1 APPENDICES
1.1 APPENDIX 1: SAMPLE SIZE CALCULATION

Sample size estimation when testing for the mean of a normal distribution (two-sided alternative). For a within subject design, the equation is:

\[ N = 2 \times \left( \frac{Z\alpha - Z\beta}{\Delta} \times SD \right)^2 \]

\( \alpha = 0.05 \) (\( Z \times 0.025 = 1.96 \))

\( \beta = 0.20 \) (\( Z \times 0.80 = 0.84 \))

\( \sigma = 210 \)

\( \Delta = 190 \text{ kcal} \)

\( N = 10 \text{ subjects} \)

Values were taken from our pilot studies. \( \sigma \) represents standard deviation,

\( \Delta \) represents the minimal difference in food intake observed between control and treatment.

\( n \) is the number of subjects needed.
1.2 APPENDIX 2: RECRUITMENT LETTER FOR PARENTS AND SCREENING QUESTIONNAIRES

1.2.1 Chapter 4: Obesity, sex and pubertal status on glycemic and appetite hormone responses to a mixed glucose and whey protein drink in adolescents

Consent Form (Care-giver)

Principal Investigator:
Dr. Jill Hamilton 416.813.5115

Co Investigators:
Dr. Harvey Anderson
Dr. Brian McCrindle
Dr. Nick Bellissimo

Study Coordinator:
Allison Jeffery 416.813.7654 x 4495
Barkha Patel 416.978.4153

Purpose of the Research:
Childhood overweight and obesity is rising faster in children than in adults in Canada. Important obesity-related complications are becoming more common in children including diabetes and heart disease risk factors. Overweight children become overweight adults, and this tracking carries the highest risk for the development of diabetes and heart disease. The reasons underlying childhood obesity are poorly understood, but we know they include a complex interaction between genetic, physiologic, behavioral and environmental factors. It is very important to understand why children
become obese so that effective prevention and treatment strategies can occur before problems develop.

The specific questions we are asking in this study are:

- What are the important factors causing overweight and obesity in children?
- What factors influence a child’s appetite and how they process food in their body?

**Description of the Research:**

For both of these visits, your child will be fasting so we will provide a meal voucher for you to purchase lunch in the hospital cafeteria. These visits are in addition to any other appointments or regular hospital visits your child may have.

**Day 1:** We will take your child’s height and weight, as well as waist and hip circumferences. We will perform two tests to measure your child’s body fat and muscle. The first is a machine called a BOD POD where your child will sit for 5-7 mins in a special chamber. The second is called BIA (bioelectrical impedance analysis). Two electrodes will be attached to your child’s right hand and right foot and a mild current that is undetectable to your child’s will measure body water composition. Neither of these tests causes discomfort.

Next, a blood sample will be taken and your child will then be given a mixed glucose and whey protein drink. If you wish, a cream that numbs your child’s skin can be put on to reduce any discomfort associated with the IV. We will take blood samples at the start of the test, and again every 15-30 minutes for 2 hours. We will leave the IV line in the arm throughout the test drawing blood as needed so that your child only receives one needle. The volume of blood collected will be 50mLs (10 teaspoons). These samples will be compared to see how the different appetite hormone and signals function in your child’s system. Before each sample is taken, your child will fill out a motivation to eat and physical comfort questionnaire.

**Potential Harms:**

We know of no harm that taking part in this study could cause your child.

**Potential Discomforts or Inconvenience:**
During the assessment, we will take blood from your child. There may be a small amount of bleeding when blood is taken from a vein and there may be slight discomfort and bruising or redness that will usually disappear in a few days. We will offer a special cream (EMLA) that can be applied to the skin to numb it and reduce the discomfort prior to the needle poke.

**Potential Benefits:**

**To individual subjects:**

There is no guarantee that your child will personally experience any benefits from joining this study. If any abnormalities are detected, we will ensure that the results are discussed with you and a copy sent to your family doctor or pediatrician. If further treatment is required, we will refer you to the appropriate pediatric specialty doctor.

You will receive a summary of your child’s results after the study is complete. We will also provide your child with a letter for volunteer hours that can be used toward high school volunteer requirements.

**To society:**

By understanding the full impact of complications of overweight (including risk for diabetes and heart disease) we can develop better tools for assessing health risk and design specific prevention and treatment strategies to prevent or reverse abnormalities before significant health problems occur.

**Confidentiality:**

We will respect your child’s privacy. No information about who your child is will be given to anyone or be published without your permission, unless the law makes us do this.

For example, the law could make us give information about your child:

- If a child has been abused
- If you have an illness that could spread to others
- If you or someone else talks about suicide (killing themselves), or
- If the court orders us to give them the study papers
SickKids Clinical Research Office Monitor or the regulator of the study may see your child’s health record to check on the study. For example, people from Health Canada Health Products and Food Branch, or Canadian Institute of Health Research may look at your records.

By signing this consent form, you agree to let these people look at your child’s records. We will put a copy of this research consent form in your patient health records.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those individuals described above) will have access to the data. This could include external research team members. Following completion of the research study the data will be kept as long as required then destroyed as required by Sick Kids policy. Published study results will not reveal your identity.

The results of the tests we describe in this form will be used only for this study.

**Reimbursement:**

We will reimburse you for all your reasonable out of pocket expenses for being in this study eg., meals, babysitters, parking and getting you to and from Sick Kids. If you stop taking part in the study, we will pay you for your expenses for taking part in the study up until that point.

We will also provide you with a thank-you gift, in recognition of your time and effort.

**Participation:**

Participation in research is voluntary. It is your choice for your child to take part in this study. You can stop at any time. The care your child gets at SickKids or another institution will not be affected in any way by whether you take part in this study. New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want your child to be in the study. During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money
from these findings, we cannot give your child any of this money now or in the future because your child took part in this study. We will give you a copy of this consent form for your records.

In some situations, the study doctor or the company paying for the study may decide to stop the study. This could happen even if the medicine given in the study is helping your child. If this happens, the study doctor will talk to you about what will happen next.

If your child becomes ill or is harmed because you took part in this study, we will treat your child for free. Your signing this consent form does not interfere with your legal rights in any way. The staff of the study, any people who gave money for the study, or the hospital are still responsible, legally and professionally, for what they do.

**Sponsorship:**

The study is funded by CIHR (Canadian Institute of Health Research). In certain situations, this study may be cancelled at the discretion of the investigator or the study sponsor even if you are benefiting personally. If this occurs, the investigator will discuss next steps with you.

**Conflict of Interest:**

There are no actual or potential conflicts of interest of members of the research team identified.

**Parental Consent:**

By signing this form, I agree that:

1) You have explained this study to me. You have answered all my questions.

2) You have explained the possible harms and benefits (if any) of this study.

3) I know what I could do instead of having my child take part in this study. I understand that I have the right to refuse to let my child take part in the study. I also have the right to take my child out of the study at any time. My decision about my child taking part in the study will not affect my child’s health care at Sick Kids.

4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my child’s medical records will be kept private except as described to me.

6) I understand that no information about my child will be given to anyone or be published without first asking my permission.

7) I agree, or consent, that my child___________________ may take part in this study.

__________________________________________________________
Printed Name of Parent/Legal Guardian                  Parent/Legal Guardian’s signature & date

__________________________________________________________
Printed Name of person who explained consent                Signature of Person who explained consent & date

__________________________________________________________
Printed Witness’ name (if the parent/legal guardian does not read English)          Witness’ signature & date

If you have any questions about this study, please call Jill Hamilton at 416.813.5115

If you have questions about your rights as a subject in a study or injuries during a study, please call the Research Ethics Manager at 416-813-5718.

Children’s Assent Form

Why are we doing this study?

Children who carry excess body weight may be at risk for future diabetes (high sugar in the blood) and heart disease (heart attacks). We want to study how the body handles sugar and how healthy the blood
vessels look and work in order to understand why some children are more at risk for these problems as adults.

**What will happen during the study?**

1) You will have some blood taken to see how your body responds to food
2) You will be asked to fill out questionnaires about your feelings and the type of foods you like to eat

**Are there good things and bad things about the study?**

You have the chance to contribute to learning (science). This may be of benefit in improving the quality of life of other patients like you.

You may feel some pain or have a small bruise when we take your blood with a needle. You can use special cream to numb the skin so the poke does not hurt as much.

**Who will know about what I did in the study?**

If we feel your health may be in danger, we may have to report your results to your doctor.

**Can I decide if I want to be in the study?**

Nobody will be angry or upset if you do not want to be in the study. We are talking to your parent/legal guardians about the study and you should talk to them about it too.

**Assent:**

The following section must be included at the end of the assent form:

I was present when ___________________________ read this form and said that he or she agreed, or assented, to take part in this study.

_________________________________________  ___________________________________
Recruitment Letter for Parents

Dear Parent

The University of Toronto is leading a cross-institutional team of researchers investigating the physiological and environmental determinants of energy intake regulation on the health of children and young adolescents. In our current work we are conducting studies aimed at understanding the controls of food intake in children, with the ultimate goal of finding ways to address the problems of overeating and obesity that are becoming a concern among those people involved in the long term health of Canadians.

We are asking the parents of girls and boys 9 to 14 years old to allow their daughter/son to take part in a research study. Their participation is quite straightforward: on four separate mornings your child will consume a sweet drink followed by a pizza lunch 30 or 60 minutes later. The study will take place on four weekend or holiday mornings at the FitzGerald Building, Department of Nutritional Sciences (150 College Street, Room 329).

There are criteria for participation that you need to be aware of, the child must:

- be between 9 and 14 years of age, and
- be healthy, and have been born at term, and
- not be taking medications.

As a reward for taking part, at each session the child will be given a free movie pass ($20 gift certificate). This study has been fully approved by the University of Toronto Health Sciences II ethics review committee. If you would like your son/daughter to participate, or to get further information beyond that provided in this letter, please contact Ms. Barkha Patel, Project Coordinator at (416) 978-4153 or Dr. G. Harvey Anderson, Professor (416) 978-1832 at the University of Toronto (Department of Nutritional Sciences).
Thank you for your support in this important research.

Sincerely,

Dr. Harvey Anderson, Department of Nutritional Sciences, University of Toronto.
Ms. Barkha Patel, Department of Nutritional Sciences, University of Toronto.
Dr. Jill Hamilton, Department of Pediatrics, Hospital for Sick Children
Dr. Scott Thomas, Faculty of Physical Education and Health, University of Toronto.

**Purpose of Research:**

The purpose of this study is to determine the effects of glucose and protein beverages on food intake regulation in NW and obese 9-14 year-old children. This experiment is being conducted through the Department of Nutritional Sciences at the University of Toronto by Dr. G. Harvey Anderson (principal investigator), Barkha Patel (project coordinator), Dr. Jill Hamilton and Dr. Scott Thomas. Your son/daughter will be required to attend four experimental sessions conducted over a 4-week period, for a total of 5 visits (4 food intake measurement sessions + 1 visit to measure physiological parameters) to the University of Toronto campus. Each visit will last approximately 90 minutes.

The purpose of our research is to develop an understanding of factors affecting the control of food intake in children. Knowing the determinants of the regulation of food intake in children will allow us to develop strategies and recommendations for the prevention of obesity.

**Procedure:**

**Appetite Assessment:**

For those parents who express interest in having their son/daughter participate, some information about the child will be requested by telephone, by the project coordinator, Patricia Mighiu. If the child was born at term, is healthy and does not receive any medications, an information session will be arranged.

During the information session, the researcher will explain the full details of the study. Parents that give consent to have their son/daughter participate will sign a consent form. The parent will receive copies of consent forms and of the study information sheet. If the child wishes to participate and signs a children’s assent form, his/her weight, height, and body fat using painless techniques, will be measured.
The boys and girls will then be asked to rank their preference for pizza that will be served as the lunch meal at each session.

The boys/girls who participate in this study, will be requested to go to the FitzGerald Building, Department of Nutritional Sciences, University of Toronto, for six individual morning sessions. These sessions will be held on weekends, over four weeks. The children will be brought to the laboratory and returned home either by parents or a research assistant, as preferred by the parents.

On each of the four test days, the children will have a standardized breakfast of cereal, milk and juice at home, either at 8:00 am or 9:00 am (the time will be consistent for each child). The children will arrive at the FitzGerald Building, either at 10:00 am or 11:00 am (but consistent throughout for each child).

Children will fast for 12 hours before breakfast and after breakfast until their arrival, except for water, which will be allowed up to one hour before their arrival.

Each child will receive one glass of sweetened Kool-aid containing carbohydrate, protein, and/or artificial sweetener. The protein drink will contain a similar amount of protein as a half-pound steak, but will be the protein in commercially available drinks. The sugar drink will contain a similar amount of sugar as common soft drinks or fruit juice. Each child will receive all drinks, one on each day in no set order.

McCain pizza and spring water (purchased at Loblaws) will be served 30 or 60 minutes after the boys/girls have consumed the drink. Children will be told that they may eat as little or as much as they like. The amount of food eaten by each child will be measured.

The boys/girls will also be requested to complete scales on which they will place a pencil mark to describe their desire to eat (“Very weak” to “Very strong”), hunger (“Not hungry at all” to “As hungry as I’ve ever felt”), fullness (“Not full at all ” to “Very full”), how much food they could eat (“A large amount” to “Nothing at all”), sweetness of the drinks (“Not sweet at all” to “Extremely sweet”). They will complete these scales during the information session, in order to become familiar with the test instruments.
The children will be fully supervised during the study sessions. They will be engaged in age appropriate entertainment (as distraction) eg: reading, puzzles, cards, before lunch. The study session will end either at 11:30 am or at 12:30 pm and after the pizza meal.

Physiological Testing:

Physiological testing will be performed on one weekend morning, separate from the food intake assessment session, at the FitzGerald Building.

Body Composition Assessment:

Bioelectrical Impedance Analysis:

Bioelectrical impedance analysis (BIA), a recently developed technique for measuring body fat content in both adults and children, is simple and painless and is an effective method for measuring body fat in children. BIA is based on measurement of electrical resistance in the body to a tiny current (that the subject cannot feel). The principle of BIA lies in that muscle mass in the body is a better conductor of electricity than fat which contains lesser amounts of water and electrolytes.

Tanner Staging Assessment:

Tanner stages are scales that assess physical development in children and adolescents, based on external primary and secondary sex characteristics, such as the size of the breasts, genitalia, and development of pubic hair. The way in which appetite is regulated is related to where children are in their pubertal development. In order to assess pubertal stage, the children will be asked to complete a questionnaire about puberty and changes in their bodies. Depending on the sex of the child, the children will be presented with cartoon pictograms of different stages of physical/sexual development (e.g., breast size, pubic hair, genitalia) and the children will be asked to pick the picture that best represents their stage of puberty. These pictograms have been used extensively in children. If for any reason the children are not willing to participate, they have the option of asking their parents to answer the questionnaire and select the pictograms for them. The children may decline the pubertal staging if they wish. Parents are welcome to discuss the reasons for including Tanner stages as part of the study or any comment or concerns with Dr. Jill Hamilton at jill.hamilton@sickkids.ca
Confidentiality:

Records relating to participants will be kept confidential in a locked cabinet in the Department of Nutritional Sciences and no disclosure of personal information of the children or parents will take place except where required by law. Participants will have a code and a number that will identify them in all documents, records and files to keep their name confidential. All data will be entered into Microsoft Excel files, available only to investigators. Each participant will have a file, also only available for investigators. All forms and printouts will be stored in the individual files – and clearly labeled. All documents will be kept for a minimum of five years following completion of the study and then securely destroyed.

Risks:

There are no risks in this study.

Benefits:

As the causes of obesity remains undefined, the potential benefits from this study will be a better understanding of the regulation food intake in children and might contribute to the prevention of obesity in children.

Questions and further information:

If you have any questions or would like further information concerning this research project, please do not hesitate to call: Barkha Patel, Project Coordinator at (416) 978-4153 or Dr. Harvey Anderson, Investigator at (416)-978-1832.

If you have questions or concerns about your rights as a research participant, please contact Marianna Richardson, Research Ethics Coordinator, Health Sciences marianna.richardson@utoronto.ca or 416-946-5806.

Dissemination of findings:

A summary of results will be made available to you to pick up after the study is completed.

Parental Consent:
I acknowledge that the research procedures described above and of which I have a copy, have been explained to me and that any questions that I have asked have been answered to my satisfaction. I know that I may ask additional questions now or in the future. I am aware that participation in the study will not involve any health risk to my child.

I understand that for purposes of the research project, if my child or I choose to withdraw from the study at any time, we may do so without prejudice.

Upon completion of each study session, my child will receive a $20 gift certificate to the theatre. The final summary and results of the study will be available for me to pick up from the Department of Nutritional Sciences, University of Toronto. I am aware that the researchers may publish the study results in scientific journals, keeping confidential my son or daughter’s identity.

I hereby consent for my child, ______________________________, to participate in this study.

___________________________________         _____________________________
(Name of parent or guardian)                                  (Signature of parent or guardian)

__________________________________           ___________________________
(Name of witness)                                                    (Signature of witness)

Date: ______________ (dd/mm/yy)

Children’s Assent Form

Purpose of Research:

The purpose of this study is to determine the effects of sweetened beverages on appetite in children. My weight, height, and body fat using painless techniques will be measured during the information visit. I will also be required to drink a sweetened beverage (within 5 minutes), complete special scales to show if I am hungry or full during each session. I will also be provided with a pizza lunch at the end of each study session (that I will eat in the Department of Nutritional Sciences, University of Toronto). All the experimental sessions will be on weekends
and school holidays, so I don’t need to be absent from school. I will be asked to fill out a questionnaire that is related to my stage of puberty (changes in my body as I grow up). I will also be asked to look at some cartoon pictures of various stages of puberty and select the picture that best resembles me.

I know that my participation in the study will not involve any health risk to me.

Also, if at any time I decide to stop participating, that will be O.K. I understand that information related to me will be kept confidential. I know that I will receive a $20 gift certificate to the theatre after completion of each study session, as a “thank you” for my participation.

“I was present when ______________________________read this form and gave his/her verbal assent.”

_____________________________

Signature

Name of the person who obtained assent:

_____________________________

Date: _________________ (dd/mm/yy)
1.2.3 Chapter 6: Effect of Television Viewing at Mealtime on Food Intake in Children

Recruitment Letter for Parents

Dear Parent

The University of Toronto is leading a cross-institutional team of researchers investigating the physiological and environmental determinants of energy intake regulation on the health of children and young adolescents. In our current work we are conducting studies aimed at understanding the controls of food intake in children, with the ultimate goal of finding ways to address the problems of overeating and obesity that are becoming a concern among those people involved in the long term health of Canadians.

We are asking the parents of girls and boys 9 to 14 years old to allow their daughter/son to take part in a research study. Their participation is quite straightforward: on four separate mornings your child will consume a sweet drink followed by a pizza lunch 30 min later with or without television viewing. They will also fill out a short questionnaire about their television viewing behaviours. The study will take place on four weekend or holiday mornings at the FitzGerald Building, Department of Nutritional Sciences (150 College Street, Room 329).

There are criteria for participation that you need to be aware of, the child must:

- be between 9 and 14 years of age, and
- be healthy, and have been born at term, and
- not be taking medications.

As a reward for taking part, at each session the child will be given a free movie pass ($20 gift certificate). In addition parents will be reimbursed for travel/parking expenses ($12). This study has been fully approved by the University of Toronto Health Sciences II ethics review committee.

If you would like your son/daughter to participate, or to get further information beyond that provided in this letter, please contact Ms. Barkha Patel, Project Coordinator at (416) 978-4153, or Dr. G. Harvey Anderson, Professor (416) 978-1832 at the University of Toronto (Department of Nutritional Sciences).
Thank you for your support in this important research.

Sincerely,

Dr. Harvey Anderson, Department of Nutritional Sciences, University of Toronto.
Ms. Barkha Patel, Department of Nutritional Sciences, University of Toronto.
Dr. Jill Hamilton, Department of Pediatrics, Hospital for Sick Children
Dr. Scott Thomas, Faculty of Physical Education and Health, University of Toronto.

**Purpose of Research:**

The purpose of this study is to determine the effects of television viewing on food intake regulation in NW and obese 9-14 year-old children. This experiment is being conducted through the Department of Nutritional Sciences at the University of Toronto by Dr. G. Harvey Anderson (principal investigator), Barkha Patel (project coordinator), Dr. Jill Hamilton, and Dr. Scott Thomas. Your son/daughter will be required to attend four feeding sessions conducted over a 4-week period, for a total of 5 visits (4 food intake measurement sessions + 1 visit to measure physiological parameters) to the University of Toronto campus. Each visit will last approximately 90 minutes. The purpose of our research is to develop an understanding of factors affecting the control of food intake in children. Knowing the determinants of the regulation of food intake in children will allow us to develop strategies and recommendations for the prevention of obesity.

**Procedure:**

**Appetite Assessment:**

For those parents who express interest in having their son/daughter participate, some information about the child will be requested by telephone, by the project coordinator, Barkha Patel. If the child was born at term, is healthy and does not receive any medications, an information session will be arranged.

During the information session, the researcher will explain the full details of the study. Parents that give consent to have their son/daughter participate will sign a consent form. The parent will receive copies of consent forms and of the study information sheet. If the child wishes to
participate and signs a children’s assent form, his/her weight, height, and body fat using painless techniques, will be measured.

The boys and girls will then be asked to rank their preference for pizza that will be served as the lunch meal at each session.

The boys/girls who participate in this study, will be requested to go to the FitzGerald Building, Department of Nutritional Sciences, University of Toronto, for four individual morning sessions. These sessions will be held on weekends, over four weeks. The children will be brought to the laboratory and returned home either by parents or a research assistant, as preferred by the parents.

On each of the four test days, the children will have a standardized breakfast of cereal, milk and juice at home, either at 8:00 am or 9:00 am (the time will be consistent for each child). The children will arrive at FitzGerald Building, either at 10:00 am or 11:00 am (but consistent throughout for each child).

Children will fast for 12 hours before breakfast and after breakfast until their arrival, except for water, which will be allowed up to one hour before their arrival.

Each child will receive one glass of sweetened Kool-aid containing sugar or artificial sweetener. The sugar drink will contain similar amount of sugar as common soft drinks or fruit juice. Each child will receive both drinks, one on each day in no set order, with or without television viewing.

McCain pizza and spring water (purchased at Loblaws) will be served 30 minutes after the boys/girls have consumed the drink. Children will be told that they may eat as little or as much as they like. The amount of food eaten by each child will be measured.

During two of the four feeding sessions a cartoon program will be running. The television show (e.g. Hannah Montana) will play for 30 – 40 min while the child eats, excluding advertisements. During the initial telephone conversation the parent will be given an overview of the content that will be shown to children during the test meal. If the parent gives their consent to allow us to show the cartoon to the child, and all other inclusion criteria are met, an information visit will be scheduled for the parent and child.
The boys/girls will also be requested to complete scales on which they will place a pencil mark to describe their desire to eat (“Very weak” to “Very strong”), hunger (“Not hungry at all” to “As hungry as I’ve ever felt”), fullness (“Not full at all ” to “Very full”), how much food they could eat (“A large amount” to “Nothing at all”), sweetness of the drinks (“Not sweet at all” to “Extremely sweet”). They will complete these scales during the information session, in order to become familiar with the test instruments.

The children will be fully supervised during the study sessions. They will be engaged in age appropriate entertainment (as distraction) eg: reading, puzzles, cards, before lunch. The study session will end either at 11:30 am or at 12:30 pm after the pizza meal.

Physiological Testing:

Physiological testing will be performed on one weekend morning, separate from the food intake assessment sessions, at the FitzGerald Building.

Body Composition Assessment:

Two methods will be used to estimate the amount of muscle and fat tissue in your child’s body.

Skinfolds:

The skinfold thickness technique is performed by pinching the skin between the thumb and forefinger and placing calipers on the fold measuring the width of thickness of the two layers of skin and subcutaneous fat underneath. The assumptions underlying the rationale of measuring skinfold thickness are that skinfold thickness is an adequate measure of subcutaneous fat (fat under the skin) and that there is a defined relationship between subcutaneous fat and total body fat.

Bioelectrical Impedance Analysis:

Bioelectrical impedance analysis (BIA), a recently developed technique for measuring body fat content in both adults and children, is simple and painless and is an effective method for measuring body fat in children. BIA is based on measurement of electrical resistance in the body to a tiny current (that the subject cannot feel). The principle of BIA lies in that muscle mass in
the body is a better conductor of electricity than fat which contains lesser amounts of water and electrolytes.

**Confidentiality:**

Records relating to participants will be kept confidential in a locked cabinet in the Department of Nutritional Sciences and no disclosure of personal information of the children or parents will take place except where required by law. Participants will have a code and a number that will identify them in all documents, records and files to keep their name confidential. All data will be entered into Microsoft Excel files, available only to investigators. Each participant will have a file, also only available for investigators. All forms and printouts will be stored in the individual files – and clearly labeled. All documents will be kept for a minimum of five years following completion of the study and then securely destroyed.

**Benefits:**

As the causes of obesity remains undefined, the potential benefits from this study will be a better understanding of the regulation food intake in children and might contribute to the prevention of obesity in children.

**Questions and further information:**

If you have any questions or would like further information concerning this research project, please do not hesitate to call: Barkha Patel, Project Coordinator at (416) 978-4153 or Dr. Harvey Anderson, Investigator at (416)-978-1832.

If you have questions or concerns about your rights as a research participant, please contact Jill Parsons, Research Ethics Officer, Health Sciences jc.parsons@utoronto.ca or 416-946-5806.

**Dissemination of findings:**

A summary of results will be made available to you to pick up after the study is completed.

**Parental Consent:**

I acknowledge that the research procedures described above and of which I have a copy, have been explained to me and that any questions that I have asked have been answered to my
satisfaction. I know that I may ask additional questions now or in the future. I am aware that participation in the study will not involve any health risk to my child.

I understand that for purposes of the research project, if my child or I choose to withdraw from the study at any time, we may do so without prejudice.

Upon completion of each study session, my child will receive a $20 gift certificate to the theatre. The final summary and results of the study will be available for me to pick up from the Department of Nutritional Sciences, University of Toronto. I am aware that the researchers may publish the study results in scientific journals, keeping confidential my son or daughter’s identity.

I hereby consent for my child, ________________________________, to participate in this study.

__________________________________________  _____________________________
(Name of parent or guardian)                          (Signature of parent or guardian)

__________________________________________  _____________________________
(Name of witness)                                      (Signature of witness)

Date: ____________(dd/mm/yy)
Children’s Assent Form

Purpose of Research:

The purpose of this study is to evaluate the effects of television viewing on appetite control in children. My weight, height, and body fat using painless techniques, will be measured during the information visit. I will also be required to drink a sweetened beverage (within 5 minutes) and complete special scales to show if I am hungry or full during each session. I will also be provided with a pizza lunch at the end of each study session (that I will eat in the Department of Nutritional Sciences, University of Toronto). All the experimental sessions will be on weekends and school holidays, so I don’t need to be absent from school.

I know that my participation in the study will not involve any health risk to me.

Also, if at any time I decide to stop participating, that will be O.K. I understand that information related to me will be kept confidential. I know that I will receive a $20 gift certificate to the theatre after completion of each study session, as a “thank you” for my participation.

“I was present when ______________________________ read this form and gave his/her verbal assent.”

____________________________
Signature

Name of the person who obtained assent:

___ __________________________

Date: __________________(dd/mm/yy)
Name: ____________________

Age: _______ years  DOB (d/m/y) _______  Term baby?  yes/no

Height: _______ cm.  Weight: _______ kg.  Normal birth weight?  yes/no

Has your child gained or lost weight recently?  yes/no  (circle correct answer)

Does your child usually have breakfast?  yes/no

Does your child like:

  milk  yes/no  cereal  yes/no

  juice  yes/no  pizza  yes/no

Is your child following a special diet?  yes/no
Does your child have food allergies or sensitivities? yes/no

Health problems? yes/no
   If yes, which problem? ____________________________

Medication/s? yes/no
   If yes, which medication/s? __________________

Education: Grade: _______ Special class? yes/no

Skipped or repeated grade? yes/no Learning difficulties/problems? yes/no

Behavioral or emotional problems yes/no
   If yes, which problem? ____________________________
   ____________________________

Include in study? yes/no
   If not, why? ____________________________

Appointment date: __________ (d/m/y)

Investigator: ________________ Date: __________ (d/m/y)
Child's Name: ____________________________

Child's Date of Birth: _______________ dd/mm/yy

Child's Sex: Male/Female (circle correct choice)

If female, has your child begun to menstruate? Yes/No

What age was your child when she had her first period? ________ years

Ethnic Background: ______________________

Mother's Name: __________________________

Mother's weight: _____ kg/lb Height: _______ cm/inches (circle unit)

Father's Name: __________________________

Father's weight: _____ kg/lb Height: _______ cm/inches (circle unit)

Subject ID: _____________________________

Investigator: ____________________________
Date: __________________________

1.2.6 CONTACT INFORMATION

Child's Name: __________________________

Address: __________________________

                                  __________________________

                                  __________________________

Home phone #: __________________________
Mother's name: ____________________________

Mother's Cell #: __________________________

Mother's work #: __________________________

Mother's e-mail: ___________________________

Father's name: ____________________________

Father's Cell #: ___________________________

Father's work #: __________________________

Father's e-mail: ___________________________

Source of referral: _________________________

Subject ID: _______________________________

Investigator: ______________________________

Date: __________________ (dd/mm/yy)
1.2.7 FOOD ACCEPTABILITY LIST

Name: ______________________________________

BREAKFAST
On each test day you will consume at home the same breakfast provided by the investigators.

Please indicate whether you will be able to consume all the following:

1 cup nonfat milk (250 mL) Yes/No (circle correct choice)
Honey Nut Cheerios (26 g) Yes/No
Junior Tropicana orange juice (236 mL) Yes/No

BEVERAGE
Please indicate whether you will be able to drink a sweet beverage if it is provided at a study session.

Yes/No

LUNCH
You will also be provided with a pizza meal the day of the study.
To enable us to provide you with a meal that you will enjoy, please indicate whether you would like to be served:

1. All pepperoni pizza (cheese, pepperoni)
2. All 3 cheese pizza (mozzarella, cheddar, parmesan)
3. A combination of pepperoni and 3 cheese pizzas (circle correct choice)

If you answered #3, and you would like a combination of both types, please indicate if you would like more:

pepperoni OR 3 cheese pizza (circle correct choice)

________________________________________________________________________

Investigator: ________________________________
PAST YEAR PHYSICAL ACTIVITY

Check all the activities that you did at least ten times in the PAST YEAR. Include times spent in school physical education classes. Make sure you include all sport teams that you participated in during the last year.

- Aerobics
- Band/Drill Team
- Baseball
- Basketball
- Bicycling
- Bowling
- Cheerleading
- Dance Class
- Football
- Garden/Yard Work
- Gymnastics
- Hiking
- Ice Skating
- Roller Skating
- Running for Exercise
- Skateboarding
- Snow Skiing
- Soccer
- Softball
- Street Hockey
- Swimming (Laps)
- Tennis
- Volleyball
- Water Skiing
- Weight Training
- Wrestling (Competitive)
- Others

List each activity that you checked above in the “Activity” box below, check the months you did each activity and then estimate the amount of time spent in each activity.

| Activity | J | F | M | A | P | A | U | U | N | L | G | P | T | V | E | C | N | D | Months Per Year | Days Per Week | Minutes Per Day |
|----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------------|--------------|----------------|

1. Modified from Aaron et al. 1995 (301).
1. Subject and test details

Name: ________________________________

Date of birth: ________________________________

Age: ________________________________

Gender: □ male □ female

Today’s date: ________________________________

2. Your weight, height, etc.

A. Current weight (kg): ________________

B. Current height (cm): ________________

C. Has your body weight been constant over the past six months?

□ yes, my weight did not change much

□ no, I lost ________ kg
no, I gained ______ kg

no, sometimes I gained weight and sometimes I lost weight

D. Have you ever had an episode of eating an amount of food that others would regard as unusually large?

☐ yes

☐ no

Please do not mark below this line

BMI (please take the age of the child into account): __________________________

<table>
<thead>
<tr>
<th>DEBQ scale</th>
<th>Raw score</th>
<th>Number of items</th>
<th>Scale score</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional eating</td>
<td></td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External eating</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restrained eating</td>
<td></td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please turn over >>>>>>
**Instructions**

Below you’ll find 20 questions about eating.

Please read each question carefully and tick the answer that suits you best.

Only one answer is allowed. Don’t skip any answer.

There are no incorrect answers; it’s your opinion that counts.

<table>
<thead>
<tr>
<th></th>
<th>Do you feel like eating whenever you see or smell good food?</th>
<th>No</th>
<th>Sometimes</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>If you feel depressed do you get a desire for food?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>If you feel lonely do you get a desire for food?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Do you keep an eye on exactly what you eat?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Does walking past a candy store make you feel like eating?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Do you intentionally eat food that helps you lose weight?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Does watching others eat make you feel like eating too?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Question</td>
<td>Response 1</td>
<td>Response 2</td>
<td>Response 3</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------------------------------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>8</td>
<td>If you have eaten too much do you eat less than usual the next day?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>Does worrying make you feel like eating?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Do you find it difficult to stay away from delicious food?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>Do you intentionally eat less to avoid gaining weight?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>If things go wrong do you get a desire for food?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>Do you feel like eating when you walk past a restaurant or fast food restaurant?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>Have you ever tried not to eat in between meals to lose weight?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>Do you have a desire to eat when you feel restless?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>Have you ever tried to avoid eating after your evening meal to lose weight?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>17</td>
<td>Do you have a desire for food when you are afraid?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Question</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>---</td>
<td>------------------------------------------------------------------------</td>
<td>----</td>
<td>-----------</td>
<td>-----</td>
</tr>
<tr>
<td>18.</td>
<td>Do you ever think that food will be fattening or slimming when you eat?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>19.</td>
<td>If you feel sorry do you feel like eating?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
<tr>
<td>20.</td>
<td>If somebody prepares food do you get an appetite?</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**PLEASE CHECK, TO BE SURE THAT YOU TICKED EVERY QUESTION.**
1.2.10  PUBERTY QUESTIONNAIRE

Would you say that your growth spurt (height):
1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

And regarding hair growth (under your arms, your pubic hair), would you say that:
1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

Have you noticed changes in your skin (e.g. acne)?
1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

FOR GIRLS:

FOR BOYS:

Have your breasts started to develop?
1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

Have you noticed that your voice has changed (lowered)?
1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

Have you started to have hair on your face?
1. there have been no changes
2. changes have barely begun
3. changes are definitely underway

*NOTE: Girls with menarche start within a year of study visit = Tanner 4, girls with menarche start over one year of study visit = Tanner 5.*
For boys and premenarchial girls, self-staging with the use of cartoons will be used.
Tanner Staging
Tanner Staging
1. When were you born? __________________________________

2. Have you had your first period? _________________________
   If you answered no, you are finished this questionnaire.

   If you answered yes, please complete the following questions.

3. How old were you when you had your first period?
   I was _____ years old when I had my first period.

4. Do you remember the day or month of your first period? ____________________

5. How long is your average menstrual cycle? (from the beginning of menstrual flow [menses] to the beginning of the next menstrual flow [menses])
   My average cycle length is _____ days.

6. Currently, for how many days do you typically experience menstrual flow each cycle?
   _____ 1 day _____ 2 days _____ 3 days _____ 4 days _____ 5 days _____ > 5+ days

7. In the past 3 months, estimate how many menstrual cycles you have had?
   I have had _______ cycles in the past 3 months

8. In the past 6 months, estimate how many menstrual cycles you have had?
   I have had _______ cycles in the past 6 months
9. In the past 9 months, estimate how many menstrual cycles you have had?
   I have had _______ cycles in the past 9 months

10. In the past 12 months, estimate how many menstrual cycles you have had?
    I have had _______ cycles in the past 12 months

11. How would you characterize your menstrual flow in the first two days of menses?

   Circle one: Heavy Moderate Light

12. Do you experience cramps during menses?

   Circle One: Always Sometimes Never

13. Do you typically experience any pain during the middle of your cycle?

   Circle one: Always Sometimes Never

14. Do you typically experience spotting or sporadic bleeding not associated with normal menstrual flow?

   Circle one: Always Sometimes Never
1.3 APPENDIX 3: STUDY DAY QUESTIONNAIRES

MOTIVATION TO EAT

PLEASANTNESS OF THE DRINK

PLEASANTNESS OF THE FOOD

SWEETNESS

TV PROGRAM ACCEPTABILITY
1.3.1 Visual Analogue Scale: Motivation to Eat

DATE: ________________________

NAME: ________________________

These questions relate to your “motivation to eat” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very               Very

WEAK __________________________ STRONG

2. How hungry do you feel?

NOT                 As hungry

Hungry __________________________ as I have

at all              ever felt

3. How full do you feel?

NOT                 VERY

Full __________________________ Full

at all

4. How much food do you think you could eat?

NOTHING                A LARGE

at all __________________________ amount
1.3.2 Visual Analogue Scale-Pleasantness

DATE: ________________________

NAME: ________________________

This question relates to the palatability of the beverage you just consumed. Please rate the pleasantness of the beverage by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How pleasant have you found the preload?

NOT ___________________________ Very pleasant

at all

pleasant
1.3.3 Visual Analogue Scale-Pleasantness

DATE: ________________________

NAME: ________________________

This question relates to the palatability of the food you just consumed. Please rate the pleasantness of the food by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How pleasant have you found the food?

NOT ___________________________ Very pleasant

at all pleasant
1.3.4 Visual Analogue Scale-Sweetness

DATE: ________________________

NAME: ________________________

Please rate the level of sweetness by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How sweet have you found the beverage?

NOT  ___________________________________________  Extremely sweet

sweet

at all
1.3.5 Visual Analogue Scale-Physical Comfort

DATE: ______________________

NAME: ______________________

These questions relate to your “motivation to eat” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How well do you feel?

NOT well ____________________________________________ Well

at all
1.3.6 Visual Analogue Scale-TV program acceptability

DATE: ________________________

NAME: ________________________

This question relates to how well you enjoyed the cartoon program. Please rate the program by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How well did you enjoy the program?

NOT well ________________________________________________ Well at all
### APPENDIX 4: Pizza Intake Sheet

Subject: ____________________________  Glucose load: ____________

Pizza preference: ____________________________________________

Time: ____________________________

<table>
<thead>
<tr>
<th>Tray 1</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tray 2</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tray 3</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
<td></td>
</tr>
</tbody>
</table>

| Water (g) |
| Water (g) |

<table>
<thead>
<tr>
<th>Tray 1</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tray 2</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tray 3</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
<td></td>
</tr>
</tbody>
</table>

| Water (g) |
| Water (g) |

Session: ________________  Session: ________________

Investigator: ________________  Investigator: ________________

Date: __________ (dd/mm/yy)  Date: __________ (dd/mm/yy)
<table>
<thead>
<tr>
<th>Tray 1</th>
<th>Pepperoni (g)</th>
<th>3 Cheese (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tray 2</td>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
</tr>
<tr>
<td>Tray 3</td>
<td>Pepperoni (g)</td>
<td>3 Cheese (g)</td>
</tr>
<tr>
<td></td>
<td>Water (g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water (g)</td>
<td></td>
</tr>
</tbody>
</table>

Session: ___________________
Investigator: ___________________
Date: ___________ (dd/mm/yy)
1.5 APPENDIX 5: Nutritional Composition of the Pizza Served at Test Meals

<table>
<thead>
<tr>
<th>Per 1 pizza</th>
<th>Pepperoni (87g)</th>
<th>Three Cheese (81 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Trans Fat (g)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>400</td>
<td>360</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
1.6 APPENDIX 6: Supplementary data from Chapter 5

Table 5.4: Experiment 1: Overall mean change from baseline average appetite scores for the pre-meal period in early pubertal and mid-late pubertal boys

<table>
<thead>
<tr>
<th>Pre-meal means (mm)²</th>
<th>Drink</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Meal at 30 min</td>
<td></td>
</tr>
<tr>
<td>Early Pubertal³</td>
<td>4.7 ± 4.0</td>
</tr>
<tr>
<td>Mid-late Pubertal⁴</td>
<td>6.9 ± 2.9</td>
</tr>
<tr>
<td>Meal at 60 min</td>
<td></td>
</tr>
<tr>
<td>Early Pubertal⁵</td>
<td>13.3 ± 2.7ᵃ</td>
</tr>
<tr>
<td>Mid-late Pubertal⁶</td>
<td>9.1 ± 2.3ᵃ</td>
</tr>
</tbody>
</table>

¹Data are means ± SEM; (n = 14; early pubertal, n = 15; mid-late pubertal). A 2-factor ANOVA was used with drink and time and as main factors and a drink by time interaction. Different superscripts are significantly different (p < 0.05). ²Average appetite pre-meal values are means of all observations before the test meal at 30 min (15 and 30 min) and the test meal at 60 min (15, 30, 45 and 60 min) minus the baseline means at 0 min.
³Average appetite was affected by time (p < 0.010), but not drink (p = 0.755) or a drink by time interaction (p = 0.958).
⁴Average appetite was affected by time (p < 0.001), but not drink (p = 0.459) or a drink by time interaction (p = 0.459).
⁵Average appetite was affected by drink (p = 0.010) and time (p < 0.010), but not a drink by time interaction (p = 0.939).
⁶Average appetite was affected by drink (p = 0.039) and time (p < 0.010), but not a drink by time interaction (p = 0.438).
Table 5.5: Experiment 2: Overall mean change from baseline average appetite scores for the pre-meal period in early and mid-late pubertal girls

<table>
<thead>
<tr>
<th>Pre-meal means (mm)²</th>
<th>Drink</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Glucose</td>
<td>Whey</td>
</tr>
<tr>
<td><strong>Meal at 30 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Pubertal³</td>
<td>5.1 ± 2.7</td>
<td>9.5 ± 2.5</td>
<td>5.8 ± 2.5</td>
</tr>
<tr>
<td>Mid-late Pubertal⁴</td>
<td>8.2 ± 2.0</td>
<td>10.5 ± 2.5</td>
<td>9.6 ± 2.7</td>
</tr>
<tr>
<td><strong>Meal at 60 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Pubertal⁵</td>
<td>5.7 ± 2.3</td>
<td>1.2 ± 2.4</td>
<td>7.9 ± 2.3</td>
</tr>
<tr>
<td>Mid-late Pubertal⁶</td>
<td>20.8 ± 2.0&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>11.5 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.5 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

¹Data are means ± SEM; (n = 13; early pubertal, n = 16; mid-late pubertal). A 2-factor ANOVA was used within each group with drink and time as main factors and a drink by time interaction. Different superscripts are significantly different (p < 0.05). ²Average appetite pre-meal values are means of all observations before the test meal at 30 min (15 and 30 min) and the test meal at 60 min (15, 30, 45 and 60 min) minus the baseline means at 0 min.
³Average appetite was affected by time (p = 0.005), but not drink (p = 0.437) or a drink by time interaction (p = 0.674).
⁴Average appetite was affected by time (p < 0.001), but not drink (p = 0.768) or a drink by time interaction (p = 0.892).
⁵Average appetite was affected by time (p = 0.003), but not drink (p = 0.056) or a drink by time interaction (p = 0.694).
⁶Average appetite was affected by drink (p = 0.031) and time (p < 0.001), but not or a drink by time interaction (p = 0.358).
Table 5.6: Sweetness and pleasantness of the drinks in early pubertal and mid-late pubertal boys and girls

<table>
<thead>
<tr>
<th>Drink</th>
<th>VAS means (mm)</th>
<th>Experiment 1 - Boys</th>
<th>Experiment 2 - Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetness</td>
<td></td>
<td>Pre-early Pubertal</td>
<td>Mid-late Pubertal</td>
</tr>
<tr>
<td>Control</td>
<td>76.5 ± 4.1a</td>
<td>79.9 ± 4.2</td>
<td>73.1 ± 6.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>66.8 ± 4.5a,b</td>
<td>76.5 ± 3.6</td>
<td>66.7 ± 6.4</td>
</tr>
<tr>
<td>Whey Protein</td>
<td>62.0 ± 4.9b</td>
<td>71.6 ± 3.4</td>
<td>78.1 ± 6.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drink Pleasantness</th>
<th></th>
<th>Experiment 1 - Boys</th>
<th>Experiment 2 - Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.5 ± 5.1a</td>
<td>73.1 ± 4.7</td>
<td>56.8 ± 7.7a</td>
</tr>
<tr>
<td>Glucose</td>
<td>72.1 ± 4.1a</td>
<td>75.8 ± 3.6</td>
<td>61.3 ± 7.1a</td>
</tr>
<tr>
<td>Whey Protein</td>
<td>55.8 ± 5.4b</td>
<td>69.5 ± 5.4</td>
<td>25.4 ± 6.4b</td>
</tr>
<tr>
<td>P</td>
<td>0.047</td>
<td>0.120</td>
<td>0.389</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM; (Boys: n = 14; early pubertal, n = 15; mid-late pubertal. Girls: n = 13; early pubertal, n = 16; mid-late pubertal). Different superscripts are significantly different, One-factor ANOVA for treatment effect followed by Tukey’s post hoc test (P < 0.05).

1.7 APPENDIX 7: SOP FOR GUT HORMONE COLLECTION

SOP for Gut Hormones Protocol (GLP-1, PYY3-36, active & total ghrelin)
1) Fill the black bucket with ice (ice is located in the metabolic kitchen). Chill your 10 ml Purple Top Vacutainer on ice. Take off ice 1-2 minutes before the blood draw.

2) Place your microtubes for whole blood on ice.

3) As soon as the blood is drawn, invert the vacutainer 10 times.

4) **GLP-1:** Within 30 seconds transfer 1800 ul of whole blood from the 4 ml purple top vacutainer into 2 ml microcentrifuge tube that contains 18 ul of DPP-4 inhibitor. Then invert that tube 3 times. Place tube on ice.

   **PYY336:** Transfer 900 ul of blood from the GLP-1 tube into a microtube that contains aprotinin labeled PYY336 (invert 3 times) and place on ice.

   **Active Ghrelin:** Transfer 800 ul of whole blood from the 4 ml purple top vacutainer in a 1.5 ml microtube and place on ice.

   **Total ghrelin/cortisol:** Transfer 800 ul of whole blood from the 4 ml purple top vacutainer into a 1.5 ml microcentrifuge tube and place on ice.

5) **GLP-1:** Transfer all the plasma into a BLUE 1.5 ml microtube labeled GLP-1. Store at -80 degrees Celsius or on dry ice.

   **SPIN ALL THE MICROTUBES FOR 15 MINUTES AT 4 DEGREES CELSIUS**
**PYY336**: Transfer all the plasma into a PURPLE 1.5 ml microtube labeled PYY336. Store at -80 degrees Celsius or on dry ice.

**Active Ghrelin**: Transfer exactly 200 ul of plasma into a GREEN microtube that contains 10 ul of 1.0N HCl solution. Then add 2.0 ul of fresh PMSF solution (10 mg/ml) to the microtube. Vortex!! Store at -80 degrees Celsius or on dry ice.

**Note**: PMSF solution MUST be made fresh the morning of the blood collection

**Total ghrelin/cortisol**: Transfer all the plasma into a YELLOW 1.5 ml microtube labeled Total ghrelin/cortisol. Store at -80 degrees Celsius or on dry ice.

**PMSF Solution**:

**Note**: PMSF solution MUST be made fresh the morning of the blood collection

You will need PMSF powder and methanol located in 329.

1) Place a 2.0 ml microtube in a weighing boat (to prevent contamination) and then onto the analytical scale in Bohdan’s office. Zero the scale.

2) Use a small spatula to carefully weigh out 7 mg (0.007g) into the microtube.

3) Remove the microtube from the scale.

4) Add 700 ul of methanol so that you have a final concentration of 10 mg/ml. Vortex the microtube and leave on ice
5) Clean the scale

**PBS Solution:**

1 tablet PBS phosphate buffered in saline in 200 ml deionized water

**Aprotinin Solution:**

For calculations see Barkha’s lab notebook

**P800 Tubes (Grey Top 2.0 mL)**

1. P800 tubes with collected blood should be gently inverted 10 times (but do not shake since it cause haemolysis) and centrifuged immediately
2. Balance the rotor of the centrifuge with P800 2.0 mL tubes filled with water.
3. **Spin at 1,100 – 1,300 g (RCF) for 10 min.**
4. Alliquote plasma immediately into 6 microtubes and keep them on dry ice.
   i. Active Ghrelin: \(200 \, \mu\text{L} \, \text{HCl} + 60 \, \mu\text{L}\) then vortex
   ii. PYY \(60 \, \mu\text{L}\)
   iii. GLP-1 \(250 \, \mu\text{L}\)
   iv. Extra \(250 \, \mu\text{L}\)
   v. Extra \(130 \, \mu\text{L}\)
5. Place all microtubes from dry ice into the appropriate boxes at -20°C in the same order as in the sample log.
1.8 APPENDIX 8: SUPPLEMENTARY MANUSCRIPTS
A premeal snack of raisins decreases mealtime food intake more than grapes in young children

Barkha P. Patel, Bohdan Luhovyy, Rebecca Mollard, James E. Painter, and G. Harvey Anderson

Abstract: The effect of a premeal snack of grapes, raisins, or a mix of almonds and raisins, compared with a water control, on food intake (FI) was examined in 8- to 11-year-old normal-weight (15th to 85th percentile) children. Children randomly received 1 of 4 ad libitum (Experiment 1: 13 boys, 13 girls) or fixed-calorie (150 kcal; Experiment 2: 13 boys, 13 girls) treatments, followed by an ad libitum pizza meal 30 min later. Appetite was measured throughout the study, and FI was measured at 30 min. The ad libitum consumption (Experiment 1) of raisins reduced pizza intake (p < 0.037), compared with water (26%), grapes (23%), and the mixed snack (15%). Cumulative energy intake (in kcal: snack + pizza) was lower after water and raisins than after either grapes or the mixed snack (p < 0.031). As a fixed-calorie (150 kcal) snack (Experiment 2), raisins reduced pizza intake, compared with water (~11%, p = 0.005), and resulted in a cumulative intake similar to water; however, both grapes and the mixed snack resulted in higher cumulative intakes (p < 0.015). Appetite was lower after all caloric ad libitum snacks (.0003) and after fixed amounts of grapes and the mixed snack (p < 0.037), compared with water. In conclusion, consumption of a premeal snack of raisins, but not grapes or a mix of raisins and almonds, reduces meal-time energy intake and does not lead to increased cumulative energy intake in children.

Key words: snacking, children, food intake, raisins, grapes, almonds.

Introduction

In addition to providing energy, snacks are important for meeting nutritional requirements in children (Sebastian et al. 2008; Johnson and Anderson 2010). Frequent snacking is a characteristic of the eating patterns of adolescents (12–19 years of age), and provides an opportunity to promote nutrient-rich, low-energy-dense snacks to improve diet quality in children (Sebastian et al. 2008). Dietary guidelines recommend the increased consumption of fruits and vegetables at meals and as snacks (Dietary Guidelines for Americans 2010; Eating Well with Canada’s Food Guide 2011). Fruit is consumed below recommended levels by children (Guenther et al. 2006; Garriguet 2007); however, few studies have examined the potential of dried fruit as a snack to fill this gap. Whole fruit is recommended over energy-dense snacks, including dried fruit, nuts, and savory snacks, because high-volume foods with a low-energy density increase feelings of satiety (Flood-Obbagy and Rolls 2009; Rolls 2010).

In recent years, dried fruit products have appeared in the market place as a preservation strategy and to provide consistent access to fruit in the form of snacks or as an addition to meals (Jangam and Mujumdar 2010). However, dried fruit accounts for less than one-tenth of the total per capita fruit consumed annually in the United States (US Department of Agriculture Economic Research Service 2012). Recent studies have suggested that dried fruit is a convenient way to fill the gap between fruit consumption...
and recommended intake to achieve healthier body weights and improved diet quality. In fact, Furchner-Evanson et al. (2010) demonstrated that a fixed-calorie snack (238 kcal) of dried plums increased satiety and elicited lower plasma glucose and insulin area under the curve than low-fat cookies in normal-weight and overweight or obese females. In addition, Farajian et al. (2010) found that a preload snack that included dried plums reduced total energy intake at a later meal and increased satiety, compared with an isoeenergetic and equal-weight bread product in normal-weight adults. Dried fruit consumption might have other health benefits: it was associated with improved diet quality and lower body weight in adults participating in the 1999–2004 National Health and Nutrition Examination Survey (Keast et al. 2011).

Raisins, the most commonly consumed dried fruit snack (Keast et al. 2011), are a source of dietary fibre (30% soluble), potassium, iron, and antioxidants (US Department of Agriculture Nutrient Database for Standard Reference 2011). The antioxidant capacity, measured by oxygen radical absorbance capacity, is generally higher in raisins, on a per-weight basis, than in grapes (Wu et al. 2004; Williamson and Carugh 2010) and could contribute to their health benefits. In one randomized crossover study (Rankin et al. 2008), overweight adults, who consumed either raisins (90 g) or an isocaloric placebo (264 kcal·day−1) for 14 days while following a low-flavonoid diet, modestly increased their antioxidant capacity with no change in inflammatory responses. Puglisi et al. (2008) found that raisins (160 g day−1 for 6 weeks) decreased the inflammatory cytokine TNF-α, potentially because of their antioxidant properties, but raisins alone or combined with walking did not affect body weight, fasting glucose, or insulin. Although body weight and total energy intake were not different in response to the interventions, a 5-day diet record indicated that raisin consumption increased the percentage of calories consumed as a carbohydrate and the amount of total dietary soluble fibre. (Puglisi et al. 2009) found that raisins and raisins combined with walking increased plasma leptin and ghrelin concentrations after 6 weeks, indicating that the higher leptin might have been a response to reduced food intake (FI) and account for the compensatory increase in ghrelin.

Although raisins are a readily available snack for increasing fruit intake, no studies have reported the effects of consumption on appetite or FI in children. Therefore, the objectives of this study were to examine the effect of a premeal snack of ad libitum (Experiment 1) or fixed-calorie (Experiment 2) raisins, grapes, and a mix of almonds plus raisins, compared with a water control, on subjective appetite, snack intake, and FI 30 min after consumption in 8- to 11-year-old children.

**Materials and methods**

**Subjects**

Boys and girls were recruited through advertisements on community bulletin boards, local newspapers, and by word of mouth. For Experiment 1, participants were recruited from June 2010 to January 2011; for Experiment 2, participants were recruited from February 2011 to August 2011. There was no overlap between experiments. Parents of interested participants were prescreened during a telephone interview that provided basic information about the study. Children were required to be 8 to 11 years of age, consume breakfast regularly, like the test foods being used in the experiments. Parents of interested participants were prescreened in 8- to 11-year-old children.

**Study design**

A within-subject, randomized, repeated-measures design was used to examine FI after consumption of an ad libitum (Experiment 1) or fixed-calorie (Experiment 2) snack of grapes, raisins, a 1:1 mix of raisins and almonds (mixed snack), or ad libitum water. Almonds, another easy-to-eat high-protein snack found to nonsignificantly decrease subjective feelings of hunger more than a cereal bar after 12 weeks in adults (Zaveri and Drummond 2009), were added to the raisins to simulate a trail mix type of snack. Children were randomly assigned to a counterbalanced treatment order using a randomized block design. On 4 separate mornings, 7 days apart, children were given access to grapes, raisins, the mixed snack, or water for 15 min. 2 h after a standardized breakfast of Parmalat fat-free skim milk (250 mL, 91 kcal), Honey Nut Cheerios (26 g, 103 kcal, donated by General Mills, Inc.), and Tropicana orange juice (236 mL, 110 kcal). Thirty minutes after eating the snacks, children were provided 30 min to eat an ad libitum pizza meal and instructed to eat until comfortably full.

**Protocol**

Procedures were similar to those reported elsewhere (Bellissimo et al. 2007a, 2007b, 2008a, 2008b; Patel et al. 2011). Children arrived at the department between 0900 and 1200, 2 h after consuming the standard breakfast at home, and were interviewed to ensure their compliance with the breakfast meal. Participants who did not consume the breakfast at the correct time were rescheduled.VAS were used to measure subjective feelings assessing motivation to eat and physical comfort, and were administered at baseline (0 min) and at 30, 45, 75, and 105 min. Immediately after consumption of the snack or meal, participants were asked “How pleasant have you found the snack (or pizza) to assess the pleasantness of the snack and the pizza lunch (the VAS ranged from not pleasant at all to very pleasant). To assess motivation to eat, 4 questions were used to measure dimensions of subjective appetite: How strong is your desire to eat? (from very weak to very strong); How hungry do you feel? (from not hungry at all to as hungry as I’ve ever felt); How full do you feel? (not full at all to very full); and How much food do you think you can eat? (from nothing at all to a large amount), as described elsewhere (Bellissimo et al. 2007a, 2007b, 2008a, 2008b; Patel et al. 2011). To assess physical comfort, participants were asked “How well do you feel?” (from not well at all to very well). Subjective measures of palatability were formatted in a similar manner (Bellissimo et al. 2007a, 2007b, 2008a, 2008b; Patel et al. 2011).

In Experiment 1, the snacks — California seedless raisins (donated by the California Raisin Marketing Board, Fresno, Calif., USA), grapes (Thompson seedless grapes purchased from the Metro grocery store, Toronto, Ont., Canada), and blanched almonds plus raisins (1:1 ratio based on weight; donated by the California Raisin Marketing Board) — were administered ad libitum to simulate a snacking occasion where uncontrolled portion sizes were available. Snacks were prepared as 100 g preweighed portions (—67 kcal for grapes, —298 kcal for raisins, —443 kcal for the mixed snack), in 3 individual containers (to a maximum of 6 containers), 1 day prior to consumption, and stored in the refrigerator. In Experiment 2, the fixed-calorie (150 kcal) snacks repre
sented the typical serving of raisins (~two 28 g boxes) that children would consume before lunch, so that the effect of the composition of the snack could be investigated independent of energy on later FI. The mixed snack provided 75 kcal from raisins and 75 kcal from almonds. The macronutrient composition of the snacks was calculated using food composition tables from the US Department of Agriculture (US Department of Agriculture Nutrient Database for Standard Reference 2011). A cup of water (~500 mL) was provided ad libitum with each of the treatments and with the pizza meal, and the children were instructed to drink until "comfortably full." Additional water was available upon request. Water was provided as a control treatment to allow an assessment of the effect of caloric snacks on cumulative FI; again, the children were instructed to drink until "comfortably full."

On test mornings, after completion of baseline questionnaires, children were individually escorted to a feeding room where they were served water and 1 of the 3 snacks or no snack (water only), and were instructed to eat until "comfortably full" (Experiment 1) or to consume the entire snack (Experiment 2) while seated for 15 min. The amount of snack and water left after the 15 min was subtracted from the initial weight to measure snack and water intake. Each snack was weighed separately, and energy consumed (in kcal) was calculated using the manufacturer's information. Pleasanthness of the snack was assessed immediately after consumption, and appetite and physical comfort were rated 15 min after consumption. After snack consumption, children engaged in child-appropriate entertainment (cards, board games, etc.) (Bellissimo et al. 2007).

Previous studies of 9- and 10-year-old children have shown that sucrose beverages (caloric content in the range of 180–360 kcal) reduce subsequent FI when the time between the beverage and test meal is short (30 min) (Black and Anderson 1994), but not when it is longer (90 min) (Anderson et al. 1989). Because children's compensation occurs at the test meal for previous ingestion of ≥180 kcal and not after time intervals greater than 60 min, a 30 min interval between the snack and the pizza meal was chosen to examine the effect of the premeal snack on later FI.

Thirty minutes after snack consumption and after rating their appetite and physical comfort, subjects were escorted to the feeding room, seated in individual cubicles, and served an ad libitum pizza lunch with ad libitum water. Children were asked to remain seated for 30 min and were instructed to eat until "comfortably full." The 2 varieties of Deep'n Delicious 5-inch pizza (~200 kcal each) used — pepperoni and 3-cheese — were donated by McCain Foods Ltd. (Florenceville, N.B., Canada). Each tray contained 3 pizzas: 2 of their first choice and 1 of their second choice. All cooked pizzas (baked 8 min at 430 °F) were cut into 4 equal pieces, weighed, and served immediately. A freshly baked tray of pizza was provided every 10 min starting 30 min after snack consumption. The amount left after the meal was subtracted from the initial weight to measure FI. Each variety of pizza was weighed separately, and energy consumed (in kcal) was calculated using the manufacturer's information. The cup of water served to the children was weighed before and after the meal to calculate the net amount ingested. Appetite, physical comfort, and pleasantness were assessed immediately and 30 min after the meal.

Eating behaviour assessment
The Dutch Eating Behaviour Questionnaire was administered to assess restrained eating (van Strien and Oosterveld 2008). Children received assistance if they had difficulty interpreting the questionnaire's language.

Statistical analyses
Statistical Analysis Software (SAS) version 9.2 (SAS Institute Inc., Carey, N.C., USA) was used for statistical analyses. Two-way repeated-measures analysis of variance (ANOVA), using the PROC MIXED procedure, was used to analyze the effects of time, treatment, and their interaction on premeal (0–45 min) and postmeal (45–105 min) subjective appetite (reported as the change from baseline) and physical comfort. One-way ANOVA, also using the PROC MIXED procedure, was used to determine the effect of the treatments on energy and water intake, caloric compensation (the amount of food compensated for after the snack), and palatability of the treatment and pizza meal. There were no sex differences, so results were pooled for boys and girls. Post hoc analysis, using the Tukey–Kramer test, was performed when treatment effects were found to be statistically significant.

An average appetite score was calculated at each time of measurement for each treatment, using the following formula: Appetite score = [desire to eat + hunger + (100 – fullness) + PFC]/4, where PFC is prospective food consumption. This reflects the 4 questions on the motivation-to-eat VAS and has been previously used (Anderson et al. 1989; Patel et al. 2011) and validated (Lluch et al. 2010). FI was determined from the total weight and energy content of the snacks and from the total energy content of the pizza consumed at the meal. Cumulative energy intake was calculated as the sum of calories and total weight consumed from the snack and pizza meal, and cumulative water intake was calculated as the amount of water consumed at the snack and pizza meal. Because grapes were the only snack that contained a large proportion of water (80%), which could affect FI, the weight of water in the grapes was added to the weight consumed at the snack. Percent caloric compensation was calculated for each person using the following formula (Bellissimo et al. 2008a; Patel et al. 2011): Caloric compensation = [(kcal consumed at the test meal after the water control – kcal consumed at the meal after the snack) / (kcal in the snack)] × 100%.

On the basis of earlier studies of children from our laboratory (Bellissimo et al. 2008a; Patel et al. 2011), we determined that a 150 kcal decrease in meal-time consumption with a sample size of 12–14 is powered to see the effect of a caloric beverage, compared with the control. Thus, there is adequate power (>80%) with 13 boys and 13 girls to detect differences between raisins and the control on FI, with a within-subject SD of 250 kcal.

Data are presented as means ± standard error (SE). Significance was considered at p < 0.05. Because appetite is related to FI (Patel et al. 2011) and palatability and water intake can affect FI, correlations between FI and appetite, palatability, and water intake were conducted using Pearson's correlation coefficient.

Results

Experiment 1: Effect of an ad libitum premeal raisin snack on satiety and FI in children

Subjects
Twenty-six children (13 girls and 13 boys) participated in Experiment 1 (Table 1). Another group of 26 children (13 girls and 13 boys) participated in Experiment 2 (Table 1). All were of normal weight (between the 15th and 85th age- and sex-specific BMI percentiles).

Snack and energy intake
The composition of the snacks consumed is shown in Table 2. Treatment affected snack, pizza, and cumulative energy intake (p < 0.001) and caloric compensation (p = 0.013, Table 3). Children consumed more calories from the mixed snack than from either grapes (84%, p < 0.001) or raisins (25%, p = 0.004), and 47% more calories from raisins than from grapes (p = 0.024). However, weight consumed from raisins and the mixed snack was less than that consumed from either water or grapes (p < 0.025). Raisins reduced pizza intake (p < 0.037), compared with water (26%, grapes (22%), and the mixed snack (15%). Cumulative energy intake (snack [kcal] + pizza [kcal]) was lower after water and raisins than after either grapes or the mixed snack (p < 0.031). Caloric compensation was −6%, −118%, and −52% for grapes, raisins, and the mixed snack, respectively.
Table 1. Baseline characteristics of test subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>10.3 ± 0.2</td>
<td>10.5 ± 0.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>35.9 ± 1.2</td>
<td>34.7 ± 1.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>143.0 ± 1.9</td>
<td>143.0 ± 1.7</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>54.7 ± 4.4</td>
<td>45.7 ± 4.4</td>
</tr>
<tr>
<td>Restrained eating†</td>
<td>1.5 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
</tbody>
</table>

Note: BMI, body mass index; Experiment 1, n = 26 (13 males, 13 females); Experiment 2, n = 26 (13 males, 13 females).
†Restrained eating was measured with the Dutch Eating Behaviour Questionnaire (van Strien and Oosterveld 2008).

Table 2. Composition of snacks.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Water</th>
<th>Grapes</th>
<th>Raisins</th>
<th>Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carbohydrate (g)</td>
<td>16.4 ± 1.0</td>
<td>72.1 ± 4.3</td>
<td>43.4 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>15.5 ± 0.9</td>
<td>67.8 ± 8.4</td>
<td>36.4 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>0.9 ± 0.2</td>
<td>4.2 ± 0.7</td>
<td>7.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.7 ± 0.1</td>
<td>3.5 ± 0.6</td>
<td>12.3 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>26.0 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Note: Mix, a mix of raisins and almonds. Composition is calculated per 100 g serving.

Table 3. Experiment 1: Effect of ad libitum treatments on snack intake, pizza intake, cumulative energy intake, and caloric compensation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Water</th>
<th>Grapes</th>
<th>Raisins</th>
<th>Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snack intake, kcal†</td>
<td>—</td>
<td>128±13a</td>
<td>188±24b</td>
<td>235±26c &lt;0.001</td>
</tr>
<tr>
<td>Snack intake, g†</td>
<td>16±134a</td>
<td>19±17a</td>
<td>63±6b</td>
<td>6±36c &lt;0.001</td>
</tr>
<tr>
<td>Pizza intake, kcal†</td>
<td>837±258a</td>
<td>798±256b</td>
<td>623±34c</td>
<td>731±25b &lt;0.001</td>
</tr>
<tr>
<td>Cumulative energy intake, kcal†</td>
<td>837±258a</td>
<td>926±262b</td>
<td>811±50c</td>
<td>966±267a &lt;0.001</td>
</tr>
<tr>
<td>Caloric compensation, %†</td>
<td>—</td>
<td>6±45a</td>
<td>118±22b</td>
<td>52±21ab 0.013</td>
</tr>
</tbody>
</table>

Note: Data are means ± SE (n = 26); Mix, a mix of raisins and almonds (children consumed 80 kcal from the raisins and 155 kcal from the almonds).
a,b,cMean values within a row with unlike superscript letters were significantly different. One-factor ANOVA was used, with treatment and time as main factors, followed by Tukey’s post hoc test (p < 0.05).
†Energy consumed at the ad libitum snack.
‡Weight consumed at the ad libitum snack (not including water consumed at the snack).
§Energy consumed at the test meal.
¶Energy consumed at the snack + energy consumed at the test meal.
Caloric compensation = [(kcal consumed at the test meal after the water control — kcal consumed at the meal after the snack)/(kcal consumed at the snack)] × 100%.

Table 4. Experiment 1: Effect of ad libitum treatments on snack-time water intake, test-meal water intake, and cumulative water intake.

<table>
<thead>
<tr>
<th>Water intake</th>
<th>Water</th>
<th>Grapes</th>
<th>Raisins</th>
<th>Mix</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>At snack-time, g†</td>
<td>16±134a</td>
<td>180±19a</td>
<td>70±14b</td>
<td>91±26b &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>At meal, g†</td>
<td>19±10c</td>
<td>197±12c</td>
<td>251±132c</td>
<td>263±26c 0.082</td>
<td></td>
</tr>
<tr>
<td>Cumulative, g‡</td>
<td>352±57a</td>
<td>377±48a</td>
<td>321±38c</td>
<td>354±46c 0.293</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data are means ± SE (n = 26); Mix, a mix of raisins and almonds (children consumed 80 kcal from the raisins and 155 kcal from the almonds).
a,b,cMean values within a row with unlike superscript letters were significantly different. One-factor ANOVA was used, with treatment and time as main factors, followed by Tukey’s post hoc test (p < 0.05).
†Weight of water consumed at ad libitum snack, including the amount of water in grapes.
‡Weight of water consumed at the test meal.
§Weight of water consumed at snack-time + water consumed at the test meal.

Table 5. Experiment 1: Overall mean change from baselineaverage appetite scores for the pre- and postmeal periods.

<table>
<thead>
<tr>
<th>Period</th>
<th>Water</th>
<th>Grapes</th>
<th>Raisins</th>
<th>Mix</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premeal</td>
<td>9.9±2.0a</td>
<td>-5.0±2.2b</td>
<td>0.1±2.8a</td>
<td>-2.2±3.3b &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Postmeal</td>
<td>-41.3±3.8a</td>
<td>-31.3±2.3b</td>
<td>-35.7±4.1ab</td>
<td>-33.7±3.5ab 0.016</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data are means ± SE (n = 26); Mix, a mix of raisins and almonds (children consumed 80 kcal from the raisins and 155 kcal from the almonds).
a,b,cMean values within a row with unlike superscript letters were significantly different. A 2-factor ANOVA was used, with treatment and time as main factors, followed by Tukey’s post hoc test (p < 0.05).
†Postmeal values are means of all observations after the test meal (30 and 45 min) minus the baseline means at 0 min.
‡Premeal values are means of all observations after the test meal (75 and 105 min) minus the premeal means at 45 min.

(p < 0.049). Fullness scores were higher after all snacks than after water (p < 0.006), and were higher after the caloric snacks at 30 min and lower at 45 min before the test meal (p = 0.029).
Scores for postmeal (75 and 105 min) minus premeal (45 min) average appetite, but not desire to eat, hunger, fullness, or PFC, were affected by treatment (p = 0.016), and scores were higher after grapes than after water (p = 0.014; Table 5). Time (p < 0.007) affected average appetite, desire to eat, hunger, and PFC. Scores were reduced by the meal at 75 min and increased toward the end of the session (at 105 min), whereas fullness increased after the meal and decreased toward the end of the session (data not shown for individual scales). Physical comfort was not affected by time, but was affected by treatment (p = 0.035), with lower scores after grapes than after raisins (p = 0.022, data not shown). There was no treatment × time interaction for any VAS.

Subjective ratings of pleasantness of the treatments and test meal
Pleasantness of the snacks differed (p < 0.001), with children rating grapes (82.6 ± 2.2) more pleasant than water (58.0 ± 5.2, p < 0.001), raisins (58.1 ± 5.2, p < 0.001), and the mixed snack (64.9 ± 4.3, p = 0.027). However, treatment did not affect pleasantness of the test meal (data not shown).

Association between subjective appetite and FI
Average appetite (r = 0.43, p = 0.027), desire to eat (r = 0.40, p = 0.044), hunger (r = 0.44, p = 0.025), and PFC (r = 0.46, p = 0.017) were positively associated with FI at 45 min, but there was a trend toward a negative association with fullness (r = -0.38, p = 0.056).

Association between water intake and FI
Neither test-meal water intake (r = 0.368, p = 0.064) nor snack-time water intake (r = 0.308, p = 0.127) were associated with pizza intake.
**Association between palatability and FI**

The palatability of snacks was not associated with pizza intake (after water \( r = -0.107, p = 0.603 \); grapes \( r = -0.163, p = 0.426 \); raisins \( r = -0.165, p = 0.426 \); and the mixed snack \( r = -0.077, p = 0.708 \)) or cumulative pizza intake (after water \( r = -0.121, p = 0.553 \); grapes \( r = -0.132, p = 0.521 \); raisins \( r = -0.252, p = 0.215 \); and the mixed snack \( r = -0.109, p = 0.596 \)).

**Experiment 2: Effect of a fixed-calorie (150 kcal) premeal raisin snack on satiety and FI in children**

### Subjects

Twenty-six children (13 girls and 13 boys) participated (Table 1) in the study. All children were of normal weight (between the 15th and 85th age- and sex-specific BMI percentiles).

### Snack and energy intake

Macronutrient composition of the fixed-calorie snacks (150 kcal) is given in Table 2. Treatment affected snack \( \text{(raisons \( r = 0.005 \)) and cumulative energy intake (caloric compensation, %)} \)

### Water intake

Treatment affected water intake at snack-time \( (p < 0.001) \) and cumulative water intake \( (p = 0.004) \), but not water intake at the meal \( (p = 0.059, \text{Table 7}) \). Water intake at snack-time was lower after raisins \( (−29%, p < 0.024) \) and the mixed snack \( (−51%, p = 0.001) \), compared with water and grapes. Cumulative water intake \( (\text{water consumed at snack [g]} + \text{water consumed at meal [g]}) \) was \( −33\% \) higher after the water, compared with raisins and the mixed snack \( (p < 0.013) \), but was not different after raisins.

### Subjective appetite and physical comfort scores

Premeal average appetite (Table 8), hunger, fullness, and PFC, but not physical comfort (data not shown for individual scales), were affected by time \( (p < 0.006) \) and treatment \( (p < 0.044) \), but there was no treatment \( \times \) time interaction. Average appetite, hunger, and PFC scores dropped after the caloric snacks at 30 min, but then increased at 45 min. Average appetite was lower after grapes and the mixed snack, but not raisins, compared with water \( (p < 0.003) \). Hunger scores were lower after the mixed snack than after water \( (p = 0.033) \), whereas PFC scores were lower after the mixed snack than after the raisins \( (p = 0.036) \). Desire to eat scores dropped after the caloric snacks at 30 min and were higher at 45 min \( (p = 0.049) \), but they were not affected by treatment. Fullness scores were higher after all snacks than after water \( (p < 0.004) \), and were higher at 30 min and lower at 45 min before the test meal \( (p = 0.017) \). Physical comfort was not affected by treatment \( (p = 0.331) \) or time \( (p = 0.546) \).

Postmeal (75 and 105 min) minus premeal (45 min) average appetite (Table 8), but not desire to eat scores were affected by treatment \( (p = 0.004) \), with higher scores after grapes than after the mixed snack \( (p = 0.003) \) and water \( (p = 0.032) \). Treatment affected hunger \( (p = 0.001) \), with higher scores after grapes than after the mixed snack \( (p = 0.003) \), raisins \( (p = 0.012) \), and water \( (p = 0.005) \). Fullness scores were affected by treatment \( (p = 0.020) \); scores were higher after the mixed snack than after grapes \( (p = 0.044) \). Treatment also affected PFC \( (p = 0.010) \), with lower scores after the mixed snack than after grapes \( (p = 0.014) \) and raisins \( (p = 0.035) \), data not shown for individual scales. Time \( (p = 0.003) \) affected average appetite, desire to eat, hunger, and PFC; scores were reduced by the meal at 75 min and increased toward the end of the session (at 105 min), whereas fullness increased after the meal and decreased toward the end of the session. Physical comfort was not affected by treatment \( (p = 0.830) \) or time \( (p = 0.798) \), data not shown.

### Subjective ratings of pleasantness of the treatments and test meal

Pleasantness of the snacks differed \( (p < 0.001) \), with children rating water \( (56.2 \pm 5.0) \) less pleasant than grapes \( (77.7 \pm 3.2, p < 0.001) \), raisins \( (71.0 \pm 5.3, p = 0.010) \), and the mixed snack \( (71.6 \pm 4.9, p = 0.007) \). However, treatment did not affect pleasantness of the test meal (data not shown).

---

**Table 6.** Experiment 2: Effect of fixed treatment on composition of the snack, pizza intake, cumulative energy intake, and caloric compensation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Water</th>
<th>Grapes</th>
<th>Raisins</th>
<th>Mix</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pizza intake, kcal ( ^{a} )</td>
<td>815±46( ^{a} )</td>
<td>775±40( ^{ab} )</td>
<td>721±39( ^{b} )</td>
<td>748±42( ^{ab} )</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>Cumulative energy intake, kcal ( ^{a} )</td>
<td>815±46( ^{a} )</td>
<td>925±40( ^{ab} )</td>
<td>871±39( ^{b} )</td>
<td>898±42( ^{b} )</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Caloric compensation, % ( ^{a} )</td>
<td>—</td>
<td>27±20</td>
<td>63±21</td>
<td>45±15</td>
<td>0.134</td>
</tr>
</tbody>
</table>

**Note:** Data are means ± SE \((n = 26)\); Mix, a mix of raisins and almonds (children consumed 75 kcal from the raisins and 75 kcal from the almonds).

\( ^{a} \) Mean values within a row with unlike superscript letters were significantly different. One-factor ANOVA for treatment effect followed by Tukey’s post hoc test \((p < 0.05)\).

\( ^{1} \) Energy consumed at the test meal.

**Table 7.** Experiment 2: Effect of fixed treatment on snack-time water intake, test-meal water intake, and cumulative water intake.

<table>
<thead>
<tr>
<th>Water intake</th>
<th>Water</th>
<th>Grapes</th>
<th>Raisins</th>
<th>Mix</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>At snack-time, g ( ^{ab} )</td>
<td>240±26( ^{b} )</td>
<td>230±15( ^{a} )</td>
<td>167±40( ^{ab} )</td>
<td>116±20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>At test meal, g ( ^{a} )</td>
<td>239±58</td>
<td>139±22</td>
<td>161±20</td>
<td>196±18</td>
<td>0.059</td>
</tr>
<tr>
<td>Cumulative, g ( ^{a} )</td>
<td>479±62( ^{a} )</td>
<td>369±32( ^{ab} )</td>
<td>328±45( ^{b} )</td>
<td>312±29( ^{b} )</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Note:** Data are means ± SEM \((n = 26)\); Mix, a mix of raisins and almonds (children consumed 75 kcal from the raisins and 75 kcal from the almonds).

\( ^{a, b} \) Mean values within a row with unlike superscript letters were significantly different. One-factor ANOVA for treatment effect followed by Tukey’s post hoc test \((p < 0.05)\).

\( ^{1} \) Weight of water consumed at snack, including the amount of water in grapes.

\( ^{2} \) Weight of water consumed at the test meal.

\( ^{3} \) Water consumed at the snack + water consumed at the test meal.

---

**Table 8.** Experiment 2: Overall mean change from baseline average appetite scores for the pre- and post-meal periods.

<table>
<thead>
<tr>
<th>Period</th>
<th>Water</th>
<th>Grapes</th>
<th>Raisins</th>
<th>Mix</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premeal (mm) ( ^{a} )</td>
<td>10.7±2.4( ^{a} )</td>
<td>3.4±1.6( ^{a} )</td>
<td>6.3±2.5( ^{ab} )</td>
<td>1.5±2.2( ^{b} )</td>
<td>0.003</td>
</tr>
<tr>
<td>Postmeal (mm) ( ^{a} )</td>
<td>−42.8±3.9( ^{a} )</td>
<td>−34.8±3.8( ^{ab} )</td>
<td>−40.6±4.3( ^{ab} )</td>
<td>−45.2±3.8( ^{a} )</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Note:** Data are means ± SE \((n = 26)\); Mix, a mix of raisins and almonds (children consumed 75 kcal from the raisins and 75 kcal from the almonds).

\( ^{a, b} \) Mean values within a row with unlike superscript letters were significantly different. A 2-factor ANOVA was used, with treatment and time as main factors, followed by Tukey’s post hoc test \((p < 0.05)\).

\( ^{1} \) Premeal values are means of all observations before the test meal (30 and 45 min) minus the baseline means at 0 min.

\( ^{2} \) Postmeal values are means of all observations after the test meal (75 and 105 min) minus the premeal means at 45 min.

---
Association between subjective appetite and FI

Average appetite at 45 min was positively associated with FI at 45 min ($r = 0.47$, $p = 0.016$), and negatively associated with fullness ($r = -0.44$, $p = 0.023$). There was a trend toward a positive association between FI and desire to eat ($r = 0.35$, $p = 0.081$) and hunger ($r = 0.37$, $p = 0.061$), but PFC ($r = 0.22$, $p = 0.286$) was not associated with FI at 45 min.

Association between water intake and FI

Neither test-meal water intake ($r = 0.164$, $p = 0.423$) nor snack-time water intake ($r = -0.063$, $p = 0.758$) was associated with pizza intake.

Association between palatability and FI

Palatability of water ($r = -0.304$, $p = 0.131$), grapes ($r = -0.207$, $p = 0.309$), raisins ($r = 0.250$, $p = 0.218$), and the mixed snack ($r = 0.026$, $p = 0.898$) was not associated with pizza intake. Palatability of water ($r = -0.171$, $p = 0.405$), grapes ($r = -0.102$, $p = 0.619$), raisins ($r = 0.339$, $p = 0.091$), and the mixed snack ($r = 0.036$, $p = 0.863$) was not associated with cumulative energy intake.

Discussion

These results add to the limited data on the effect of incorporating dried fruit into the diet, and suggest that raisins can be recommended as a strategy for increasing fruit consumption. Although they are energy dense, they did not result in increased FI, compared with grapes, but instead resulted in decreased FI. Both ad libitum (Experiment 1) and fixed-calorie (Experiment 2) pre-meal snacks of raisins alone suppressed FI at a pizza meal, compared with the water control, and in Experiment 1 they reduced appetite. Ad libitum intake of raisins also reduced FI, compared with grapes and the mixed snack in Experiment 1. Most important, cumulative [snack (kcal) + meal (kcal)] energy intake after raisins was not different than it was after water, whereas grapes and the mixed snack increased cumulative intake above the water control in both experiments.

The reduction in appetite and later FI after raisins is consistent with recent reports of dried plum consumption. A snack including dried plums (40 g) reduced total energy intake at a later meal more than an isoenergetic control (white bread (70 g) and 30 g of low-fat (10% fat) cheese) in normal-weight individuals (Farajian et al. 2010). Similarly, a fixed snack (238 kcal) of dried plums increased satiety more than low-fat cookies in normal-weight and overweight and obese females (Furchner-Evanson et al. 2010).

Neither appetite nor FI was predicted by the volume or energy density of the snacks. Grapes were lower in energy density (–0.67 kcal·g⁻¹), compared with raisins (–2.99 kcal·g⁻¹) and the mixed snack (–4.41 kcal·g⁻¹), but cumulative energy intake was lowest after raisins. This is consistent with reports showing that energy intake is primary over volume in delineating FI (Gray et al. 2002). In males, FI after a high-volume and high-energy-dense soup preload was lower than after soup of low volume and low energy density, soup of low volume and high energy density, or soup of high volume and low energy density, which did not differ from one another (Gray et al. 2002). Likewise, children consumed less energy at a meal after high-energy-dense beverages sweetened with sucrose or aspartame plus low glucose maltodextrin than after beverages low in energy (water or aspartame) (Birch et al. 1989). Similarly, after a high-energy preload (56 g muffin and a 250 mL orange drink (1628 kJ)), children consumed less than after a low-energy preload (56 g muffin and a 250 mL orange drink (783 kJ)) (Cecil et al. 2005). Furthermore, when the energy density of a food was strongly manipulated by the weight of water, it was not associated with energy intake (Westerterp-Plantenga 2001), which may explain why grapes, despite their high volume and low energy density, did not lower meal-time pizza intake and resulted in increased cumulative FI compared with raisins.

Cumulative energy intake was higher after grapes and mixed snacks than after water in both experiments, showing a lack of complete compensation in meal energy intake for the energy contained in these snacks. This is not surprising because, with the exception of protein snacks (Akhavan et al. 2010), foods with low caloric content (100–300 kcal) eaten before or between meals resulted in increased cumulative energy intake, compared with an equivalent weight of water or beverage with artificial sweeteners (Anderson et al. 2004; Akhavan and Anderson 2007). However, raisins did not lead to higher cumulative intakes when given either ad libitum or in a controlled amount. Thus, raisins may be a preferred snack to help control appetite and prevent excess intake during later meals in children. However, further studies are needed to determine if this effect is sustained when the duration between snacks and meals is longer, which is more representative of snacking behaviour (Johnson and Anderson 2010), and if consumed as a customary snack replacement at other times of the day or on a daily basis.

Macronutrient composition of the snacks did not appear to be a factor in their effect on FI (Experiment 2). The fixed snack of raisins and grapes contained similar amounts of each macronutrient, but less fat and protein than the mixed snack (Table 2), yet both the mixed snack and grapes, but not raisins, resulted in higher cumulative intake. When almonds were added to raisins in both experiments, carbohydrate content decreased and fat and protein content increased; however, like grapes, which are high in carbohydrate (sugars), they did not decrease FI. Similarly, dietary fibre was an unlikely factor because all the snacks had similar amounts.

Differences in water intake (–126 mL in Experiment 1; –188 mL in Experiment 2) during the snack treatments did not explain the effects of the treatment on FI. Snack-time water intake was highest after grapes in Experiment 1 because of the water content of the grapes. However, there was no correlation between water intake after any of the snacks and meal-time energy intake. Although water content in the grapes failed to lower FI or cumulative energy intake, it may explain why the children reported greater subjective discomfort after the pizza meal following grapes; volume and water content of the grapes may have delayed stomach emptying (Westerterp-Plantenga 2001). Total water intake among the caloric treatments was similar throughout the experimental period, with partial compensation at the test meal for the water content of the snacks. However, meal-time energy intake did not correlate with water intake at the meal.

Pre-meal average appetite did not predict changes in FI in either experiment. However, in contrast to previous studies showing increases in appetite and decreases in fullness in children given caloric beverages (Bellissimo et al. 2008a, 2008b; Patel et al. 2011), pre-meal average appetite scores were lower and fullness higher after the solid snacks, with the exception of raisins in Experiment 2. This suggests that when solid, but not liquid, snacks are given prior to a meal, children are able to assess subjective feelings of fullness and hunger. Furthermore, average appetite, desire to eat, and hunger were positively associated with FI at 45 min, and postmeal scores decreased after the pizza meal in both experiments, consistent with other studies in children (Anderson et al. 1989; Bellissimo et al. 2007a, 2008a, 2008b; Patel et al. 2011), suggesting that they understood the VAS. PFC in Experiment 1, but not Experiment 2, was positively associated with FI at 45 min, suggesting that after a fixed snack, this is a weak predictor of FI in children.

Palatability of food affects caloric intake in free-living adults (de Castro et al. 2000) and in laboratory studies (Yeomans et al. 1997), but this was not a factor in our study. Although grapes were rated the most pleasant, compared with all other snacks in Experiment 1, and water was rated the least pleasant in Experiment 2, snack palatability was not correlated with meal-time pizza intake or cumulative energy intake in either experiment (data not shown).
Although this study adds to the limited literature on the potential benefits of snacking in children, there are some limitations to the current experiments. First, because of the short-term nature of this study and the fact that we did not examine the effect of overall daily compensation from the snacks, the results cannot be generalized to longer-term studies or overall daily caloric intake. Second, the snacks we used might not be representative of what children prefer or have access to in the absence of supervision. Third, it is not possible to generalize our results to overweight or obese children, because all children in both experiments were of healthy body weight. However, as with grapes, raisins provide valuable nutrients for children, such as dietary fibre, antioxidants, vitamin C, potassium, and iron (Williamson and Carughi 2010; US Department of Agriculture Nutrient Database for Standard Reference 2011), and their consumption may improve the diet quality in children.

In conclusion, consumption of a premeal snack of raisins, but not grapes or a mix of raisins and almonds, reduces meal-time energy intake and does not lead to increased cumulative energy intake in children.

Acknowledgements

We would like to thank the parents and children enrolled in the study for their participation and the volunteers who assisted in the execution of the study: Balarajan Pragatheesh, Meaghan R. Boddy: Michelle Lai, and Nina Simeunovic. This study was supported by an operating grant from the California Raisin Marketing Board. All authors, except J.E.P, who is the consulting director for research of the California Raisin Marketing Board, declare no conflicts of interest. The funding sources had no input into the design, implementation, analyses, or interpretation of the data.

References


An After-School Snack of Raisins Lowers Cumulative Food Intake in Young Children

Barkha P. Patel, Nick Bellissimo, Bohdan Luhovyy, Lorianne J. Bennett, Evelyn Hurton, James E. Painter, and G. Harvey Anderson

Abstract: Snacks are an important part of children's dietary intake, but the role of dried fruit on energy intake in children is unknown. Therefore, the effect of *ad libitum* consumption of an after-school snack of raisins, grapes, potato chips, and chocolate chip cookies on appetite and energy intake in twenty-six 8- to 11-y-old normal-weight (15th to 85th percentile) children was examined. On 4 separate weekdays, 1 wk apart, children (11 M, 15 F) were given a standardized breakfast, morning snack (apple), and a standardized lunch. After school, children randomly received 1 of 4 *ad libitum* snacks and were instructed to eat until "comfortably full." Appetite was measured before and 15, 30, and 45 min after snack consumption. Children consumed the least calories from raisins and grapes and the most from cookies (*P* < 0.001). However, weight of raisins consumed was similar to potato chips (about 75 g) and lower compared to grapes and cookies (*P* < 0.009). Raisins and grapes led to lower cumulative food intake (breakfast + morning snack + lunch + after-school snack) (*P* < 0.001), while the cookies increased cumulative food intake (*P* < 0.001) compared to the other snacks. Grapes lowered appetite compared to all other snacks (*P* < 0.001) when expressed as a change in appetite per kilocalorie of the snack. *Ad libitum* consumption of raisins has potential as an after-school snack to achieve low snack intake prior to dinner, similar to grapes, compared to potato chips, and cookies in children 8 to 11 y old.

Keywords: children, food intake, raisins, snacking

Practical Application: Children do not consume an adequate amount of fruit and commonly consume snacks that tend to be high in energy and fat, suggesting a need to identify healthy snacks that contribute to nutrient intake, suppress appetite, and reduce caloric intake at later meals. Raisins, the most commonly consumed dried fruit snack, and grapes, may be used to increase fruit intake in children. Results indicate that an after-school snack of raisins, similar to grapes, contributes to lower daily energy intake, making them a nutrient-rich snack for children.

Introduction

Snacks are an important part of children's dietary intake. Although increased consumption of snacks by children and adolescents is suggested to contribute to overweight (Francis and others 2003; Kant 2003; Nicklas and others 2003), a comprehensive review did not find snacking to be causally associated with body weight (Johnson and Anderson 2010).

In addition to providing energy, snacks are necessary for children to meet their nutritional requirements (Sebastian and others 2008; Johnson and Anderson 2010). The time between lunch at school and dinner at home is a critical period in a child's daily nutrient intake (American Academy of Pediatrics 2010). The majority of snacks served in after-school environments are low in nutrient density and high in energy, including those with added sugar (such as cookies) or salty snacks (such as potato chips) (Mozaffarian and others 2010). However, after-school snacking may be an opportunity to offer nutrient-rich snacks, such as fruit, which is normally consumed below recommendations by children (Guenther and others 2006; Garnier 2007).

Nutritional guidelines for after-school programs consistently endorse serving whole fruit and limiting snacks high in sugar, fat, and calories, but currently no guidelines exist for the inclusion of dried fruit as an after-school snack (Beets and others 2011). Whole fruits may be promoted over more energy-dense snacks, despite their low energy density (Rolls 2010), because food volume increases feelings of fullness (Flood-Obbagy and Rolls 2009). Although energy-dense, raisins, the most commonly consumed dried fruit snack, (Keast and others 2011) are a source of dietary fiber (30% soluble), antioxidants, potassium, and iron (US Dept. of Agriculture Nutrient Database for Standard Reference 2007), and dried fruit consumption was associated with improved diet quality and lower body weight in adults participating in the Natl. Health and Nutrition Examination (NHANES) survey of 1999 to 2004 (Keast and others 2011). A study in adults showed that 1 cup of raisins per day, alone or combined with walking, had no effect on body weight, fasting glucose, or insulin (Puglisi and others 2008),
but increased leptin and ghrelin levels after 6 wk (Puglisi and others 2009), suggesting improved balance of appetite-regulating hormones.

In children 8 to 11 y old, premeal snacks of raisins given ad libitum lowered cumulative energy intake at an ad libitum meal 30 min later compared to grapes and a mix of raisins and almonds, and was similar to water. In contrast, grapes and the mixed snack resulted in higher cumulative energy intakes compared to water (Patel and others 2012). These findings support a role for raisins as a satiating premeal snack, but it is unclear how raisins given as an after-school snack may affect energy consumed over a child’s day. No studies have reported the effect of after-school snacking on raisins compared to other commonly consumed snacks on energy intake in children.

Therefore, the objective of this study was to examine appetite and energy intake following ad libitum consumption of an after-school snack of raisins, grapes, potato chips, and chocolate chip cookies in children 8 to 11 y old.

Materials and Methods

Subjects

Normal-weight boys and girls (11 M, 15 F; 8 to 11 y) were recruited by word of mouth and through advertisements in the local newspaper. Baseline characteristics are reported in Table 1. The Univ. Research Ethics Board of Mount Saint Vincent Univ. approved this study.

To participate, children had to be normal weight (between 15th and 85th percentile for age and sex) based on the Centers for Disease Control and Prevention growth charts (Ogden and others 2002), born at full-term and at a normal birth weight. Individuals dieting, taking medication, and with any significant learning, behavioral, or emotional difficulties were excluded to ensure rigorous compliance to all aspects of the study design. Telephone interviews were conducted initially with a parent of the potential participant. When a child met the study requirements, a screening was made at the Applied Human Nutrition Dept., where informed consent was obtained from a parent and assent was obtained from the child. Participants’ heights (m) were measured using a stadiometer and weight (kg) was recorded from a digital scale while subjects wore light clothing. The children were told that the purpose of the study was to examine children’s snack preferences. They were familiarized with visual analogue scale (VAS) questionnaires used in the study and the parent and child were given a tour of the facility in order to minimize apprehension during the 1st test visit.

Study design

A within-subjects repeated measures design was used to measure children’s energy intake and subjective appetite following consumption of 4 ad libitum snacks. On 4 separate weekday afternoons (between 3:30 pm and 4:00 pm), in random order 1 wk apart, children were given grapes, raisins, potato chips, or chocolate chip cookies to consume (within 15 min). A standardized breakfast of Baxters® fat-free skim milk (250 mL, 90 kcal), Cheerios® (28 g, 100 kcal) and Tropicana Orange Juice® (236 mL, 110 kcal), a morning snack (1 medium apple, 72 kcal), and lunch consisting of a turkey sandwich on white bread with lettuce, tomato, mayonnaise, and mustard (369 kcal) and Baxters® 2% milk (250 mL, 130 kcal) were consumed on test days prior to the child’s arrival to the laboratory. The standardized intake was designed to provide 54% and 58% of Health Canada’s estimated energy requirements for each boy and girl, respectively, adjusted for age, body weight, height, and physical activity level (Dietary Reference Intake Tables 2011). The rationale for the controlled intake during the day was to account for variability in energy intakes of the children.

Table 1–Baseline characteristics of test subjects.*

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>All children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>10.1 ± 0.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>33.1 ± 1.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.39 ± 0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.0 ± 0.3</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>51.9 ± 5.3</td>
</tr>
<tr>
<td>DEBQ®</td>
<td>1.6 ± 0.1</td>
</tr>
</tbody>
</table>

*Data are means ± SEM; n = 26; (11 boys and 15 girls).

Dutch eating behavior questionnaire.

Effect of raisins on snack intake . . .

Materials and Methods

Subjects

Normal-weight boys and girls (11 M, 15 F; 8 to 11 y) were recruited by word of mouth and through advertisements in the local newspaper. Baseline characteristics are reported in Table 1. The Univ. Research Ethics Board of Mount Saint Vincent Univ. approved this study.

To participate, children had to be normal weight (between 15th and 85th percentile for age and sex) based on the Centers for Disease Control and Prevention growth charts (Ogden and others 2002), born at full-term and at a normal birth weight. Individuals dieting, taking medication, and with any significant learning, behavioral, or emotional difficulties were excluded to ensure rigorous compliance to all aspects of the study design. Telephone interviews were conducted initially with a parent of the potential participant. When a child met the study requirements, a screening was made at the Applied Human Nutrition Dept., where informed consent was obtained from a parent and assent was obtained from the child. Participants’ heights (m) were measured using a stadiometer and weight (kg) was recorded from a digital scale while subjects wore light clothing. The children were told that the purpose of the study was to examine children’s snack preferences. They were familiarized with visual analogue scale (VAS) questionnaires used in the study and the parent and child were given a tour of the facility in order to minimize apprehension during the 1st test visit.

Study design

A within-subjects repeated measures design was used to measure children’s energy intake and subjective appetite following consumption of 4 ad libitum snacks. On 4 separate weekday afternoons (between 3:30 pm and 4:00 pm), in random order 1 wk apart, children were given grapes, raisins, potato chips, or chocolate chip cookies to consume (within 15 min). A standardized breakfast of Baxters® fat-free skim milk (250 mL, 90 kcal), Cheerios® (28 g, 100 kcal) and Tropicana Orange Juice® (236 mL, 110 kcal), a morning snack (1 medium apple, 72 kcal), and lunch consisting of a turkey sandwich on white bread with lettuce, tomato, mayonnaise, and mustard (369 kcal) and Baxters® 2% milk (250 mL, 130 kcal) were consumed on test days prior to the child’s arrival to the laboratory. The standardized intake was designed to provide 54% and 58% of Health Canada’s estimated energy requirements for each boy and girl, respectively, adjusted for age, body weight, height, and physical activity level (Dietary Reference Intake Tables 2011). The rationale for the controlled intake during the day was to account for variability in energy intakes of the children.

Protocol

Participants arrived at Evaristus Hall, Dept. of Applied Human Nutrition, between 3:30 pm and 4:00 pm, after consuming the standardized breakfast at home and standardized snack and lunch at school, whereby compliance was assessed with a questionnaire. Upon arrival, children completed VAS measuring their subjective appetite and physical comfort (Bellissimo and others 2007a, 2007b, 2008a, 2008b; Bozinski and others 2009; Patel and others 2011; Tamam and others 2012). VAS for pleasantness and sweetness of the snacks were administered immediately after snack consumption. Motivation-to-eat VAS, which measure dimensions of subjective appetite (Stubbs and others 2000), was composed of 4 questions: (1) How strong is your desire-to-eat? (“very weak” to “very strong”); (2) How hungry do you feel? (“not hungry at all” to “as hungry as I’ve ever felt”); (3) How full do you feel? (“not full at all” to “very full”); and (4) How much food do you think you can eat? (prospective food consumption, PFC) (“nothing at all” to “a large amount”). Children were instructed to read each question and place an “x” along the 100-mm line depending on their current feelings. Physical comfort was assessed by “How well do you feel?” (“not well at all” to “very well”). Pleasantness of the snack was assessed by “How pleasant have you found the snack” (“not pleasant at all to very pleasant”), while sweetness of the snack was assessed by “How sweet have you found the snack?” (“not sweet at all to very sweet”), and both VAS were administered immediately after snack consumption.

Participants were escorted into the taste panel room and individually seated in their own cubicle, free of external cues, and served 1 of the 4 ad libitum test snacks, which were assigned in random order. They were also provided with a 500-mL bottle of natural spring water (Nestlé® Pure Life®) and instructed to eat and drink until they were “comfortably full” while seated for a 15-min duration.

The 4 snacks were raisins (California seedless; donated by California Raisin Marketing Board), grapes (Dole; purchased at Sobey’s), potato chips (Lays Classic, Lays Canada), and chocolate chip cookies (Chips Ahoy!, Mr. Christie). Snacks were served in 500-mL clear plastic containers (about 210 kcal) provided as 65 g raisins, 301 g grapes, 45 g cookies, and 38 g potato chips. Participants were initially provided with 1 container and additional containers of snacks were available upon request during the 15-min measurement interval. The amount of snack and water left after 15 min was subtracted from the initial weight to measure snack and water intake. Each snack was weighed separately and the energy consumed (in kcal) was calculated. The macronutrient composition of the snacks was calculated using food composition data.
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Tables from the US Dept. of Agriculture (US Dept. of Agriculture Nutrient Database for Standard Reference 2007). At 15 min, children completed VAS for palatability, sweetness, motivation-to-eat, and physical comfort, which were repeated at 30 and 45 min.

Eating behavior assessment

The Dutch Eating Behaviour Questionnaire was administered to assess restrained eating (van Strien and Oosterveld 2008). Children received assistance in case of difficulty interpreting the questionnaire’s language.

Statistical analyses

Energy intake, water intake, and sweetness and pleasantness of the snacks were analyzed by the MIXED model procedure in SAS 9.2 (SAS Inst. Inc., Carey, N.C., U.S.A.), using a one-way repeated measures analysis of variance (ANOVA). Two-way repeated measures ANOVA using the MIXED model procedure was used to analyze the effects of time, treatment, and their interaction on appetite and physical comfort. Results were pooled for boys and girls since there were no gender differences. Post hoc analysis by the Tukey–Kramer test was performed when treatment effects were found to be statistically significant. An average appetite score was calculated at each time of measurement for each treatment by the formula:

\[
\text{appetite score} = \frac{\text{[desire-to-eat} + \text{hunger} + (100 - \text{fullness}) + PFC]}{4},
\]

which reflects the 4 questions on the motivation-to-eat VAS as used previously (Woodsend and Anderson 2001; Anderson and others 2002; Anderson and Woodsend 2003) and validated by Lluch and others (2010). Cumulative food intake was calculated from the sum of calories consumed from the breakfast, morning snack, lunch, and ad libitum snack.

On the basis of an earlier study investigating raisins as a snack before a meal (Patel and others 2012), a sample size of 26 is powered to see an approximate 135 kcal difference in cumulative intake after the raisins compared to the grapes with a within-subject SD of approximately 180 kcal.

Data are presented as means ± standard error of mean (SEM). Significance was considered at \( P < 0.05 \). Correlations on dependent measures were conducted by use of Pearson’s correlation coefficients.

Results and Discussion

Intake, energy density, and composition of snacks

Treatment affected snack intake (\( P < 0.001 \)), cumulative food intake (\( P < 0.001 \)), and water intake (\( P = 0.003 \), Table 2). Snack intake (kcal) was lowest after raisins and grapes (\( P < 0.001 \)) and highest after cookies (\( P < 0.040 \)), compared with all other snacks. However, children consumed less weight from raisins and potato chips, compared to grapes and cookies (\( P < 0.009 \)), and less weight from cookies compared to grapes (\( P < 0.001 \)). Similarly, cumulative food intake (kcal) was lowest after raisins and grapes (\( P < 0.001 \)) and highest after cookies (\( P < 0.011 \)), compared to all other snacks. There are no other published reports of the effect of dried fruit as snacks on energy intake in children. However, the reduction on cumulative intake after the raisin snack is consistent with a study showing that a preload including dried prunes reduced total energy intake at a later meal compared to an isocaloric and equal weighed bread product preload in normal-weight adults (Farajan and others 2010).

Neither energy density nor volume predicted the effect of the snack on cumulative energy intake. The energy densities of raisins (3.04 kcal/g), chips (5.58 kcal/g), and cookies (4.68 kcal/g) were higher than for grapes (0.69 kcal/g), but grapes and raisins had similar effects on cumulative food intake. These findings are consistent with reports showing that there is a disconnect between preload volume and food intake (Gray and others 2002). In males, food intake following a high-volume and high—energy-dense soup preload was lower compared to after soups of low-volume and low—energy density or low—volume and high—energy density or high—volume and low—energy density, which did not differ from one another (Gray and others 2002). Although energy density of grapes was low, the role of water content is unclear. Water intake was highest after grapes compared with all other snacks (\( P < 0.033 \)) due to the high water content of the grapes. Previous studies show that when water was the dominant component of a food’s energy density, energy intake was not related to the energy density of that food (Westerterp–Plantenga 2001). However, water content in the grapes may have helped to lower snack and cumulative energy intake, similar to a study showing that a low—energy-dense soup preload lowered lunchtime energy intake by women compared to a similar serving of calories from a casserole and casserole with water (Rolls and others 1999). In addition, weight of the snack consumed was not explained by energy content, since children consumed the same weight from raisins and potato chips (Table 2).

Macronutrient composition of the snacks may have been a factor affecting cumulative food intake (Table 3). Sugars regulate physiological satiety signals in normal—weight adult men and children and suppress food intake when given 30 min prior to a meal (Birch and Deysner 1986; Rodin 1990; Anderson and others 2002;
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Figure 1—Absolute scores for average appetite (A), desire-to-eat (B), hunger (C), fullness (D), and prospective food consumption (E).
Bellissimo and others (2008a). The carbohydrate (sugars) content of raisins was associated with an increase in leptin levels after 6 wk of raisin consumption (Puglisi and others 2009). The children consumed similar amounts of carbohydrate, primarily as the sugars glucose and fructose from the grapes (about 43 g carbohydrate/254 g of grapes) and raisins (about 55 g carbohydrate/75 g of raisins), but the greatest amount from the cookies (about 69 g carbohydrates/108 g of cookies) (Table 3), which led to the greatest cumulative food intake compared to the other snacks.

Furthermore, protein, fat and starch, but not dietary fiber, differed among the snacks (Table 3). The amount of potato chips (74 g) and cookies (108 g) consumed had the greatest amounts of protein, fat and starch, while all the snacks had similar amounts of dietary fiber, which is unlikely to have been a factor on food intake. However, the greater amount of calories consumed from the cookies and resultant higher cumulative food intake may be due to its saturated fat content. Snacks with higher fat content have been shown to increase cumulative energy intake in children (Birch and others 1993), and foods high in fat are less satiating than high carbohydrate foods (Holt and others 1995). Children (3 to 16 y) also exhibit a strong preference for fatty, sugary, and savory foods (Cooke and Wardle 2005), and this may have influenced consumption of the snack.

Pleasantness and sweetness of snacks

Palatability is a determinant of children's energy intake (Fisher and Birch 1995; Rasmussen and others 2006; McClain and others 2009). Sweetness and pleasantness differed among snack treatments (P = 0.024, Table 4). Sweetness following consumption of chocolate chip cookies was significantly higher than potato chips (P = 0.016). Raisins (P < 0.001) and grapes (P < 0.045) were rated less pleasant compared to potato chips and cookies, while grapes were rated more pleasant than raisins (P = 0.004). Palatability of grapes (r = 0.087, P = 0.673), potato chips (r = 0.046, P = 0.823), and cookies (r = 0.014, P = 0.945) was not associated with snack intake. However, palatability of raisins (r = 0.527, P = 0.006) was positively associated with snack intake. Overall palatability of the snacks was not associated with average snack intake (r = -0.084, P = 0.683). Sweetness of grapes, (r = -0.067, P = 0.746), raisins (0.323, P = 0.108), potato chips (r = 0.106, P = 0.604), and cookies (r = 0.129, P = 0.530) was not associated with snack intake. Therefore, the inclusion of highly palatable snack foods that children frequently consume (including chips and cookies) (Cooke and Wardle 2005) may have contributed to the relatively lower ratings for raisins.

Subjective appetite scores

In contrast to previous studies showing increases in appetite and decreases in fullness in children after liquid preloads (Bellissimo and others 2008a, 2008b; Patel and others 2011), appetite scores were lower and fullness higher after the solid snacks, irrespective of composition (P < 0.001; Figure 1A). This suggests that children are better able to assess subjective feelings of fullness and hunger after solid foods compared to liquids. For individual appetite scores, snack was not a factor, but desire-to-eat, hunger, and PFC decreased and fullness increased over time (Figure 1B to E). Since children felt similarly full after each treatment despite differences in caloric intake of the snacks, appetite data were reported as the change from baseline per kcal of treatment consumed. When expressed as a change in appetite per kilocalorie of the snack, an effect of treatment was observed for average appetite (P < 0.001; Figure 2), desire-to-eat (P = 0.001; data not shown), hunger (P < 0.001, data not shown), fullness (P = 0.001, data not shown), and PFC (P = 0.002, data not shown). All snacks reduced average appetite, but appetite following consumption of the grapes was lowest during the study measurement period compared with all other snacks, suggesting that grapes increased satiety after the ad libitum snack, possibly due to its water content. This may have implications on later food intake.

Baseline (Time 0) average appetite (r = 0.598, P = 0.001), desire-to-eat (r = 0.399, P = 0.043), hunger (r = 0.447, P = 0.022), and PFC scores (r = 0.671, P < 0.001) were positively, and fullness scores (r = -0.505, P = 0.008) negatively, associated with snack intake. Physical comfort was affected by treatment only (P = 0.037; Figure 1), but post hoc analysis revealed only a trend for lower scores after raisins compared to grapes (P = 0.064).

The effect of after-school snacks on cumulative food intake is important in promoting foods that will enhance satiety and lower caloric intake in children. However, there are some limitations to the current experiment. First, the daily intake to the snack was controlled. This was done to account for any variability in energy intake between children that may have obscured the consumption of the ad libitum snack. Second, it is not possible to generalize our results to overweight/obese children, since all participating children were normal body weight. Third, the snacks used may not represent what children like or to which they have access when a supervisor is absent. Fourth, mealtime energy intake at dinner was not measured due to timing of the afternoon snack interfering with family schedules, since all of the children were brought from school to the department by their parents and were unable to stay.
for a 2nd meal. Thus, we cannot assume that cumulative food intake would remain lowest after grapes. As previously shown, a raisin snack prior to lunch reduced energy intake at a meal given 30 min later and did not increase cumulative energy intake compared to water (Patel and others 2012). Future studies should address how an after-school snack/meal full of vegetables, fruits and dairy as well as whole grains help to control weight and appetite in children (Azadbakht and others 2011).

Conclusions

In addition to promoting satiety (Puglisi and others 2009), raisins provide valuable nutrients for children, such as dietary fiber, antioxidants (Camire and Dougherty 2003), vitamin C, potassium, and iron. Thus, raisins may be recommended to increase fruit intake, but future studies are required to determine if an after-school snack of raisins leads to better management of energy intake in children. Ad libitum consumption of raisins has the potential as an after-school snack to achieve low snack intake prior to dinner, similar to grapes, compared to potato chips and cookies in children 8 to 11 y old.

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