OREGON HEALTH & SCIENCE UNIVERSITY

Food photography method to measure dietary intake in healthy controls and subjects with urea cycle disorders

By

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SPECIFIC AIMS

Significance

Diet assessment is an essential component of medical nutrition therapy (MNT). However, current diet assessment methods, including 3-day diet records or multi-pass 24-hour recalls, have proved to be unreliable at gathering accurate dietary intake data for subjects in free-living environments. Without extensive training, subjects often fail to estimate correct portion sizes or provide incomplete or illegible records. For individuals with intellectual disabilities, keeping detailed dietary intake information can be especially burdensome. These inconsistencies can sometimes result in physiologically implausible estimations of the number of calories or nutrients consumed. In many instances, estimations differ widely from actual intake. Inaccurate dietary information can change research conclusions, impact providers' Medical Nutrition Therapy (MNT) interventions, and may negatively affect subjects' health, particularly those with strict dietary limitations due to an inherited metabolic disorder.

Successful management of metabolic disorders often involves diet prescriptions that limit the intake of particular nutrients. Documentation of current dietary intake relative to biochemical measures of metabolites assists providers in determining an appropriate diet prescription and helps patients implement recommendations. Accurate dietary intake records can prevent adverse effects by ensuring individuals with metabolic disorders are not over-consuming specific foods leading to metabolic sequelae of their disorder or under-consuming specific foods leading to nutrient deficiencies. For some disorders, overconsumption of specific nutrients could lead to permanent neurological damage, while inadequate intake of other nutrients could impair growth, development, and health maintenance.

Restriction of dietary protein is a key intervention in the treatment of Urea Cycle Disorders (UCDs). Limited protein consumption minimizes the risk of hyperammonemia and its neurological consequences. However, restriction of high protein foods such as meat and dairy increases the risk for micronutrient deficiencies, especially calcium, vitamin D, vitamin B12, and iron. A more reliable dietary assessment method would improve the ability to accurately measure protein intake in individuals with UCDs and provide insights on current micronutrient intake levels. With greater confidence, recommendations could be appropriately tailored to restrict protein consumption, increase intake of micronutrients, or indicate whether vitamin and mineral supplements are needed.

Digital food photography is a promising method of dietary assessment for individuals with UCDs. This method eliminates the need for participants to estimate portion sizes, captures all components of a meal eaten, and decreases the burden of food documentation for subjects and their caregivers. With the incorporation of photos sent to researchers in real-time, this method minimizes any errors due to inaccurate recollection. Subjects can recall and relay details of meals within hours of photo capture. As a result, researchers are supplied with complete dietary intake information, assisting in nutrient quantification. A team of researchers at OHSU is currently collecting information using a novel food photography method in patients with UCDs. This thesis includes a control data set for comparison.

Study Objective

This study will compare a novel food photography method against traditional 3-day pen and paper diet records for 4 healthy individuals and 3 individuals with a UCD. This study aims to compare dietary intake data from healthy control subjects and individuals with UCDs. Results from this study will aid in the development of a larger study comparing the food photography method against 3-day diet records among individuals with UCDs.

We have modified a food photography application previously developed by bioengineers at OHSU for the type 1 diabetes population and renamed the modified version mFood. Subjects will participate in the study for 2 weeks, during which they will complete a traditional 3-day diet record and document food intakes using the mFood app. We will then compare estimated average nutrient intakes from the mFood app and 3-day diet records. Average micronutrient intakes of calcium, vitamin D, vitamin B12, and iron from the two methods will be compared to Dietary Reference Intakes (DRI), Additionally, protein intake in healthy controls will be compared to the DRI, while for subjects with UCDs, protein intake will be compared to individual protein prescriptions. This will provide insight into how nutrient consumption estimated with each method (3-day diet record versus food photography) compares to recommended or prescribed intakes. Finally, we will compare all data gathered from healthy controls to data gathered from individuals with UCDs and evaluate any differences. This study will allow us to:

1. Specific Aim 1: To compare estimated protein intakes from 3-day diet records and mFood application data to the DRI for protein for healthy control subjects and protein prescriptions for subjects with UCDs.

   Hypothesis: The food photography method will show estimated protein intakes closer to the DRI for protein (for healthy controls) or protein prescriptions (for subjects with UCDs) than 3-day diet records.

2. Specific Aim 2: To compare estimated micronutrient intakes of calcium, vitamin D, vitamin B12, and iron from 3-day diet records and mFood application data to micronutrient RDAs for healthy control subjects and individuals with UCDs.

   Hypothesis: The novel food photography method will show estimated intakes of calcium, vitamin D, vitamin B12, and iron closer to micronutrient RDAs than 3-day diet records for both healthy controls and individuals with UCDs.

This study proposes to evaluate a novel method using food photography by comparing it to traditional dietary intake evaluation methods. Information from this study can guide the implementation of the novel food photography method in special populations where accurate dietary intake records are crucial.

BACKGROUND

Urea Cycle Disorders

Urea cycle disorders (UCDs) are rare inherited metabolic diseases that result in deficiencies in one or more enzymes that make up the urea cycle. These enzymes include carbamoyl phosphate synthetase 1 (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), and arginase 1 (ARG1). Transporter proteins, including the mitochondrial ornithine-citrulline antiporter (ORNT1) and the CPS1-activating enzyme N-acetyl glutamate synthase (NAGS), are also essential to the urea cycle. The urea cycle is responsible for converting toxic ammonia, a byproduct of protein catabolism, into urea, which can be excreted from the body. Without the urea cycle to control ammonia levels in the body, hyperammonemia can occur. Hyperammonemia can lead to cerebral edema and has many neurological consequences, including lethargy, decreased appetite, vomiting, hyperventilation, hypothermia, ataxia, disordered sleep, confusion, irritability, delusions, and neurologic posturing. Patients with untreated UCDs can experience seizures, become comatose and die.[1] All UCDs, except OTC enzyme deficiencies, are autosomal recessive inborn errors of metabolism, and the incidence of UCDs is around 1 in 35,000 births.[2] UCDs can be diagnosed using prenatal or neonatal screening tests or can be identified due to neurological symptoms presenting during infancy to later into adulthood.[1] Those with early onset of the disease experience hyperammonemia in the first 28 days of life. These individuals have a high risk of mortality, and those who survive are likely to experience neurological defects. [3] One study observed that out of 26 children who had survived early-onset urea cycle disorders, 79% had mental retardation, 46% had cerebral palsy, 4% were blind, 17% had epilepsy, and 46% had multiple other neurodevelopmental disabilities.[4] Those with late-onset usually have partial enzyme deficiencies and experience less severe neurological symptoms. [3,5]

The current dietary treatment for UCDs is dietary protein restriction and supplementation of essential amino acids (EAA), vitamins, and minerals. Providers give protein recommendations based on patient age, diagnosis, and clinical status. Protein levels begin as a combination of protein from intact, natural sources and from formulas with essential amino acids. THE FAO/WHO/UNU 2007 "safe levels of protein intake" can be used to guide energy and protein intake recommendations. Recommended protein intake can then be adjusted based on growth, ammonia levels, and plasma amino acids. UCDs range in severity; individuals may have variations in tolerance to dietary protein. Therefore, the degree of protein restriction is personalized for each individual to prevent protein deficiency and body protein catabolism while ensuring adequate growth and development.[6]

EAA supplementation is essential when tolerance of protein from foods and natural supplements is too low to ensure subjects have normal growth and metabolic activity. One approach is to provide 20-30% of total protein as EAA supplements, divided over 3-4 main meals.[7] For those with ASS and ASL enzyme deficiencies, treatment includes supplementation of L-arginine. For those with CPS1 or OTC enzyme deficiencies, L-citrulline is supplemented.[8] Individuals with UCDs are also at risk for iron, zinc, copper, calcium, and cobalamin deficiencies, and these micronutrients may also need to be supplemented. Adequate energy intake is also necessary to avoid catabolism and hyperammonemia. Individuals with higher mobility will have higher energy requirements. Long fasting periods should also be avoided, and bedtime snacks are often used to prevent overnight catabolism.[6]

Accurate dietary intake records are important in managing UCDs for a number of reasons. Since protein recommendations and tolerance can vary between individuals and through different life stages, accurate dietary intake monitoring is critical to prevent under or overconsumption of protein and adequate energy intake. They can also assist providers in determining how to adjust protein recommendations. Finally, reliable dietary intake records can assist researchers in drawing valid conclusions about treatment strategies for UCDs.

Measurement Errors Associated with Dietary Assessment

Accurately measuring dietary intake has been challenging for researchers, as all dietary assessment tools are subject to random and systematic measurement errors. Random errors are a result of variability in dietary intake. Subjects may have differences in day-to-day dietary intake, especially when comparing weekdays to weekends. Systematic errors occur when the parameter being measured is consistently different than the actual value. Systematic errors in dietary assessments can be a result of self-reported dietary data. For example, subjects may underreport food that they see as "unhealthy" but over-report foods that they see as "healthy." [9]

Additionally, subjects may have difficulty accurately reporting portion sizes. Studies have found that subjects better estimate portion sizes for foods with defined shapes, such as potatoes or cucumber slices, but overestimate portion sizes for amorphously shaped foods, such as curries or stews.[10] Systematic errors also may occur as a result of interviewer bias. Asking probing questions about foods consumed may lead subjects to recall dietary intake inaccurately.[9] While there are techniques for minimizing the errors associated with dietary assessment methods, it is impossible to completely eliminate the risk of these errors occurring.

Common Methods for Measuring Dietary Intake

One method for measuring dietary intake is the 24-hour dietary recall. For this method, the subject recounts all foods and beverages consumed for 24 hours. The subject can complete the recall themselves, or the recall can be led by a trained interviewer. A limitation of this method is that it relies on subject memory, leading to recall bias. Recall bias can result in oversights in foods or amounts of food eaten, and this method can be especially troublesome in UCD patients with cognitive impairments. 24-hour recalls also do not capture day-to-day variation. When researchers use this type of dietary assessment method, they often use multiple recalls to identify trends in dietary intake. However, multiple recalls can be burdensome on both subjects and researchers.[11]

Another dietary assessment method is the food frequency questionnaire. This tool collects data on the frequency, and sometimes portion size, of foods and beverages consumed over a month to a year. Some limitations are that the questionnaire may not list all foods consumed by the subject, leading to missing or inaccurate data. Longer questionnaires listing a greater number of foods can also decrease a participant's food intake recall accuracy. Again, in patients with cognitive impairment, this method may not accurately measure dietary intake.[11]

The most commonly used dietary assessment method for individuals with urea cycle disorders is a food record. This method collects data on foods and beverages consumed over multiple days. It is self-administered and usually completed at home during or after mealtimes. Some limitations to this method are that data quality may decrease as the number of days reported increases. Food records are also subject to reactivity. Self-administration can result in variations between actual and reported intake. Reasons for this include the inability to estimate portion sizes accurately, forgetting to document foods or embarrassment of foods or amounts eaten.[11]

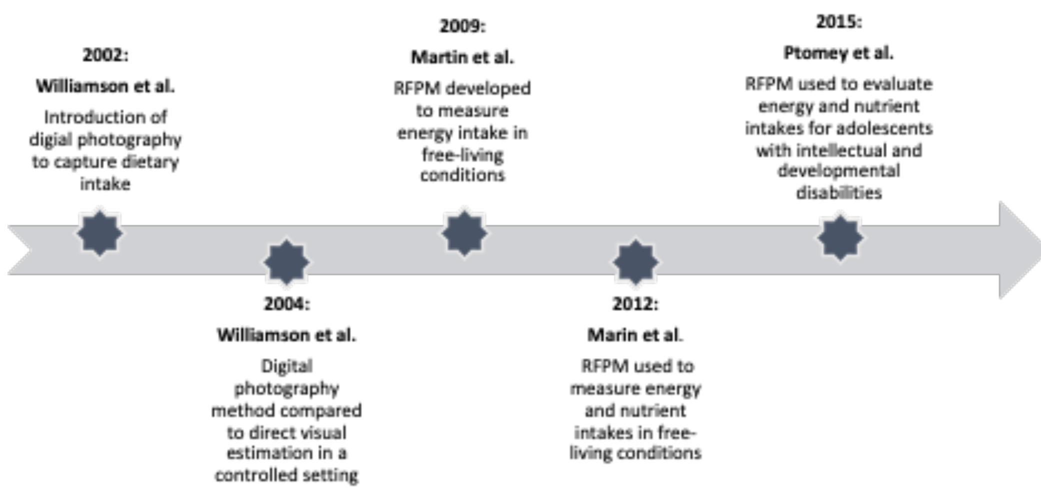
Another method of measuring dietary intake is the weighed food record. This method is often utilized when subjects consume food under observation because it requires the subject or researcher to weigh all food before consumption and plate waste after a meal. This method can be very effective in determining food and nutrient intake because it eliminates the estimation of portion sizes and reliance on subject recall. This method can also take into account preparation methods' effects on nutrients in meals. However, significant training is needed to reduce errors in intake estimation. Additionally, weighed food records can be very intrusive for subjects in free-living conditions because subjects are more aware of how much they are eating at the time of the meal. This could cause a subject to consume more or less, leading to inaccuracies in "usual" dietary intake.[12]

The gold standard for validating different measures of energy intake in free-living individuals is doubly labeled water (DLW). This method measures total daily energy expenditure, which is equivalent to energy intake during a period of energy balance. Total daily energy expenditure is estimated based on the rate of elimination of CO_2 from the body after a subject is orally dosed with water that contains stable isotopes of deuterium (H^2) and oxygen-18 (O^{18}).[13] This method is simple, non-invasive, and highly effective, making it the preferred method for accurately measuring energy expenditure. However, there are limitations to this method. The isotopes used are extremely expensive, and sophisticated technology is needed to complete the measurement. This makes the entire process expensive and limited to institutions that possess the necessary equipment. This method of validation also assumes that energy expenditure is equal to intake and the subject is in energy balance. If a subject's weight fluctuates, energy expenditure may not reflect energy intake. Additionally, because of growth in children, dietary intake may not necessarily equal energy expenditure. Finally, this method does not provide information on intakes of specific micro and macronutrients, such as protein, in foods consumed. Therefore, the utility of DLW is primarily to validate other methods for complete capture of total energy intake rather than as an independent method to measure dietary intake on its own.[14,15]

For individuals with urea cycle disorders, detailed and precise documentation of dietary intake is essential for the treatment and prevention of hyperammonemia. This population would highly benefit from a more appropriate and accurate way to document dietary intake.

Using Digital Photography to Measure Dietary Intake

The newest advancement in dietary assessment tools is the integration of digital photography. Digital photographs allow for real-time documentation of mealtimes, reducing errors from recall bias. This method is less intrusive compared to traditional recall methods because participants can document multiple foods and portion sizes in one photo. Additionally, food documentation is not limited to food lists, and participants can identify all foods consumed in real-time. While this method may require participants to provide additional information regarding foods shown in photographs, it is overall less burdensome. Digital photographs can reduce errors in portion size estimation because portion sizes can be predicted by a qualified team or a Registered Dietitian instead of the subject or caregiver. In addition to documenting food intake, digital photographs also allow participants to document mealtimes and settings, which can provide additional information to aid in dietary assessments.[16]



Williamson et al. first introduced the use of digital photography as a method to assess dietary intake in 2002. In this study, the aim was to evaluate changes in food selections, food intake, and body weight in U.S. soldiers. Digital photographs were integrated to minimize the impact of the study on usual eating behaviors, and photos were taken using a digital video camera connected to a computer. The research team took photographs of food trays before and after subjects ate to measure food selections and food intake. Registered dietitians became familiar with recipes and cooking techniques for each item that was available to subjects on the menu. Registered dietitians compared reference photographs of standard portion sizes to photographs of subjects' trays. Three research associates trained on using the visual estimation method for assessing dietary intake assisted in classifying standard portion sizes for foods; however, no other efforts were made to assess the validity of using this method to assess dietary intake. They did find that there was good reliability for estimating dietary intake between registered dietitians who classified food types and portion sizes; however, more evidence was needed to determine the validity of these estimations.[17]

In 2004, this same group assessed the validity of the digital photography method by comparing it to the direct visual estimation method for estimating food intake. They noted that up to this point, weighed food records were the most accurate method for measuring food intake; however, this method had proven to be time-consuming, costly, and intrusive. Food items with ten different portion sizes were prepared in a cafeteria. Reference foods in typical portion sizes were weighed and used as reference foods for both the direct visual estimation and digital photography method. Research associates were trained to use either the visual examination method or the digital photography method. They found that both methods slightly overestimated food intake, but, overall, both methods were highly correlated with weighed foods.[18] The digital photography method proved to be a reliable and valid tool for estimating food intake for subjects in a controlled setting.

Martin et al. developed the remote food photography method (RFPM) in 2009 to determine the use of the digital photography method in free-living populations. The RFPM uses camera-enabled phones to take and send food photos, which allows subjects to take photos of their foods and send them to the research team in real-time. This contrasts with previous studies by Williamson et al., which used a trained team and standard camera equipment to take photos. Two pilot studies developed methods that were used in the main study to investigate the reliability and validity of using the RFPM. In pilot study 2, participants occasionally neglected to take photos of their food at mealtimes. This information could not be obtained until the camera-enabled devices were returned at the end of the study. In response, researchers adopted ecological momentary assessment (EMA) technology, which reminded participants to take photos of their food. EMA technology sends automated reminders and messages to participants. This ultimately minimizes bias from prospective recall because missing data is easily recognized and corrected quickly because food images are sent in real-time. These findings guided the development of the RFPM.[15]

In the main study by Martin et al., participants used the RFPM to take photos of foods selected, plate waste, and food labels. They were also asked to respond to four to six EMA prompts that reminded them to send food photos to researchers. In addition, subjects recorded meals using traditional pen-and-paper methods in case of technology failure. Weighed energy intake acted as an objective measure of dietary intake, and it was measured in a laboratory and natural environments with pre-weighed food. The main difficulties in obtaining data from these methods were technical errors, poor-quality photos, missing photos, and protocol violations, which included subjects not eating the food provided. However, it was found that the RFPM was reliable over the three days of testing and provided reliable estimates of energy intake in both laboratory and free-living conditions. After statistical analysis, energy intake estimated with the RFPM highly correlated with weighed energy intake for dine-in laboratory meals, take-out laboratory meals, and meals eaten in free-living conditions. For participants who ate meals in the laboratory and in free-living conditions, the RFPM significantly underestimated energy intake. They also found that there was not a significant association between body weight and the RFPM method, suggesting this method was not subject to weight bias. While there was some underestimation error, it was very small compared to the large errors associated with self-reported dietary intake methods. 93.6% of participants also stated that they would rather use the RFPM method than traditional pen-and-paper methods.[15]

The RFPM was used again in 2012 by Marin et al. to estimate both energy and nutrient intakes. In addition to analyzing the accuracy of the RFPM, the team investigated the best EMA method for increasing data quality among participants. In this study, EMA was integrated with the RFPM through email & text prompts sent to participants, reminding them to input data. It was found that sending customized prompts to participants around individual mealtimes increased the accuracy of the RFPM when compared to standard prompts sent to all participants at standardized times of the day. Participants were required to use the RFPM for one week in free-living conditions. Total energy expenditure via doubly labeled water was used as an objective measure to assess the validity of the RFPM in free-living settings. They found that in free-living conditions, the RFPM underestimated energy intake by only 3.7% when compared to total energy intake by doubly labeled water. It was also concluded that the RFPM did not lead to significant under or overeating. As another measure of the accuracy of the RFPM in a controlled setting, laboratory buffet meals were used to measure energy and nutrient intakes directly. Foods were weighed for an objective measure of intake. Estimated intake did not differ significantly between weighed foods and the RFPM. There were no significant differences in recorded protein, carbohydrate, fat, vitamin c, calcium, sodium, or fiber for weighed foods compared to the RFPM. However, the RFPM significantly overestimated measurements of vitamin A and cholesterol.[19] The findings of this study give evidence of the applicability of using the RFPM to estimate both energy and macronutrient intakes in cognitively functioning adults. Therefore, dietary assessment using digital photography is an appropriate method for estimating protein intake and applies to this study's aims.

Since subjects in our study may have cognitive impairments, it was necessary to investigate if the RFPM would be applicable when used by this population. In 2015, Ptomey et al. evaluated the use of digital photography to estimate dietary intake in adolescents with intellectual and developmental disabilities. This study assessed the ability of digital photographs to improve estimations of energy and macronutrient intake compared to traditional 3-day diet records. For this study, twenty adolescents ages 11 to 18 were given mobile devices with a camera and taught how to capture photos. They were instructed to take photos of food and beverages before and after consumption for three days without help from a parent or guardian. Participants also completed 3-day pen-and-paper diet records with help from a parent or guardian if necessary. A registered dietitian first reviewed the paper proxy-assisted diet records and estimated portion sizes of foods using a portion guide. Then, they separately reviewed images taken by participants to obtain additional information on the foods and beverages consumed. The most common difference between the proxy-assisted diet records and photos was incorrect estimations of portion sizes from participants and their proxies. Compared to 3-day paper diet records, photo-assisted diet records showed significantly higher estimated energy, protein, carbohydrate, and fat intakes. All macronutrients were over-reported in equal percentages of total energy intake. Even proxy-assisted food records may underreport the amounts of foods actually consumed by adolescents with intellectual and developmental disabilities. While this population needed assistance with traditional paper 3-day diet records, adolescents with intellectual and developmental disabilities were able to capture images of their food at all mealtimes using the digital photograph method without the help of a parent or guardian.[20] The literature supports the ability of our subjects to use the RFPM to capture dietary intake. It also indicates that the RFPM may lead to more accurate dietary data, even when a parent or guardian is not providing assistance.

Previous studies have demonstrated the applicability of integrating digital photographs into diet assessment methods, but this has not been previously investigated among individuals with UCDs. Digital photographs may improve the accuracy of both energy and nutrient intake data compared to traditional methods. As previously discussed, inadequate or excessive protein intake can negatively affect this population, while adequate calories are essential for healthy growth and development. The digital photograph dietary assessment method can assist patients and providers in closely monitoring intakes of dietary protein. This method may also be less intrusive and burdensome to this population, who frequently monitor their dietary intake. Previous studies have shown that the digital photography method can allow these individuals to easily document food intake in free-living conditions for later interpretation by a trained professional. Finally, this method can be used to improve dietary intake data in adolescents and adults, as previous studies have shown successful results in both age groups. Overall, the data is promising regarding the potential advantages of digital photography in assessing protein and energy intake in our population of interest.

METHODOLOGY

STUDY DESIGN

This is a crossover study designed to collect and compare dietary intake information from a traditional method, a 3-day food diary, and our mFood app in healthy normal control adults and adults with UCDs. We compared grams of protein and micronutrient levels of calcium, vitamin D, vitamin B12, and iron with the two methods. For this study, 4 healthy adult subjects and 3 individuals with UCDs were recruited. Each subject participated in one virtual or in-person training session and six days of dietary intake data collection in their free-living environments. Participants documented food intake via 3-day diet records and the mFood digital food photography app. Dietary intake was recorded for three consecutive days: two weekdays and one weekend day. Intake documentation for the 3-day diet records was on the same days of the week as the mFood app documentation, exactly 1 week apart. Data from 3-day diet records and the mFood application were then compared. Finally, data from both methods in healthy controls and individuals with UCDs were also compared.

STUDY POPULATION

Recruitment

We recruited 4 healthy adult subjects and 3 individuals with UCDs to participate in this study. Individuals with UCDs were recruited from the UCDC longitudinal study and were trained at Children's National Hospital in Washington D.C. Healthy controls were recruited from the general population and trained at OHSU in Portland, Oregon. Interested participants contacted the investigator or study coordinator and were sent a packet of information which included a cover letter and a consent form prior to their initial study visit.

Inclusion and Exclusion Criteria

For subjects to be included in the study, they must own a personal smartphone, be at least 18 years old, and have a rudimentary understanding of English for instructions and commands on the mFood app. Healthy subjects were excluded if they had any serious acute or chronic medical condition that may interfere with the interpretation of study results. Healthy subjects were also excluded if they were following a special diet or other dietary intervention.

STUDY MEASUREMENTS AND PROCEDURES

Digital Food Photography Application

A group of bioengineers at OHSU have recently developed a phone application utilizing the RFPM to measure dietary intake in individuals with type 1 diabetes. The engineer that developed the application and our team have modified it to be applicable to our study's objectives and named the modified version mFood. Subjects used this application to capture photos of all meals and snacks consumed for 2 weekdays and 1 weekend in their free-living environment. The application also allows subjects to add descriptions of the foods consumed, including ingredients, restaurants, brand names, and formula types. Subjects also included a standardized card in all photos to assist the research team in estimating portion sizes. The mFood app was programmed to send out notifications to subjects around personalized mealtimes, reminding subjects to capture photos of foods and beverages consumed. If a subject forgot to take a photo of a meal, they had the option to manually enter a description of what they ate for the mealtime forgotten. Photos were sent in real-time to a research server and accessed by the study team daily. Messages via email were then sent to subjects for further clarification on foods eaten if needed.

Three Day Diet Records

Subjects were given a traditional, pen and paper 3-day diet record to complete for 2 weekdays and 1 weekend day and were asked to document all foods and beverages consumed. Subjects returned the diet record via email or mail. Subjects were provided a pre-addressed stamped envelope if they wished to return the record via mail. Once returned, 3-day diet records were reviewed for completeness, and a study coordinator reached out to the subject if more clarification was needed on brand names, portion sizes, ingredients, restaurants, etc.

Study Encounters

Healthy subjects had one virtual encounter for consenting and training through Oregon Health and Science University. Individuals with UCDs had an in-person encounter at Children's National Hospital in Washington, D.C. At this encounter, a team member gathered height and weight data. They also trained subjects on using the mFood app and completing 3-day diet records. A team member instructed all subjects to program the mFood application on their phone to send reminder notifications at individualized meal and snack times. For individuals with UCDs, a caregiver was able to assist the subject in documenting food intakes via the two methods.

Training

There were 2 training objectives of the first study encounter:

1. mFood App: Subjects were guided in downloading and navigating the mFood app. Subjects were taught how to take photos before and after meals, using proper lighting and angles of photos. They were instructed to include the standardized card in all photos. Subjects were also taught how to provide descriptions of meals and how to receive and respond to messages by the study team regarding foods in photos. A handout was also created with step-by-step instructions on navigating and using the application. This handout was sent home with participants to assist in data collection. (See **Appendix A**).
2. 3-Day Diet Record: Subjects were given a 3-day diet record and taught how to complete it. Subjects were instructed to return the 3-day diet record via email or pre-addressed shipping label.

Follow Up Contact

Subjects were contacted via email *during* the mFood Application period if further clarification was needed on food photos or descriptions. They were contacted *after* completing and returning 3-day diet records via email or phone if clarification on documented intakes was needed.

Schedule of Study Procedures

Subjects documented dietary intake for 3 consecutive days: 2 weekdays and 1 weekend. Intake documentation for the 3-day diet records was on the same days of the week as the mFood app documentation. Subjects were randomized to either complete the 3-day diet records or mFood app photos first, and documentation began on either a Thursday or a Sunday to meet weekday and weekend requirements. Subjects received a reminder on the projected start date of both their mFood data collection and 3-day diet record collection period from the study team.

DATA COLLECTION

mFood Digital Food Photography Analysis

Photos from the mFood app were sent in real-time to a research server and accessed by the study team daily. A team member interpreted the data and documented intakes in a secure online storage location. Any clarification data gathered from email was documented, dated, and initialed by a team member.

3-Day Diet Record Analysis

After completed 3-day diet records were received via mail or email, a team member documented intakes in a secure online storage location. Any clarification data gathered from phone or email was documented, dated, and initialed by a team member.

Nutrient Analysis Software

Data from 3-day diet records and the mFood application was quantified using Metabolic Pro and Food Processor nutrient analysis software. Metabolic Pro was specifically designed for metabolic nutritionists and includes information on specialty formulas and supplements, while Food Processor contains nutrient information on a wide selection of foods and brands. These food analysis tools are applicable to the UCD population. Using a combination of the two software in this study increased the accuracy of data quantification when comparing healthy control data and data from subjects with UCDs. Data taken from Metabolic Pro and Food Processor was stored as a Microsoft Excel file.

Data Storage

All data was stored as Microsoft Excel files on a secure OneDrive folder. All stored data contained only a subject's ID number to maintain confidentiality. Enrollment IDs given to subjects upon enrollment were in the format of XX1, XX2, etc., where XX is the 2-digit site number (10 for OHSU and 20 for Children's National Hospital), followed by the patient identifier number (1-4). For example, healthy subject 1 from OHSU was given ID number 101.

STATISTICAL METHODS

Outcome Variables

<u>Primary variables</u>: The primary variables of this study are documented intakes of protein, calcium, vitamin D, vitamin B12, and iron averaged over the 3 days from both the 3-day diet records and the mFood application. Intakes estimated from 3-day diet records were compared to the estimates from the mFood application.

<u>Secondary variables</u>: Average estimated protein intakes from 3-day diet records and the mFood application were compared to individual DRIs for healthy subjects and individual protein prescriptions for those with UCDs. Additionally, average estimated calcium, vitamin D, vitamin B12, and iron intakes from 3-day diet records and the mFood application were also compared to DRIs to determine if there were statistically significant differences.

<u>Tertiary variables</u>: Secondary Outcomes were compared between the two populations. Average estimated intakes from the 3-day diet record, the mFood application, and their correlation to recommended or prescribed intakes were compared between healthy control subjects and individuals with UCDs. Outcome variables are summarized in **Table 1**.

Table 1	Variables being Compared		
	Healthy Controls	Subjects with UCDs	Statistical Method
Primary Outcomes	**3-Day Diet Records:** Average estimated intakes of protein, calcium, vitamin D, vitamin B12, and iron ⇳ **mFood App:** Average estimated intakes of protein, calcium, vitamin D, vitamin B12, and iron	**3-Day Diet Records**: Average estimated intakes of protein, calcium, vitamin D, vitamin B12, and iron ⇳ **mFood App:** Average estimated intakes of protein, calcium, vitamin D, vitamin B12, and iron	Paired t-tests
Secondary Outcomes	**3-day Diet Records**: Average estimated protein intakes **mFood App:** Average estimated protein intakes ⇳ **Individual DRIs** (0.8 grams/kg)	**3-Day Diet Records**: Average estimated protein intakes **mFood App**: Average estimated protein intakes ⇳ **Individual protein prescriptions**	Dunnet's test for multiple comparisons (as part of an ANOVA test)
	3-Day Diet Records: Average estimated intakes of calcium, vitamin D, vitamin B12, and iron **mFood App**: Average estimated intakes of calcium, vitamin D, vitamin B12, and iron ⇳ **Micronutrient RDAs**	**3-Day Diet Records**: Average estimated intakes of calcium, vitamin D, vitamin B12, and iron **mFood App**: Average estimated intakes of calcium, vitamin D, vitamin B12, and iron ⇳ **Micronutrient RDAs**	
Tertiary Outcomes	**Healthy Controls**: Secondary outcomes ⇳ **Subjects with UCDs**: Secondary Outcomes		Unpaired t-tests

Method of Analysis

The research team performed statistical analyses for subjects who completed three days of diet records and three days of food documentation on the mFood application. Average protein intake from 3-day diet records and mFood application data was documented as absolute grams, grams/kg body weight, and a percentage of the DRI (healthy controls) or as a percentage of individual protein prescription (UCD subjects). Average calcium, vitamin D, vitamin B12, and iron intakes from 3-day diet records and the mFood application data were documented as a percentage of DRI and as milligrams, micrograms, or International Units (IUs). Data was summarized as means and standard deviations. For primary outcomes, individual paired t-tests were performed to compare data from 3-day diet records and the mFood application for protein, calcium, vitamin D, vitamin B12, and iron. For secondary outcomes, Dunnet's test for multiple comparisons (as part of an ANOVA test) was used to compare estimated intakes from 3-day diet records and the mFood application to micronutrient DRIs, protein DRI, or individual protein prescriptions. For this test, DRIs were set as a control variable. Finally, for tertiary outcomes, individual unpaired t-tests were used to compare data from the mFood app for healthy controls to data from the mFood app for individuals with Urea Cycle Disorders. Individual unpaired t-tests were also used to compare data from the 3-day diet record from healthy controls to data from 3-day diet records from UCD subjects. For all tests, results with a p-value less than 0.05 was considered statistically significant.

RESULTS

CHARACTERISTICS OF STUDY SAMPLE

Four healthy subjects and 3 subjects with UCDs were included in this study. Three of the healthy subjects were female, and one was male. All healthy participants were 25 years old and had an average BMI of 22.6 ± 0.9 kg/m^2. Three subjects with UCDs were included (aged 31 ± 16.7 years). Two UCD subjects were male, and one was female. The average BMI of UCD subjects was 24.9 ± 0.6 kg/m^2. Characteristics of the study sample are outlined in **Table 2**.

Table 2 Characteristics of Study Sample

	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Gender*	
					Male	Female
Healthy Subjects (N=4)	25 ± 0	149.21 ± 16.1	67.98 ±12.6	22.6 ± 0.9	1 (25%)	3 (75%)
UCD Subjects (N=3)	31 ± 16.7	163.97 ± 6.3	67 ± 6.1	24.9 ± 0.6	2 (66.7%)	1 (33.3%)

*Note: Gender is reported as a number (percent). All other values represent mean ± SD.

HEALTHY CONTROLS

Primary Outcomes: mFood Application vs. 3-Day Diet Records

Estimated average intakes for healthy control subjects are shown in **Table 3**. There were no statistically significant differences in protein, calcium, vitamin D, iron, or vitamin B12 intakes between the mFood app and 3-day diet records.

Table 3 Healthy Controls: mFood app vs. 3-Day Diet Records

		Dietary Assessment Method		
		mFood App (mean ± SD)	3Day Diet (mean ± SD)	p-value
Protein	g/day	84.65 ± 16.5	84.65 ± 27.8	0.99
	g/kg	1.25 ± 0.1	1.34 ± 0.6	0.77
	%DRI*	155 ± 14.3%	168.02 ± 69.7%	0.78
Calcium	mg/day	745.16 ± 535.4	775.35 ± 309.2	0.94
	%DRI	74.42 ± 0.5%	77.57 ± 0.3%	
Vitamin D	IU/day	62.80 ± 40.4	85.25 ± 98.7	0.74
	%DRI	14.36 ± 0.1%	14.21 ± 0.2%	
Iron	mg/day	16.16 ± 8.6	14.53 ± 2.8	0.79
	%DRI	109.19 ± 0.6%	102.26 ± 0.3%	
Vitamin B12	mcg/day	2.86 ± 1.0	2.19 ± 0.8	0.56
	%DRI	118.54 ± 0.4%	91.87 ± 0.3%	

*Note: DRI for protein for healthy subjects is 0.8 g/kg.

Secondary Outcomes: Estimated Nutrient Intakes vs. DRIs

Estimated average intakes from the mFood app and 3-day diet records for healthy control subjects were compared to nutrient DRIs using Dunnet's test for multiple comparisons, and the p-values are shown in **Table 4**. The following values were used as DRIs: 0.8 g/kg of protein, 1000 mg/day of calcium, 600 IU/day of vitamin D, 8 mg/day of iron (men) or 18 mg/day of iron (women), and 2.4 mcg/day of vitamin B12. There were significant differences between protein (g/kg) intakes estimated using the mFood app when compared to the DRI ($p=0.012$); however, there were no significant differences between estimated protein intakes from the 3-day diet record and the DRI. There were also significant differences in estimated nutrient intakes of vitamin D from the two methods when compared to the DRI for vitamin D (mFood app $p=0.0003$, 3-day diet record $p=0.00048$). Both methods showed intakes much lower than the DRI; however, the mFood app estimated lower vitamin D intakes (62.80 ± 40.4 IU/day) than the 3-day diet record (85.25 ± 98.7 IU/day). No significant differences were found for estimated calcium, iron, and vitamin B12 intakes between the mFood app and the 3-day diet records when compared to DRIs.

Table 4 Healthy Controls: Estimated Nutrient Intakes vs. DRIs

Nutrient	mFood App vs. DRI	3-Day Diet Record vs. DRI
	p-value	p-value
Protein (g/kg)	0.012	0.29
Calcium (mg/day)	0.66	0.45
Vitamin D (IU/day)	0.0003	0.00048
Iron (mg/day)	0.94	0.99
Vitamin B12 (mcg/day)	0.70	0.87

Healthy Control Estimated Average Intakes vs. DRIs

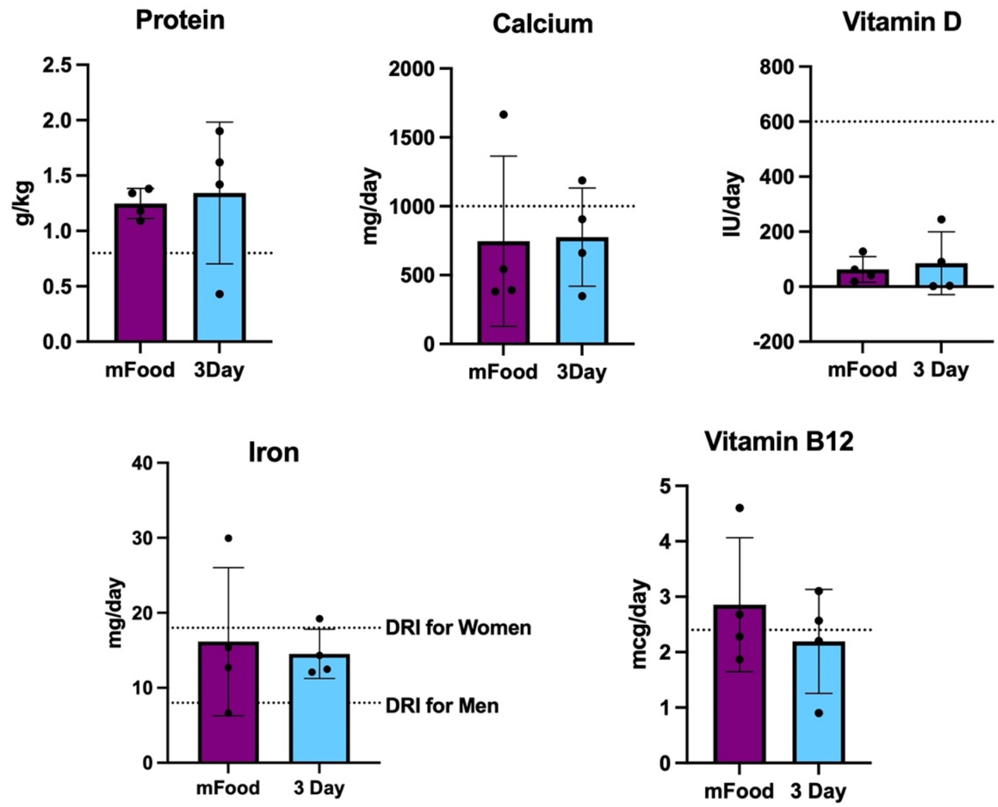


Figure 1 The figures above compare estimated average intakes for healthy subjects. Data for this figure was obtained using paired t-tests. Estimated intake averages from the mFood app are shown in dark purple, and estimated intake averages from 3-Day Diet Records are shown in light blue. The dotted horizontal line indicates the DRI for each nutrient. For iron, the DRI for males is 8 mg/dL, and the DRI for females is 8 mg/dL.

UCD SUBJECTS

Primary Outcomes: mFood Application vs. 3-Day Diet Records

Estimated average intakes for UCD subjects using the mFood app and 3-day diet records are shown in **Table 5**. There were no significant differences in estimated intakes of protein, calcium, vitamin D, iron, and vitamin B12. The nutrient with the greatest non-significant variation in estimated intake was protein.

Table 5 UCD Subjects: mFood App & 3-Day Diet Records

		Dietary Assessment Method		
		mFood App (mean ± SD)	3Day Diet (mean ± SD)	p-value
Protein	g/day	74.45 ± 25.09	53.59 ± 14.65	0.17
	g/kg	1.11 ± 0.32	0.79 ± 0.16	0.17
	% Protein Prescription*	121.44 ± 24.89 %	87.27 ± 3.62%	0.17
Calcium	mg/day	982.36 ± 564.29	1026.79 ± 42.55	0.93
	%DRI	98.04 ± 56.28%	103.01± 5.21%	
Vitamin D	IU/day	3160.57 ± 1592.27	3968.10 ± 944.15	0.36
	%DRI	526.76 ± 265.38%	661.35 ± 157.36%	
Iron	mg/day	24.81 ± 18.5	14.28 ± 10.04	0.25
	%DRI	282.51 ± 253.33%	169.06 ± 135.21%	
Vitamin B12	mcg/day	32.12 ± 33.28	31.3 ± 32.61	0.39
	%DRI	1294.69 ± 1326.19%	1261.56 ± 1299.19%	

*Note: Protein prescription differs for each subject. Subject 201's protein prescription was 0.9-1.1 g/kg, subject 202's protein prescription was 0.8 g/kg, and subject 203 is asymptomatic and does not have a protein prescription. For subject 203, 0.8 g/kg (DRI for healthy subjects) was used.

Secondary Outcomes: Estimated Nutrient Intakes vs. DRIs

Estimated average intakes from the mFood app and 3-day diet records for UCD subjects were compared to nutrient DRIs. The following values were used as DRIs for UCD subjects: 1000 mg/day of calcium, 600 IU/day of vitamin D, 8 mg/day of iron (men) or 18 mg/day of iron (women), and 2.4 mcg/day of vitamin B12. For protein, individual protein prescriptions were used instead of the DRI. P-values generated from Dunnet's test for multiple comparisons are shown in **Table 6**. Significant differences were found for estimated intakes of protein from 3-day diet records compared to the protein prescription for each participant (p=0.017). No other significant differences were found between estimated intakes from both methods and DRIs. However, vitamin D had the greatest non-significant differences between estimated intakes from 3-Day Diet Records and the DRI (p=0.06).

Table 6 UCD Subjects: Estimated Nutrient Intakes vs. DRIs

Nutrient	mFood App vs. DRI	3-Day Diet Record vs. DRI
	p-value	p-value
Protein* (g/kg)	0.47	0.017
Calcium (mg/day)	0.99	0.65
Vitamin D (IU/day)	0.23	0.06
Iron (mg/day)	0.64	0.94
Vitamin B12 (mcg/day)	0.48	0.48

*Note: For protein, estimated intakes were compared to individual protein prescriptions for each subject instead of the DRI.

UCD Subjects Estimated Average Intakes vs. DRIs

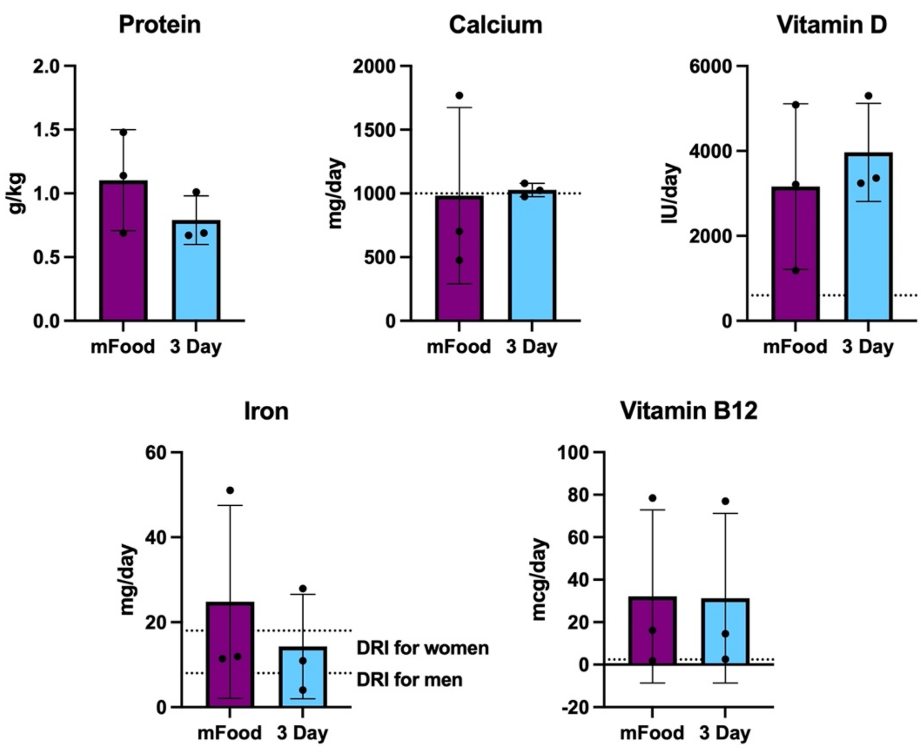


Figure 2 The figures above compare estimated average intakes for UCD subjects. Data for this figure was obtained using paired t-tests. Estimated intake averages from the mFood app are shown in dark purple, and estimated intake averages from 3-Day Diet Records are shown in light blue. The dotted horizontal line indicates the DRI for each nutrient. For protein, the DRI (Protein Prescription) for UCD subjects varies. For iron, the DRI for males is 8 mg/dL, and the DRI for females is 8 mg/dL.

HEALTHY CONTROLS VS. UCD SUBJECTS

Tertiary Outcomes

Unpaired T-tests were used to compare estimated average intakes from the mFood app and 3-day diet records between the healthy control subjects and UCD subjects, and the results are shown in **Table 7.** There were significant differences in estimated vitamin D intake between healthy control subjects and UCD subjects for the mFood app and 3-day diet records (p=0.021, p=0.001). For all other nutrients, no significant differences were found between the two subject groups for either nutrient intake estimation method. **Figures 3** and **4** show these comparisons.

Table 7 Healthy Control Subjects vs. UCD Subjects

Nutrient	mFood App	3-Day Diet Record
	p-value	p-value
Protein (g/kg)	0.52	0.22
Calcium (mg/day)	0.65	0.29
Vitamin D (IU/day)	0.021	0.001
Iron (mg/day)	0.52	0.97
Vitamin B12 (mcg/day)	0.20	0.19

mFood App Estimated Average Intakes

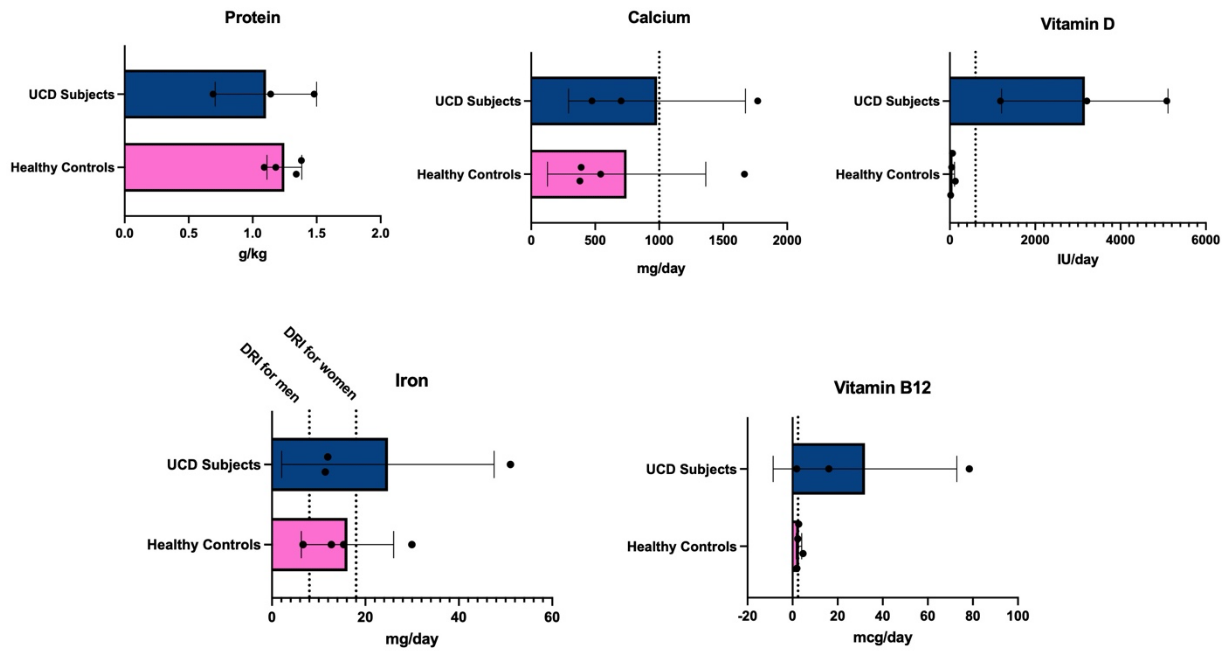


Figure 3 The figures above compare estimated average intakes from the mFood app. UCD Subjects are shown in dark navy, and healthy controls are shown in light pink. Data for this figure was obtained using unpaired t-tests. The dotted horizontal line indicates the DRI for each nutrient. For protein, the DRI (Protein Prescription) for UCD subjects varies, while the DRI for healthy controls is 0.8 g/kg. For iron, the DRI for males is 8 mg/dL, and the DRI for females is 8 mg/dL.

3-Day Diet Record Estimated Average Intakes

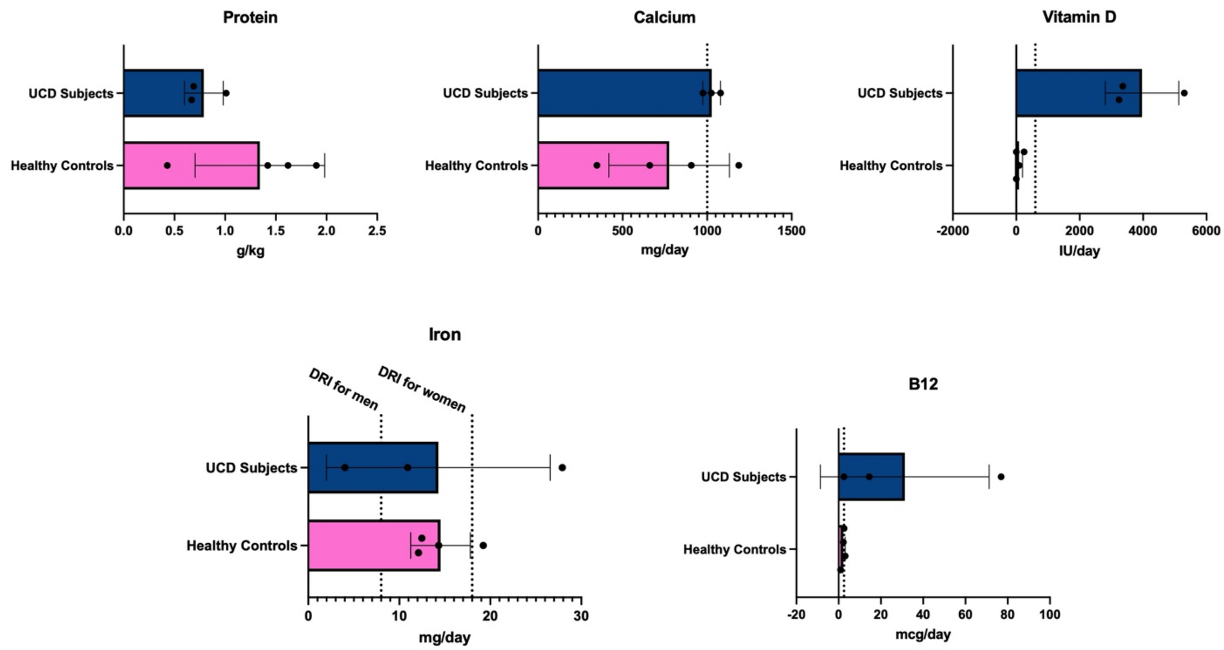


Figure 4 The figures above compare estimated average intakes from 3-day diet records. UCD Subjects are shown in dark navy, and healthy controls are shown in light pink. Data for this figure was obtained using unpaired t-tests. The dotted horizontal line indicates the DRI for each nutrient. For protein, the DRI (Protein Prescription) for UCD subjects varies, while the DRI for healthy controls is 0.8 g/kg. For iron, the DRI for males is 8 mg/dL, and the DRI for females is 8 mg/dL.

USER PREFERENCE

Subjects were given a User Preference Survey after using both the mFood app and the 3-day diet record to assess satisfaction with both methods. This survey included statements about the mFood app and 3-day Diet Records, and subjects were asked to rate these statements from 1 (Strongly Disagree) to 6 (Strongly Agree). Using a score range of 1-5 would allow subjects to choose 3 as a neutral rating, indicating they did not agree or disagree with the statement. However, by using a score range of 1-6, subjects had to determine if they agreed or disagreed with the survey's statements to a certain degree. Overall, three out of four healthy subjects preferred using the mFood app to track dietary intake. The one healthy subject who preferred the 3-day diet record attributed their preference to the technical difficulties experienced while using the mFood app. However, there was no significant difference in satisfaction scores for healthy subjects ($p=0.33$). One UCD subject failed to turn in a user preference survey. The two other UCD subjects preferred using the mFood app over traditional 3-day diet records, and there was a significant difference in satisfaction scores (p=0.004). Average scores for the mFood app and 3-day diet records are shown in **Table 8,** and the User Preference Survey can be viewed in **Appendix B**.

Table 8 User Preference Survey

	Healthy Control Subjects	UCD Subjects
The mFood app was easy to use…		
to record types and amounts of foods and ingredients eaten.	4.8 (±0.83)	3.5 (±0.5)
to record meals I skipped**.**	4.7 (±1.25)	4 (±0)
to record meals when I forgot to take a picture.	3.3 (±1.48)	4 (±0)
to take photos of my meals.	4.5 (±1.12)	5 (±0)
to remember to take photos of my meals *before* and *after* I ate because of the reminder messages.	4.3 (±1.09)	4 (±1.0)
because the app training prepared me to take photos.	5.3 (±0.83)	4 (±1.0)
and I liked using the app.	4.0 (±1.41)	4 (±2)
Average Score for the mFood App	**4.4 (±1.14)**	**4.1 (0.69)**
The 3-day diet record was easy to use…		
to record types of foods and ingredients eaten.	4.8 (±1.09)	3 (±0)
to record amounts of foods eaten.	3.5 (±1.12)	3 (±0)
to record skipped meals**.**	3.0 (±0.71)	3 (±0)
to report the date and time I ate meals.	4.5 (±2.06)	3 (±0)
Average Score for the 3-Day Diet Record	**3.9 (±1.24)**	**3 (±0)**
p-value	0.33	0.004

Note: Subjects answered questions as a number between 1 and 6. Averages and Standard Deviations are shown for each question.

1= Strongly Disagree, 6= Strongly Agree

DISCUSSION

Estimated Nutrient Intakes

The findings of this study indicate there were few significant differences in estimated nutrient intakes between the mFood app and the 3-day diet record for both healthy subjects and controls. The nutrient with the most variation between subject groups and dietary assessment methods was vitamin D. Estimated vitamin D intakes were significantly lower in healthy controls than UCD subjects for both the mFood app and 3-day diet records. This can be attributed to the fact that all three UCD subjects included in this study took daily vitamin D supplements of 2000 IU or greater, while healthy subjects had no micronutrient supplementation. In healthy subjects, estimated vitamin D intake from both the mFood app and the 3-day diet record was significantly lower than the DRI. For UCD subjects, estimated vitamin D intakes from both dietary assessment methods were not significantly different than the DRI. This may suggest that it is difficult to meet DRI requirements for vitamin D without supplementation; however, subjects can also meet their vitamin D requirements from sun exposure rather than through dietary intake. All nutrient intake estimation methods, including food photography and traditional 3-day diet records, do not account for vitamin D synthesis from sun exposure; therefore, it is impossible to determine if subjects are meeting vitamin D requirements from dietary assessment methods alone. Webb et al. noted that individuals only need 17 minutes of unobstructed sun exposure to meet vitamin D requirements. Although, the ability to meet vitamin D requirements from sun exposure varies based on geographic location and should be considered when assessing dietary vitamin D needs.[21]

Protein intake for healthy subjects was greater than the DRI. This is consistent with expected results, as healthy Americans tend to consume more protein than the DRI (0.8 g/kg). After examining average protein intakes from National Health and Nutrition Examination Survey (NHANES), 2001–2014, it was found that females aged 19-30 consumed an average daily intake of 1.14 g/kg ideal body weight, and males aged 19-30 consumed an average daily intake of 1.45 g/kg ideal body weight.[22] Our study estimated protein intakes from the mFood app and 3-day diet records as 1.25 (±0.01) g/kg and 1.34 (±0.6) g/kg, respectively, which was consistent with NHANES survey data. Estimated protein intakes for healthy subjects using the mFood app were significantly higher than the DRI ($p = 0.01$); however, 3-day diet records did not show significant differences between estimated intakes and the DRI ($p = 0.29$). It is important to also note the differences in measurement variation between the two methods. 3-day diet records had a relatively large variation (SD = ±0.6), while the mFood app had a smaller variation (SD = ±0.1). This may suggest that the mFood app is a more precise measure of estimated protein intake than 3-day diet records.

Another interesting finding was that for UCD subjects, 3-day diet records showed significantly different protein intakes than individual protein prescriptions ($p=0.017$), while the mFood app showed no significant differences in estimated intakes compared to protein prescriptions ($p=0.47$). For individuals with UCDs, adhering to individual protein prescriptions is critical to minimizing the side effects of the disease.[7] Therefore, the findings of this study show that UCD subjects' actual protein intakes may not be consistent with the expected intake. The digital photography method has shown to be a dependable tool for estimating protein intakes in previous studies. In a study by Martin et al., visual estimation of protein intake from food photos was consistent among three registered dietitians estimating intake.[15] Martin et al. also documented the reliability and validity of the food photography method for estimating protein intake when compared to direct nutrient intake of laboratory buffet meals.[19] Future studies examining the validity of the food photography method and 3-day diet records in UCD subjects are needed to further investigate discrepancies in actual protein intake compared to individual protein prescriptions.

Estimated nutrient intakes of calcium, iron, and vitamin B12 did not differ between subject groups and were not significantly different than DRIs. High-protein animal products, such as meat and dairy, are often rich in calcium, iron, and vitamin B12. Since healthy control subjects do not limit their protein intake, high-protein animal products may have helped them meet micronutrient DRIs. While individuals with UCDs often limit their intake of high-protein animal products, multivitamin supplementation can also assist in meeting micronutrient DRIs. Two of the three UCD subjects in this study consumed daily multivitamin supplements, and this may account for the observed results.

Dietary Assessment Methods

Ease of nutrient estimation and quantification by the study team differed between the mFood app and 3-day diet records. Descriptions of food ingredients and preparation methods on the mFood app were less detailed than 3-day diet records. This could be attributed to subjects' beliefs that a less detailed description was needed if they included a photo of their intakes. However, even with a photo, further clarification on foods entered on the mFood app was often necessary and was obtained via follow-up messages sent to subjects. The food photos alone assist in obtaining a more accurate estimation of portion sizes; however, it was often difficult to determine the ingredients consumed without a detailed description. Detailed descriptions on the mFood app (similar to those on 3-day diet records) combined with meal photos may lead to more accurate estimated intakes overall. Additionally, for certain meals, it was difficult to confidently determine portion sizes from the photos provided on the mFood app. The option to add multiple photos of the same meal at different angles and depths may have eased portion size estimation by the study team. A common complaint from subjects was the app's inability to log more than one photo for each mealtime. For example, if a subject consumed their morning coffee at a different time than their breakfast, they were unable to log both meals under "breakfast." The ability to add multiple photos under each mealtime may have also increased the accuracy of estimated intakes. These technical difficulties influenced user satisfaction with the mFood app.

The user preference survey, which was given to all subjects after using the mFood app and 3-day diet records, indicated that healthy control subjects did not prefer one method over the other, while UCD subjects preferred the mFood app over traditional pen and paper 3-day diet records. Individuals with UCDs on protein prescriptions often undergo regular monitoring of dietary intake, suggesting they are more familiar with traditional dietary assessment methods.[7] Therefore, UCD subjects' preference for the mFood Application is consistent with the literature and supports the study team's belief that the digital food photography method eases the burden of dietary assessment for subjects and/or is easier to use than traditional 3-day diet records.[23] A possible reason for preference of the mFood app over traditional 3-day diet records is the integration of reminders sent from the mFood app. Individuals with UCDs may experience cognitive impairments related to their disease, making it difficult to remember to document food intakes. The mFood app has the ability to remind subjects to document intakes, and the integration of the dietary intake record onto a smartphone allows subjects to easily document foods if they are away from home. In contrast, if a subject forgets to bring their pen and paper 3-day diet record with them when eating a meal away from home, they may easily forget to document food intake. Future studies examining the applicability of the mFood app in a larger population of UCD subjects with varying ages and degrees of cognitive impairment would provide more evidence to support UCD subjects' preference for the digital photography method over traditional 3-day diet records.

Limitations

This study had several limitations. First, the sample size for both the healthy controls and individuals with UCDs was small. Small sample sizes affected the statistical power and limited the study's ability to account for potential outliers in the data. Secondly, the healthy control group lacked age and weight diversity, as all subjects were 25 years old and had normal BMIs. Ease of use of the mFood app may differ in an older population. Previous studies have also shown that under or over-reporting may be present for over or underweight individuals. A systematic review by Wehling and Lusher found that underreporting of dietary intake was significantly associated with a BMI >30 kg/m^2.[24]

Technical difficulties in the mFood app also occurred during the data collection period. There were several occasions when meal descriptions and photos logged by users were not accessible by the study team. In order to obtain this lost information, subjects were contacted via follow-up questions. Obtaining mealtime information after the meal was eaten may have influenced reporting accuracy.

Conclusion

This study supports my initial hypothesis that the food photography method would show estimated protein intakes closer to the protein prescription for UCD subjects when compared to 3-day diet records. However, this study fails to support this same hypothesis for healthy subjects. Additionally, this study also fails to support the hypothesis that the food photography method would show estimated intakes of calcium, vitamin D, vitamin B12, and iron closer to micronutrient RDAs than 3-day diet records for both healthy controls and individuals with UCDs. While healthy controls' vitamin D intakes were significantly different from the DRI, this was true for both the food photography method and traditional 3-day diet records. Due to the small sample size of this study and lack of variation in subject characteristics, future studies are needed to further investigate these hypotheses and determine the applicability of using digital food photography, more specifically the mFood app, to assess dietary intake in free-living subjects with Urea Cycle Disorders.

<u>APPENDIX</u>

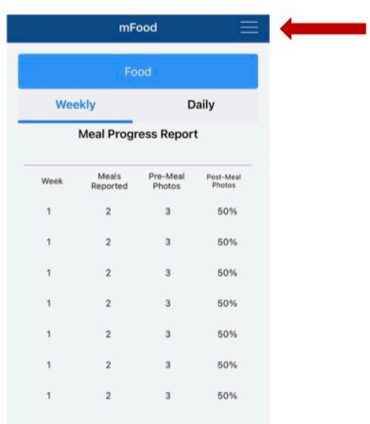
Appendix A: mFood Phone Application Instructions

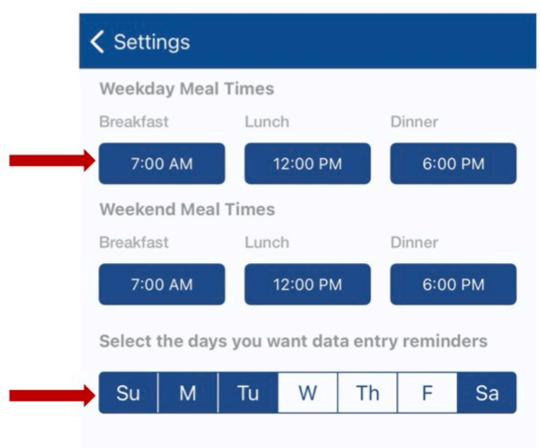
mFood Phone Application
TRACKING DIETARY INTAKE

Purpose: Tracking dietary intake accurately helps us to assess how much and what food you eat during our 3-day evaluation period.

<u>Getting Started:</u>

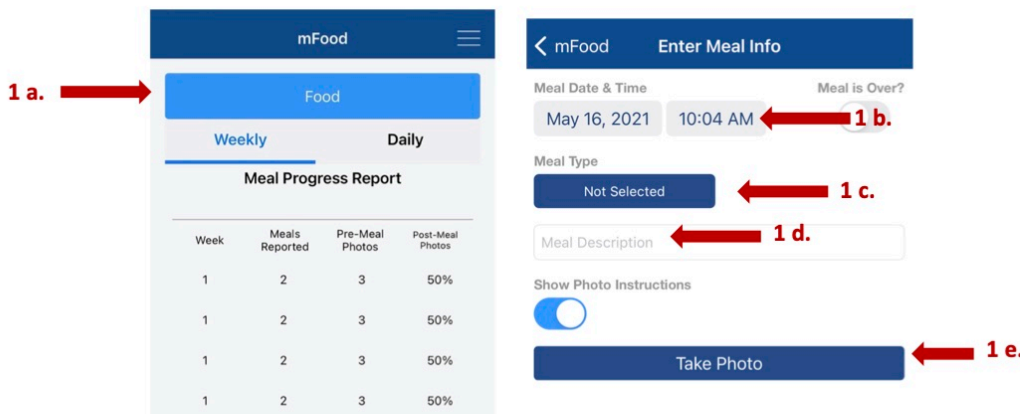
- Logging In
    1. Open mFood App and Log-In using provided username and password.
- Enable notifications and camera on your phone
    1. A window should pop-up allowing you to enable both of these settings
        i. Notifications allow us to send you reminders to document your meals
        ii. Camera is required so you can take photos of your meals
    2. This can also be changed in “Settings and Alerts”
- Customize your mealtimes and days of the week
    1. Click the 3 lines (on the iphone app) or 3 dots (on android app) in the upper right-hand corner of your screen
    2. Select ” Settings and Alerts”
    3. Choose what times you eat breakfast, lunch and dinner on the weekdays and on the weekends
    4. Select 3 days for which you are entering meals
    5. Click “Update”





Entering Food

1. Start a meal entry
    a. You can start a meal entry by clicking the blue "Food" button at the top of the page
    b. Confirm that the date and time of the meal is correct under "Meal Date & Time"
    c. Choose meal type (Breakfast, Morning Snack, Lunch, etc.) by clicking the button under "Meal Type"
    d. Provide a meal description with as much detail as possible. Include portion sizes and ingredients.
    e. Select "Take Photo"

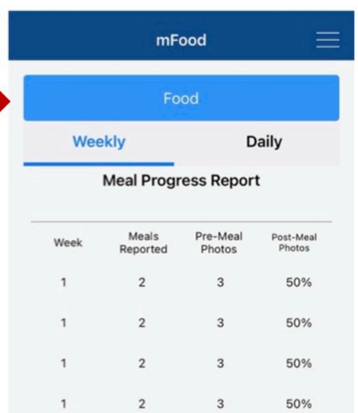


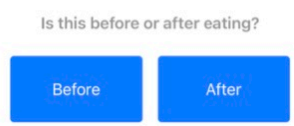
2. Take your "Before" photo
    a. Place the reference card flat on the surface next to your plate and/or cup.
    b. Hold the Camera about an arm's length distance away at a 45-degree angle from the plate. Camera should not be close to the plate
    c. ALL plates, cups, and wrappers should be seen clearly in the viewfinder screen. You may tap the camera screen to bring the objects into focus and use the flash if you are in a dimly lit area.
    d. Make sure the ensure food photo reference card is also included
    e. The "before" photo will be saved under the "Daily" tab
    f. Eat your meal as you normally would

Before **After**

3. Next, record an "After" photo of any food that you did not consume
    a. Click the blue "Food" button at the top of the page
    b. Select the "After" Button
    c. Remember to include your reference card and follow the same photo instructions as you did when taking the "Before" photo.
    d. Select "Take" and take a photo of your plate, including any food you did not consume.

3 a.

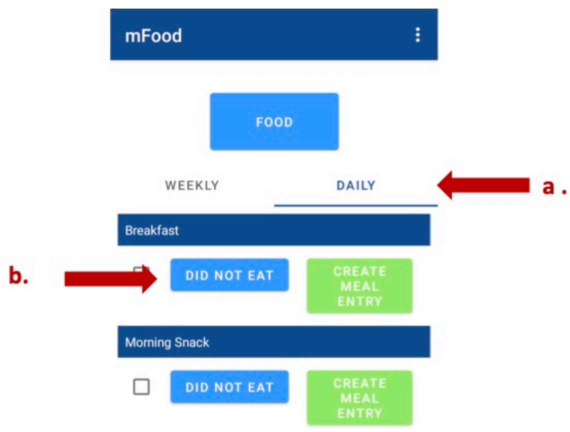




3 b.

What if I did not eat anything for a meal time?

1. Documenting a missed mealtime.
    a. If you did not eat anything during a meal time, Click “Daily” to see all the meal times for the day
    b. Click the “Did Not Eat” button
    c. Click “Yes” to create a Did Not Eat entry



Appendix B: User Preference Survey

mFood User Preference Survey

Study ID ______________________________ Date: ______________________________

In this study you used a smartphone app (mFood) to take and send photos of foods you ate during the study period. Below is a brief survey that will help us understand your satisfaction with this app. Please take a few minutes to complete this survey by marking an "X" in the box that best fits your opinion. There are no wrong or right answers. Your opinion is important to us! Thank you for your feedback.

The mFood app was easy to use…	Strongly Disagree 1	2	3	4	5	Strongly Agree 6	N/A
1. to record **types and amounts** of foods and ingredients eaten.							
2. to record meals I **skipped**.							
3. to record meals when I **forgot to take a picture.**							
4. to **take photos** of my meals.							
5. to remember to take photos of my meals *before* and *after* I ate because of the reminder messages.							
6. because the app training prepared me to take photos.							
7. and I liked using the app.							

8. Please provide any other comments about the **mFood app** here:

____

____

____

Final Version 1.0 11 June 2021
Adapted from a survey by Louisiana State University Pennington Biomedical Research Center

We also asked you complete pen and paper, **3-day diet records** to record your food intake. For this method we asked you to write down what you consumed, the ingredients, how much you ate, and the date and time you ate it. Please take a few minutes to complete this survey by marking an "X" in the box that best fits your opinion. There are no wrong or right answers. Your opinion is important to us!

The 3-day diet record was easy to use…	Strongly Disagree 1	2	3	4	5	Strongly Agree 6	N/A
9. to record **types** of foods and ingredients eaten.							
10. to record **amounts** of foods eaten.							
11. to record **skipped meals**.							
12. to report the **date and time** I ate meals.							

13. Please provide any other comments about the **3-day diet record** here:

____

____

____

	3-day Diet Record	mFood App
14. Which method did you like best for **recording meals ate**?		
15. Which method did you like best for recording **ingredients in foods eaten**?		
16. Which method did you like best for recording **amounts of each food consumed**?		

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17. Please provide any additional comments below:

____

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Thank you for your feedback!

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