Acute effects of dietary oatmeal on serum levels of N-acyl-phosphatidylethanoleamines and their metabolites.

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Acute effects of dietary oatmeal on serum levels of N-acyl-phosphatidylethanoleamines and their metabolites.

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Table of Contents:

1.0 Background

Obesity and its multitude of comorbidities represent a growing problem in the US and around the world. To date, dietary interventions have been largely unsuccessful in promoting prolonged weight loss, and many patients undergo medical and/or surgical approaches to lose weight and prevent obesity-associated diseases. Improved understanding of biochemical mechanisms in obesity and specific dietary factors that modulate obesity may have profound health benefits for millions of people. N-acyl-phosphatidylethanolamine (NAPEs) and their active metabolites, N-acyl-ethanolamides (NAEs) are lipid satiety factors that are normally biosynthesized in the intestinal tract in response to food intake(1, 2). While NAPEs are endogenously synthesized by mammals in their intestinal tract, many other organisms, including plants, also biosynthesize NAPEs. Reduced levels of NAPEs and NAEs have been found in obese humans and in response to chronic high fat diet(3), suggesting that increasing plasma NAPE and NAEs levels may be beneficial to obese individuals trying to lose weight or to keep off weight gain after losing weight.

The vast majority of studies on effects of diet on NAPE and NAE levels, and of the effects of these compounds on obesity and disease have been performed on rodent models; human studies are lacking. We have convincing evidence (see below in Preliminary Studies) that increasing plasma NAPEs, either by inducing bacteria-mediated NAPE production in the intestines or by direct IP injection, slows weight gain in mouse models of obesity(3). We therefore hypothesize that increasing plasma NAPE and NAE levels may induce satiety in human subjects, preventing weight gain and possibly improving weight loss in obese individuals.

To test this hypothesis, we need safe and reliable means of elevating plasma NAPEs in human subjects. As modulating bacterial expression of NAPEs in humans is not currently feasible, we sought dietary means to increase plasma NAPEs. As detailed below, we evaluated various foods as NAPE and NAE sources, and we found that oat bran has high levels of both. In mice, a single dose of oat bran was sufficient to raise plasma NAPE levels by approximately 50% over two hours. Thus, dietary oatmeal may be a safe and effective way to raise NAPE levels.

2.0 Rationale and Specific Aims

General Approach: In this pilot trial, we will study the effect of typical meal-sized doses of oatmeal on blood levels of NAPEs and their metabolites. Healthy volunteers will be fed a weight-based dose of oatmeal, and serial blood measurements will be collected to determine the subsequent change in serum NAPEs. From preliminary mouse data, we hypothesize that a 0.8g Oatmeal per kg mass (1-2 standard servings) will provide sufficient endogenous NAPE to double serum levels from baseline.

2.1 Rationale and Strategy

Patient population: The current study will help to establish the acute effects of moderate sized servings of oatmeal on serum NAPE levels. If successful, information gained in the current study will be used to study the effect of dietary changes on NAPEs in obese and lean individuals. Thus, we will exclude patients with obesity (BMI>or= 30) or other known metabolic diseases. We will also exclude patients with known gastrointestinal disorders that may affect absorption of nutrients.

Food choice: Based on our labs screen of various foods, we identified oatmeal as a valid source of NAPEs. Our prior studies in mice using synthetic NAPE and with bacteria biosynthesizing NAPE suggest that a dose of store-bought instant oatmeal sufficient to deliver 0.135 mg NAPE per kg body should be sufficient to double plasma NAE levels from baseline. For a 100 kg person, 80 grams of dry regular instant oatmeal (2 servings) provides this 0.135 mg / kg dose. (The dose of dry oatmeal per person = (body weight in kg / 100 kg)* 80 g dry oatmeal. Thus for 50 kg person, 40 g dry oatmeal (1 serving) provides the required dose.) Oatmeal will be served unsweetened only with water to control for confounding effects of any sweeteners or additives.

Study Duration: Our studies in mice show a peak in blood NAPE levels within 90 min following oatmeal consumption. We therefore expect that serial collection over two hours will be sufficient to detect change from baseline.

Choice of measurements: Mass spectroscopy is an efficient and reliable method to measure NAPEs and their metabolites. Our laboratory is experienced in mass spectrometry and has access to all necessary machines and reagents. We will also measure blood glucose in blood samples, as this is easily measurable and may be important clinically in future experiments.

2.2 Specific Aims

Obesity and its associated comorbidities are increasing in prevalence. Dietary or medical interventions to prevent and treat obesity have been only moderately effective and difficult for patients to implement. N-acyl-phosphatidylethanolamine (NAPEs) and their active metabolites, N-acyl-ethanolamides (NAEs) are lipid satiety factors that may play a role in losing weight and in maintaining weight loss.

Overarching Hypothesis: Pharmaceutical and dietary methods to increase NAPE levels will promote satiety and aid in non-surgical weight loss.

Specific Hypothesis: Our preliminary data have shown that oatmeal is rich in endogenous NAPEs, and a relatively modest serving of oatmeal leads to a doubling in NAPE and NAE levels in mice. We hypothesize that similar body-mass based serving of regular oatmeal will lead to a doubling of serum NAPE and NAE levels in humans.

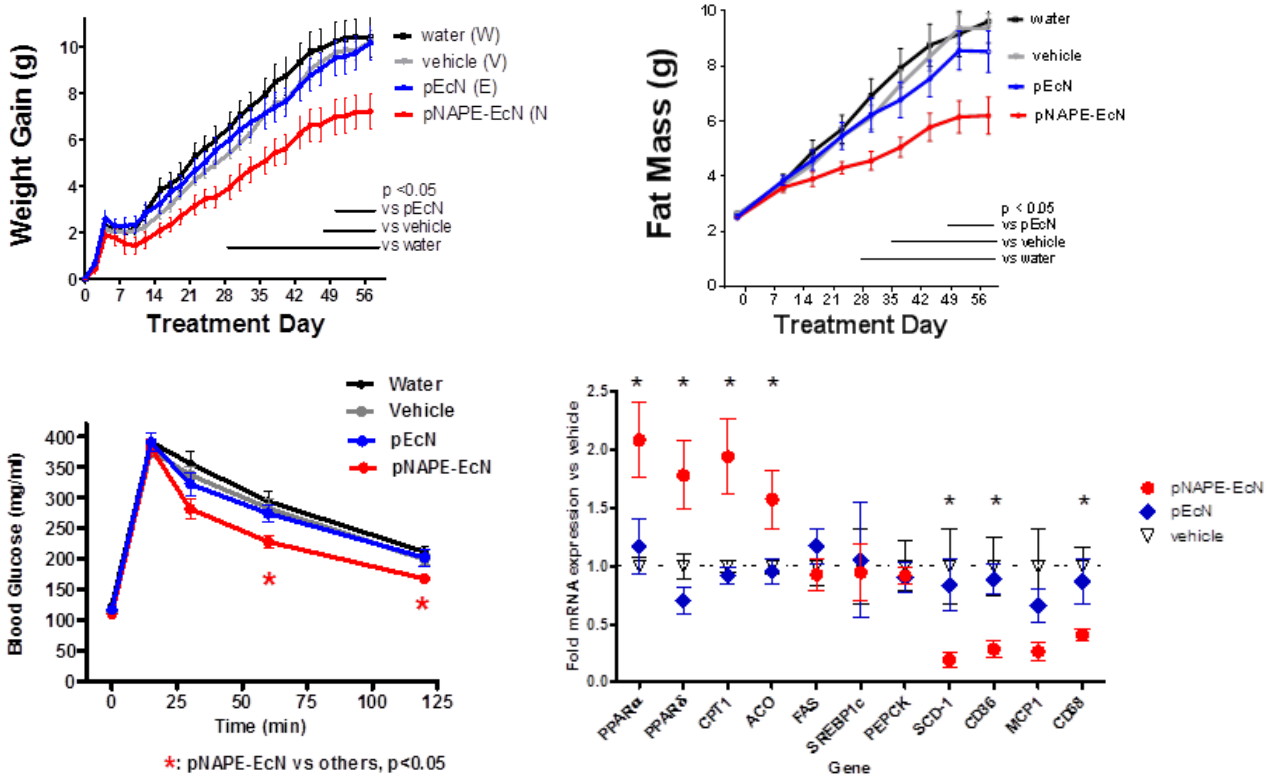
Aim 1: To determine the acute effects of dietary oatmeal on serum levels of NAPEs and NAEs in humans. To meet this aim, we will serve 10 healthy subjects a weight-based serving of oatmeal while drawing blood serially over two hours. Serum NAPE and NAE levels will be measured by mass spectrometry.

3.0 Animal Studies and Previous Human Studies

Our lab has published results showing that administration of E. coli that are engineered to produce NAPEs attenuates high-fat diet induced obesity. We transformed C31-DE3 laboratory strain of E. coli with At1g78690, an *N*-acyltransferase from *Arabidopsis thaliana* that catalyzes the synthesis of NAPEs(4). These bacteria (pNAPE-EcN) were then administered to high-fat diet fed (HFD) C57BL6 mice via drinking water for 8 weeks, during which time food intake, weight gain, and body composition were monitored. pNAPE-EcN administration attenuated weight gain (Fig 1a), decreased fat mass (Fig 1b), and

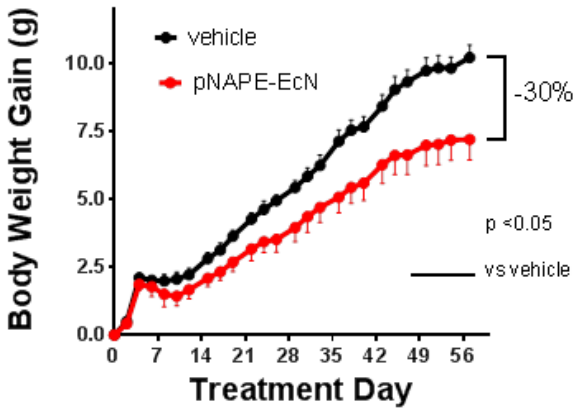
attenuated post-prandial glucose excursions (Fig 1c). It also induced expression of genes encoding for fatty acid oxidation but not fatty acid synthesis and reduced expression of inflammatory genes in the liver (Fig 1d). Furthermore, it decreased serum cholesterol, inhibitied development and necrosis of atherosclerotic lesions, and inhibited development of fatty-liver disease (data not shown). See (5).

Fig 1a-d. pNAPE-EcN attenuates HFD-induced weight gain and fat mass, decreases post prandial blood glucose excursions, and induces expression of genes encoding for fatty acid oxidation but not fatty acid synthesis and reduces expression of inflammatory genes in the liver.



Oral purified NAPE showed similar results with regards to weight gain, though the effects were far less potent than with use of pNAPE-EcN bacteria (Fig 2a vs 2b). We found that levels of NAPEs varies widely between different mouse diets (Fig 3), which led us to hypothesize that different dietary sources may be enriched in NAPEs compared to others. Fig 4 shows varying NAPE concentrations in various natural foods. Based on these results, we chose oatmeal as a potentially effective source of dietary NAPEs. When mice were fed a 0.8g/kg dose of dry oatmeal, we saw a 50% increase in plasma NAPE over the subsequent 2 hours, whereas feeding similar caloric amounts of applesauce had no such effect (Fig 5).

Fig 2a-b. Oral NAPE is less potent than pNAPE-EcN, even at high doses (200mg/kg).



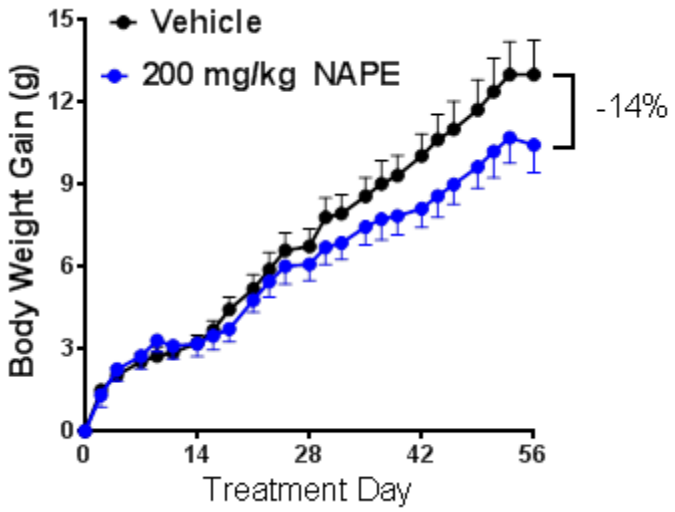


Fig 3. NAPE levels vary widely by mouse diet.

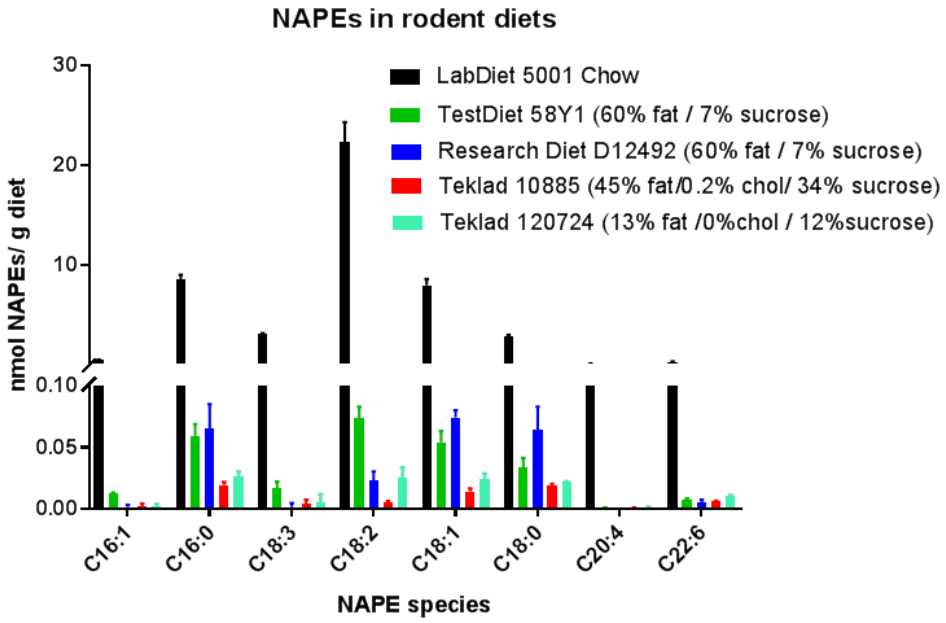


Fig 4. NAPE content of various foods.

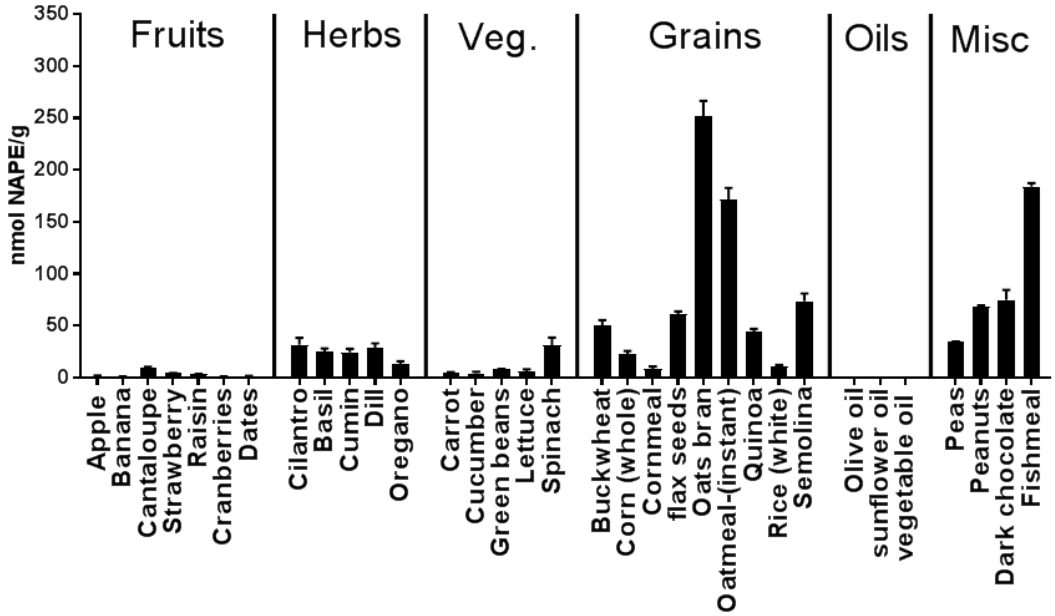
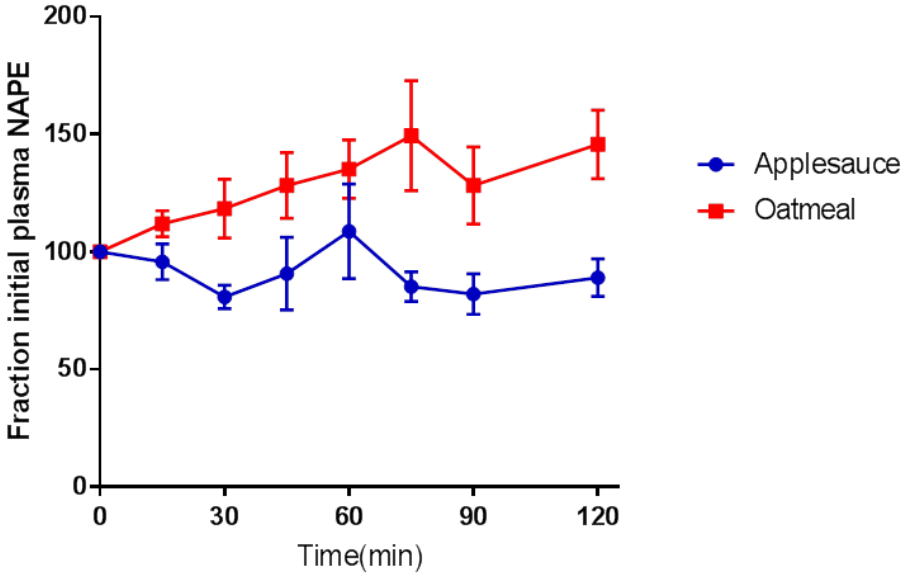


Fig 5. Effect of dietary oatmeal on plasma NAPE in mice.



4. Inclusion/Exclusion Criteria

There is no known effect of age, sex, or race on NAPE absorption, so we will enroll any interested patient who meets the following criteria:

Inclusion Criteria	Exclusion Criteria
• Age: ≥ 18 years • *BMI: 20-30 kg/m^2*	• BMI>30 or <20 • History of type 1 or 2 diabetes • History of coronary artery disease • History of oatmeal allergies • History of irritable bowel syndrome • History of inflammatory bowel disease • History of celiac disease • History of hyper/hypo-coagulability • History of food intolerances • History of malabsorption

5.0 Screening and Enrollment

Study participants will be recruited by word of mouth among colleagues and acquaintances of the study investigators. Given the low number of subjects, we anticipate that this will be sufficient. Subjects will meet initially with Dr. Wright for a medical history and physical to determine eligibility.

6.0 Study Procedures

Participants will present after overnight fast to the CRC. They will have IV catheter inserted and baseline blood obtained (5ml). They will then be fed the above calculated dose of oatmeal, and blood will be drawn at 30, 60, 90, and 120 minutes (5ml each time). Blood glucose will be measured at each timepoint, and remaining blood will be taken to Dr. Davies' lab for mass spectrometric analysis of NAPEs and NAEs. All samples and data will be deidentified at time of collection for participant confidentiality.
Measures:

- -Bloodwork:
    - Glucose: POC glucose will be measured using clinical glucometer at time of blood sampling.
    - Serum NAPE and NAE levels: Samples will be centrifuged and serum NAPE and NAE levels measured by mass spectrometry in the Davies laboratory.
- Anthropometrics:
    - Height (m) and Weight (kg) measured on calibrated digital equipment will be used to calculate BMI.

7.0 Risks

- The participant will be required not to eat or drink, except water, after 10 pm on the night before testing visit. This may cause the participant to have a headache or a feeling of weakness, or the participant may become irritable.
- Blood draw: Blood draws can cause redness, soreness, bleeding or bruising at the needle stick site. The CRC nurses will be careful and use sterile technique. Sometimes people feel faint. The nurse may put some cream (called EMLA) on the skin to numb the area so the participant will not feel the needle stick as much. The numbing cream may make the skin change color, but this is rare.
- Patients may be allergic/intolerant to oatmeal or trace contaminants in oatmeal product.
- Risks that are not known: There are no known risks beyond those mentioned above. If new information is discovered that may affect the risks or benefits of this study, the participant will be told so that he/she can decide whether or not to remain in the study.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

All serious and unanticipated adverse events or problems involving risks to subjects that may possibly be or are known to be related to the research activity will be reported promptly to the IRB office per Vanderbilt University IRB Policy. The PI will ensure proper data and safety monitoring for the materials and data used for the purposes of this study.

9.0 Study Withdrawal/Discontinuation

Subjects may choose not to participate in this research study. This decision will not alter the routine care administered by the physician or the risks associated with standard of care procedures. Additionally, the participant will be removed from this study if the investigator does not think it is in best interest for the subject to be in this study. If the participant is removed from the study he/she will be told the reason why.

10.0 Statistical Considerations

Serum NAPE and NAE levels will be compared with each individuals baseline level, with statistical significance of mean increase over baseline determined by students t test.

11.0 Privacy/Confidentiality issues

All efforts, within reason, will be made to keep personal information in the participant's research record confidential. Careful safeguards are in place, and confidentiality will be maintained by coding data and blood samples using only a number to identify the data. The number assigned will be specific to this study and will not be related to other personal identifiers such as medical record number, telephone number, social security number or initials. The identification number will only be known to the study staff. The record linking the study number with the participant's name will be maintained by Dr. Wright, Dr. Davies and the study team. It will be kept in a locked research office and in a locked file cabinet. Computer data will be password-protected.

The Sponsor and/or Vanderbilt may share the participant's information, without identifiers, to others or use it for other research projects not listed in this form. The Sponsor, Vanderbilt, Dr. Wright, Dr. Davies and the study team will comply with any and all laws regarding the privacy of such information. There are no plans to pay the participant for the use or transfer of this de-identified information.

12.0 Follow-up and Record Retention

Follow-Up: Since this study only requires a single visit to the clinical research center, no further follow-up will be required.

Record Retention: The study results will be kept in the participant's research record for at least six years after the study is finished. No research data will be put in the subjects medical record.

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