospective food consumption, or urge to eat were detected between conditions for the time period prior to the test lunch. Directly prior to the test lunch, when energy intake was equal in both conditions, hunger ratings were higher in the lower-EF condition. Test lunch energy consumption was also higher in the lower-EF condition. Comparison of pre-and post-test lunch satiety ratings showed that participants rated hunger, prospective food consumption, and urge to eat significantly lower in the lower-EF condition post-test lunch, while no difference was observed in the higher-EF condition (24). The findings of Speechly et al. suggest that higher eating frequency may suppress hunger and subsequent intake. In another crossover study, healthy male subjects consumed isoenergetic meals as either two eating occasions spaced three hours apart, or six hourly eating occasions on two different testing occasions. No significant difference was observed between conditions in terms of subjective appetite ratings (25).

Although few controlled trials have addressed the issue, findings from observational studies suggest some link between eating frequency and health-related outcomes. The majority of epidemiological studies show evidence of an inverse relationship between eating frequency and body weight, body fatness, and other markers for disease risk (3-5, 7, 64-74). Some have detected a positive relationship (75-77) and still others have reported mixed results based on subject demographics including age and gender (78, 79). However, major shortcomings exist in the available research, including underreporting of intake, unclear definition of terms such as “meals” and “snacks,” and inconsistencies across studies, leaving this issue largely unresolved. Moreover, none of these observational studies directly measured appetite, so a relationship between eating frequency, satiety, and health outcomes could not be determined.

This study had several notable strengths. Firstly, we used a reliable tool to assess appetite, which has been used in numerous other studies and the measurement properties of the tool are thought to be very reliable (80). Second, the total energy and volume of food was exactly the same for the high-EF and low-EF test meals; the total food provided was simply divided into one or two eating occasions. This ensured that the subjective appetite ratings did not differ between conditions based on any possible differences in palatability. Participants were compliant with the protocol and there were no drop-outs. There are also limitations. This study assessed the short-term (approx. four hours) effects of alterations in eating frequency on subjective appetite ratings in a laboratory environment. Manipulations in eating frequency in a free-living population may not have similar effects on appetite over a longer period of time. Other researchers have measured satiety either on a daily basis in a long-term crossover (19), or have incorporated measurement of energy intake at a test meal in the study design(24). Future investigations will benefit from a combination of longer-term intervention periods, daily satiety ratings, and appetite testing sessions with *ad libitum* test meal energy intake measurement. Further, findings from this investigation and others will ultimately be translated into recommendations for optimal eating frequency. More frequent subjective measures of appetite in a naturalistic setting and measurement of outcomes such as quality of life should be used to determine whether altering eating frequency is a realistic behavioral change. Finally, the study population was largely female, and with normal BMI. Results may therefore not be generalizable to the overweight and obese population, who may benefit most from this research.

Conclusion

Results from this investigation indicate that increased eating frequency is not associated with decreased appetite over a short time period. Thus, although it is often recommended as a means by which to control intake, the “grazing,” “nibbling,” or “snacking,” meal pattern may not result in decreased

appetite or weight control. In fact, it has been suggested that increased eating frequency may actually predispose individuals to unintended weight gain due to increased opportunities for intake. Health professionals should use caution when making recommendations for eating frequency until further research is conducted.

Table 1. Participant characteristics by gender (n=12)^{1,2}

	Males (n=4)	Females (n=8)	Overall (n=12)
Age (y)	25.0 (4.7)	28.13 (8.41)	27.08 (7.30)
Body Mass Index (kg/m ²)	22.4 (1.2)	24.3 (4.18)	23.67 (3.52)
% total body fat	25.5 (2.8)	35.15 (6.17)	31.93 (7.00)
Waist/hip ratio	0.84 (0.05)	0.80 (0.06)	0.82 (0.06)
Systolic blood pressure (mmHg)	118.5 (9.1)	112.5 (8.54)	114.5 (8.83)
Diastolic blood pressure (mmHg)	76.3 (3.2)	72.5 (4.24)	73.8 (4.2)

¹ Data collected during participant's first clinic visit.

² Values are means (Standard Deviation; SD).

Table 2. Participant diet composition by Body Mass Index (n=12) ^{1,2}

	Normal Weight (BMI 18-24.9) (n=10)	Overweight (BMI ≥ 25) (n=2)	Overall (n=12)
Total daily energy intake (kcal)	2164 (422)	2538 (560)	2227 (442)
Carbohydrate (g)	263 (46)	269 (75)	264 (48)
Carbohydrate (% energy)	48 (4)	41 (1)	47 (5)
Protein (g)	98 (30)	102 (30)	99 (29)
Protein (% energy)	18 (3)	16 (0.7)	17 (3)
Fat (g)	80 (17)	114 (15)	86 (21)
Fat (% energy)	33 (3)	40 (4)	34 (4)
Fiber (g)	26 (8)	35 (24)	28 (11)

¹ Data obtained from seven-day food record.

² Values are means (Standard Deviation; SD).

Table 3. Composition of meals/snacks during satiety testing sessions (n=12) ¹

	Low-EF	High-EF
Total kcals	547 (221)	540 (229)
% Carbohydrate	58.4 (11.7)	58.1 (11.4)
% Protein	14.6 (2.7)	14.6 (2.9)
% Fat	30.0 (12.5)	30.3 (12.0)
Fiber (g)	8.0 (4.0)	7.8 (3.9)

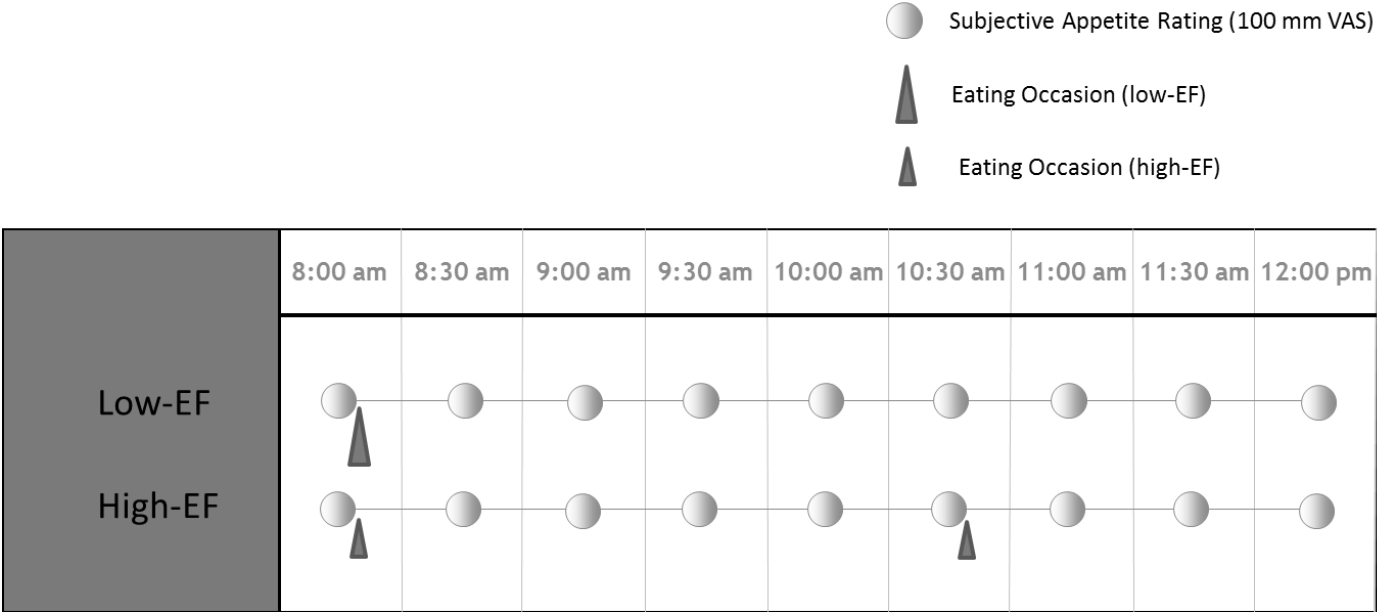
¹ Values are means (Standard Deviation; SD).

Table 4. Anthropometric measures and vital signs by study condition (n=12) ¹

	Low-EF	High-EF
Body weight (kg)	70.4 (14.0)	70.3 (13.8)
Waist/hip ratio (cm)	0.79 (0.06)	0.80 (0.05)
Systolic Blood pressure (mmHg)	106 (6.9)	112 (7.8)
Diastolic Blood pressure (mmHg)	72 (4.2)	71 (4.2)

¹Data collected prior to baseline appetite measurements at appetite testing sessions.

Figure 2. Timing of appetite testing sessions¹.



¹Participants completed subjective appetite ratings using 100-mm Visual Analog Scales every 30 minutes. In the low-EF condition, there was one eating occasion, and in the high-EF condition, there were two eating occasions.

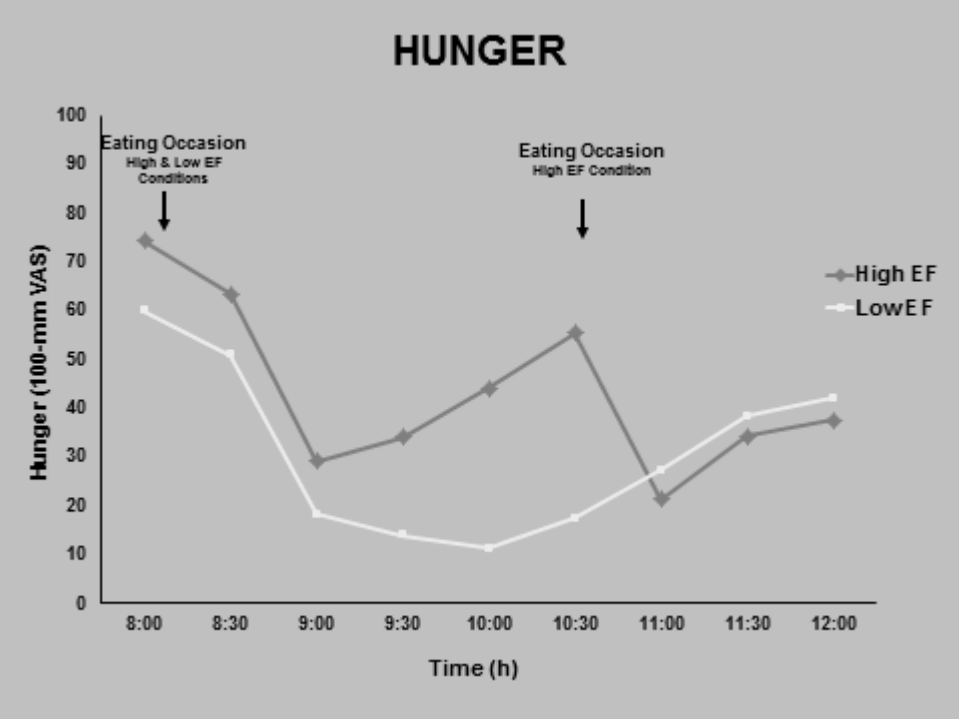
Figure 3. 100-mm Visual Analog Scale (VAS)¹.

How hungry do you feel right now?

NOT hungry at all	_____	Extremely hungry
----------------------	-------	---------------------

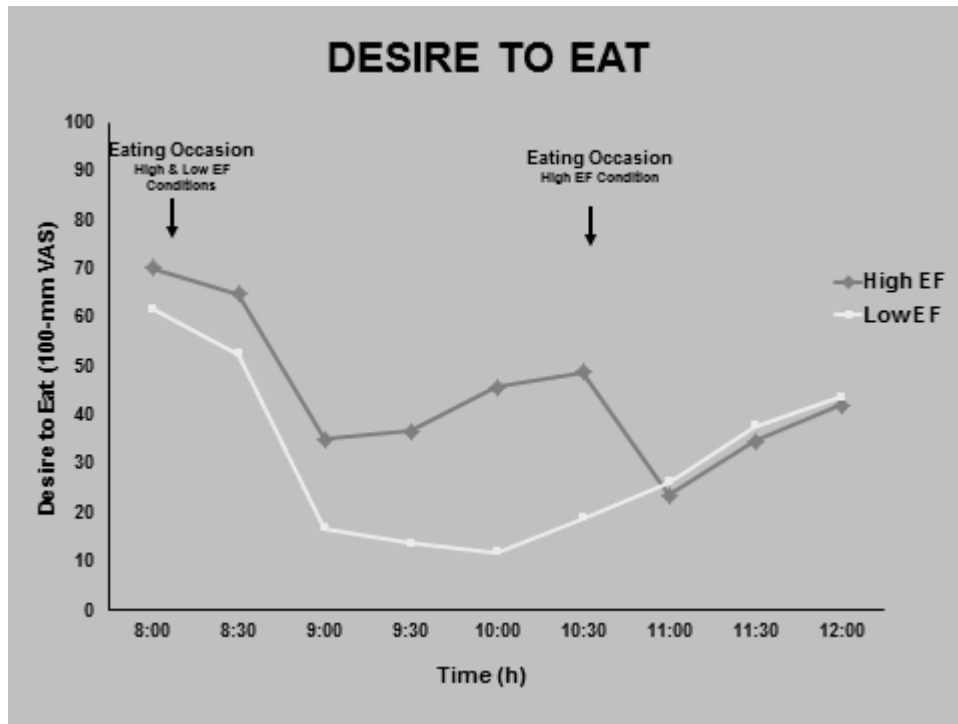
¹Participants rated hunger, desire to eat, fullness, thirst, and nausea every 30 minutes from baseline through 12:00 pm using semi-anchored 100-mm Visual Analog Scales. VAS were presented as a booklet with one scale per page and participants used a pen to place a single mark on the horizontal bar at the point which best described their feeling at the time of each rating.

Figure 4a. Temporal profiles of the mean hunger as a function of study condition (High-EF and low-EF)¹.



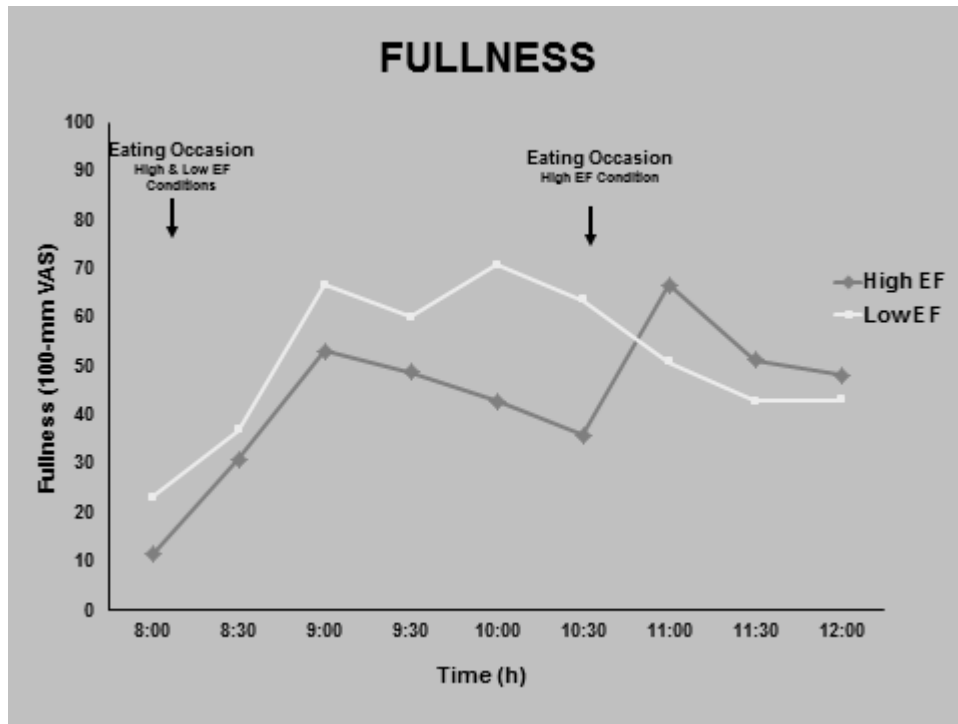
¹Subjective appetite ratings were collected using a semi-anchored Visual Analog Scale (VAS) presented in a booklet every 30 minutes throughout the testing session.

Figure 4b. Temporal profile of the desire to eat as a function of study condition (High-EF and low-EF)¹.



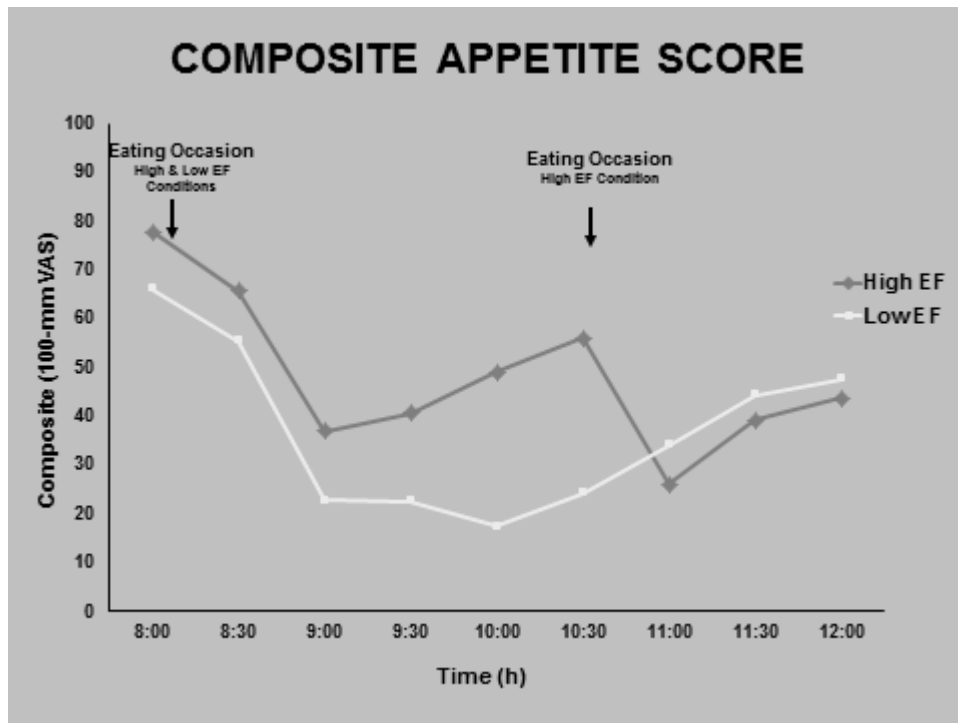
¹Subjective appetite ratings were collected using a semi-anchored Visual Analog Scale (VAS) presented in a booklet every 30 minutes throughout the testing session.

Figure 4c. Temporal profile of fullness as a function of study condition (High-EF and low-EF)¹.



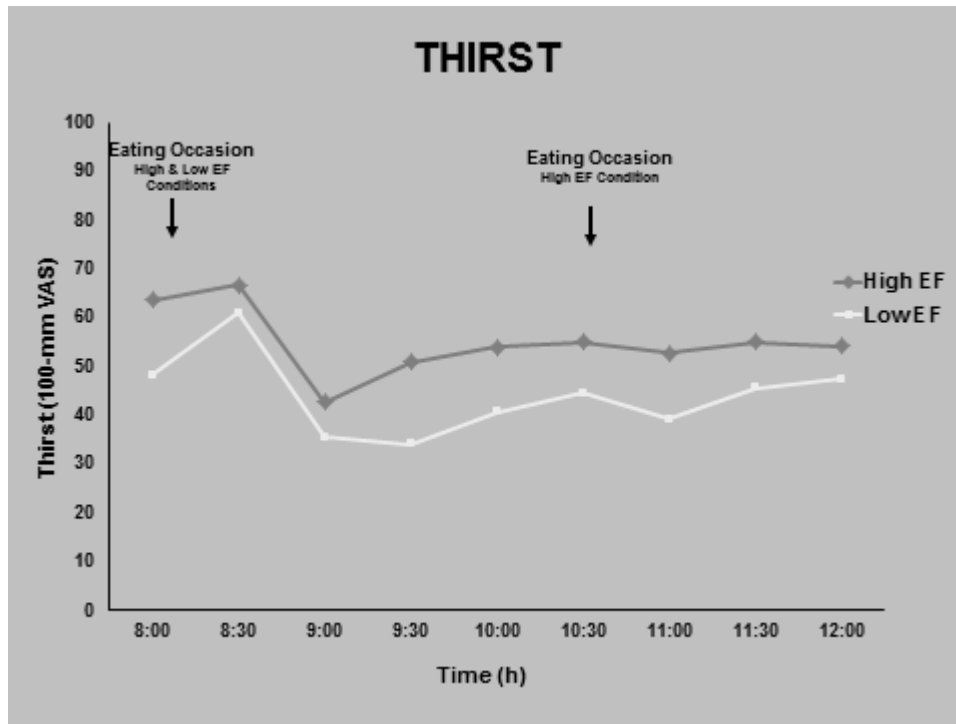
¹Subjective appetite ratings were collected using a semi-anchored Visual Analog Scale (VAS) presented in a booklet every 30 minutes throughout the testing session.

Figure 4d. Temporal profile of the composite appetite score as a function of study condition (High-EF and low-EF)¹.



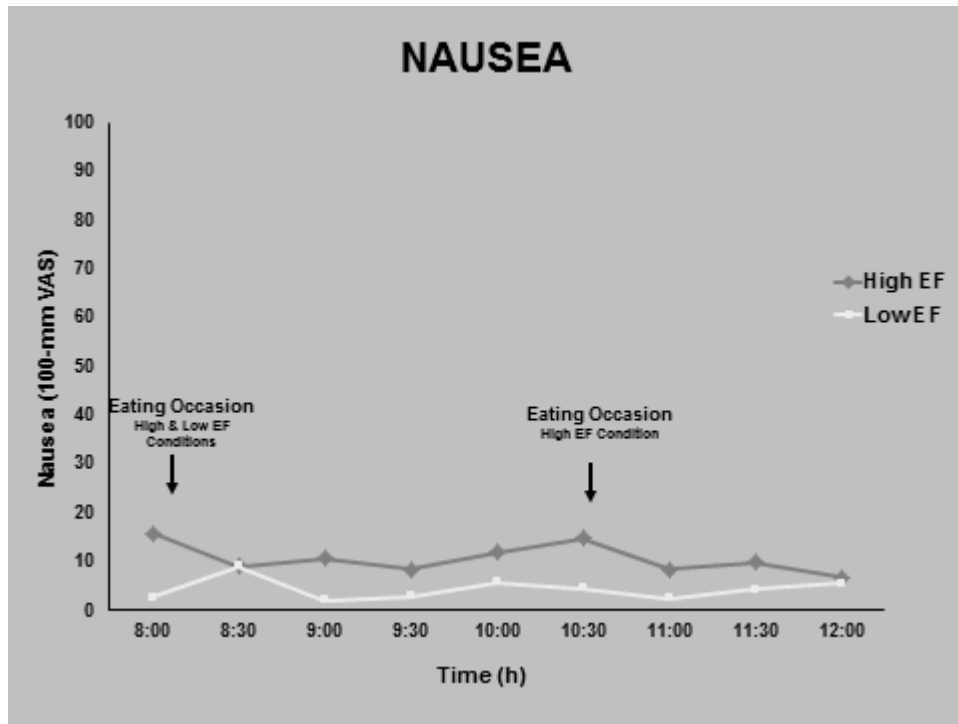
¹Subjective appetite ratings were collected using a semi-anchored Visual Analog Scale (VAS) presented in a booklet every 30 minutes throughout the testing session.

Figure 4e. Temporal profile of thirst as a function of study condition (High-EF and low-EF)¹.



¹Subjective appetite ratings were collected using a semi-anchored Visual Analog Scale (VAS) presented in a booklet every 30 minutes throughout the testing session.

Figure 4f. Temporal profile of the desire to eat as a function of study condition (High-EF and low-EF)¹.



¹Subjective appetite ratings were collected using a semi-anchored Visual Analog Scale (VAS) presented in a booklet every 30 minutes throughout the testing session.

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Appendix

Example Meal Plan Checklist

Welcome to the MAGS online Meal Plan Checklist!

The online Meal Plan Checklist uses WebQ, an online survey tool supported by Learning and Scholarly Technologies at the University of Washington. Use of WebQ has been approved by the Human Subjects Division at the University of Washington for use in research studies like this one. This is a confidential survey, and the information you enter will be stored separately from your email address.


Please contact the MAGS study coordinator at MAGS@fhcrc.org or (206)667-4760 if you have any questions or problems as soon as possible.

Please click "Next" to complete today's Meal Plan Checklist.

8:00 am


6 oz. lowfat vanilla yogurt, cold

- yes
- no, I didn't eat
- no, I substituted something else

 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:


1/4 cup granola, cold

- yes
- no, I didn't eat
- no, I substituted something else

 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:


1/2 cup frozen blueberries, microwaved

- yes
- no, I didn't eat
- no, I substituted something else

 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

8 fl. oz. orange juice, cold

- yes
- no, I didn't eat
- no, I substituted something else

 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

1 whole wheat english muffin, toasted

- yes
- no, I didn't eat
- no, I substituted something else
- If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

1 tbsp peanut butter, spread on muffin

- yes
- no, I didn't eat
- no, I substituted something else
- If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

2:00pm

2 slices whole wheat bread, cold

- yes
- no, I didn't eat
- no, I substituted something else
- If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

2 oz. deli sliced turkey meat, cold

- yes
- no, I didn't eat
- no, I substituted something else
- If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

4 slices roma tomato, cold

- yes
- no, I didn't eat
- no, I substituted something else
- If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

1 oz. sliced cheddar cheese, cold


- yes
- no, I didn't eat
- no, I substituted something else
- If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

1 leaf iceberg lettuce, cold

yes

no, I didn't eat

no, I substituted something else


 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

1 tbsp regular mayonnaise, cold

yes

no, I didn't eat

no, I substituted something else


 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

1 tsp. yellow mustard, cold

yes

no, I didn't eat

no, I substituted something else


 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

8 fl. oz. canned tomato soup, microwaved

yes

no, I didn't eat

no, I substituted something else


 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

1 cup green grapes, cold

yes

no, I didn't eat

no, I substituted something else

 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:


8:00 pm

2 slices 14" thick crust pepperoni pizza, warm

yes

no, I didn't eat

no, I substituted something else

 If you made a substitution, please indicate the amount and type of food you substituted, and what time you ate it here:

Skipped foods

Were there any eating occasions or specific foods today that you didn't eat?

No, I did not skip any eating occasions or specific foods

If you skipped any eating occasions or specific foods today, please indicate why:

Physical activity

Was your level of physical activity normal today?

yes

no

If no, please indicate how your physical activity today was different from normal:

¹Example Meal Plan Checklist for a participant in the low-EF “Meals” condition, in which three eating occasions were assigned at 8:00 am, 2:00 pm, and 8:00 pm. Completed Meal Plan Checklists were submitted daily to the study coordinator.