CHAGNING HABITS AND LIFESTYLES IN OLDER INDIVIDUALS

Registration: NCT02777918

Date of Document: 26.07.2018

**INCREASING EGG AND DIETARY PROTEIN INTAKE IN COMMUNITY-DWELLING OLDER ADULTS USING RECIPES AND SINGLE-USE HERB / SPICE PACKETS: A RANDOMIZED CONTROLLED TRIAL**

## METHODS

The study used a parallel group, randomized controlled intervention design, where community-dwelling older adults were randomized to receive or not receive high-protein egg-based recipes and herb/spice packets for 12 weeks, and egg intake, protein intake, and measures of health and well-being were measured at baseline, at the end of 12 weeks and after a further 12 weeks.

### Participants

Older adults were considered for inclusion in the study if they were: 55 years and over; living in the community; not allergic to eggs; not knowingly suffering from hypercholesterolaemia or familial hypercholesterolaemia; not knowingly suffering from known renal insufficiency; not having undergone chemotherapy or radiotherapy in the last 6 months; not suffering from any condition, or receiving treatment that affected eating or sense of flavour; were able to give consent and complete all questionnaire measures; and did not have a pacemaker or defibrillator. These inclusion criteria allowed inclusion of volunteers within our target population (older adults living in the community), while ensuring against adverse health consequences as a result of a change in eggs or dietary protein consumption (British-Heart-Foundation, 2015; Pedrini, Levey, Lau, Chalmers, & Wang, 1996) or involvement in the study, and valid outcome assessments (Jcobsen et al., 1993; Steinbach et al., 2009; Van Cutsem & Arends, 2005).

### Sample size:

Data from the National Diet and Nutrition Survey (NDNS) show that UK adults over 65 years old have a mean daily protein intake of 69.8g (SD = 20.3) (Bates et al., 2014). Assuming a threshold to stimulate muscle protein synthesis of 25-30g protein per meal (Paddon-Jones & Rasmussen, 2009) for three meals per day, daily intake should be at least 75-90g per day, an increase of on average 12.7g of protein per day. Assuming no change in the control group over the intervention period, and an alpha of 5% for a power of 0.8, the required sample size to detect a 12.7g increase in protein intake in the intervention group equals 41 participants per group. Taking into account a possible drop-out of 20%, we aimed to recruit 50 participants per study group.

### Recruitment:

Participants were recruited via contact lists of individuals who have volunteered in previous studies; organisations that ran group meetings within the target population, e.g. bridge clubs; and by flyers and posters at local community buildings, such as museums and libraries. All volunteers were initially screened for suitability by telephone or email, and they were sent a participant information sheet before coming to the University for the baseline test session. Screening was repeated at this test session, and consent completed. Participants were not informed of the aim of the study, but were instead told the project was studying habits and lifestyles in older adults.

### Randomisation:

All eligible volunteers were randomized into one of two groups: intervention and control, following consent. Randomisation was stratified per ten year age group (55-64, 65-74, 75-85, 85+) and by earlier involvement in related research [ref], using blocks of 6-10 participants, with an exception that couples were always allocated to the same group. Randomization was undertaken by a researcher with no direct contact with participants (KMA).

### Intervention

Community-dwelling older adults randomized to the intervention group received six high-protein egg-based recipes and herb/spice packets every two weeks to their home address, for twelve weeks. Recipes were intended to provide ideas for consuming eggs in different ways and using different flavours, to increase variety in the overall diet. Each recipe contained one or two eggs, and provided 25-30g of protein per meal.

The 36 different recipes were gained from the website www.eggrecipes.co.uk, developed by the British Egg Industry Council (British-Egg-Industry-Council, 2016), selected as suitable for breakfast / brunch as well for other meals, and amended to contain 25-30g of protein. Participants in the intervention group received six sets of six recipes. Each set of recipes included a variety of egg preparations (e.g. fried eggs, scrambled eggs, omelettes); a variety of preparation times and methods (hob, oven, microwave); meat, fish and vegetarian dishes; and a variety of traditional (bacon, cheese, ham) and foreign flavours e.g. Indian, Turkish, Moroccan, Italian. Several of the recipes were developed by chefs known from UK television. Recipes were printed in large clear text on glossy A5 recipe cards, to aid durability and simulate supermarket recipe cards, and included preparation time and nutritional composition, with the protein content highlighted in bold. All recipes mentioned the dish was high in protein, and recommended consumption for breakfast or brunch. Recipe cards were pilot tested with individuals in the target age group, prior to use, and the text on several recipes was altered for face validity as a result. A list of all recipes can be found in Supplementary Materials I.

All herb/spices mentioned in the recipes were also provided for one preparation of the dish as part of the intervention. Herbs/spices were provided to reduce disuse of the recipes through ingredient expense. Other ingredients were not provided to result in an intervention that could be manageable in a real life scenario.

**Control**

Participants in the control group did not receive any recipes, herbs or spices, or the recipe feedback forms, but received all other documentation and undertook all tests sessions in the same manner as the intervention group.

**Outcomes**

Our primary outcomes were egg intake, protein intake (total), protein intake from animal-based foods and adverse events. Secondary outcomes were lean body mass and various functional measures of lean body mass. Variables of potential influence on these outcomes were also assessed, to include various demographic and lifestyle characteristics, energy intake, BMI, usual physical activity levels, health-related quality of life, reasons for eating / not eating eggs, and recipe feedback (intervention group only) Morton et al., 2017). Some of these additional variables were included in analyses to control for potential confounding, some were used only to describe the sample and some were used to investigate further any reasons for intervention success or failure.

**Primary Outcomes:**

**Egg intake:** Egg intake was measured using two measures: an egg consumption Food Frequency Questionnaire (FFQ) measure [ref] and the egg section of a validated FFQ [SCG-FFQ, Clark, 2017; Masson et al., 2003]. The egg consumption FFQ requested frequency of consumption of 18 preparations of egg: boiled eggs (hot), hard boiled eggs (cold), fried eggs, scrambled eggs, poached eggs, omelettes, scotch eggs, quiches/savoury flans, egg sandwiches, egg salad, custards, meringues, sweet flan/crème caramel, duck/quail's eggs, raw eggs, egg yolk separate from the white, and egg white separate from the yolk. For each type of egg, participants were asked to complete number of measures (e.g. 1 egg, 1 slice) consumed per day (1, 2, 3, 4, 5+) on number of days per week, using response options 1 - 7 days per week, once or twice per month, or rarely / never. Responses were converted to number of eggs eaten per month, where the category 'rarely or never' was counted as 0 times eaten per month, 'once or twice per month' as 1.5 times, and 1 to 7 days per week, as 4, 8, 12, 16, 20, 24, and 28 times per month, respectively. All foods were considered to contribute equal portions of eggs, excepting the egg dishes custards, meringues, and sweet flan / crème caramel, where each consumption was considered as 0.5 portions, because a standard portion tends to be less than one egg.

The SCG-FFQ is a validated FFQ, developed by the Scottish collaborative group, measuring dietary intake over the last two-three months (Clark, 2017; Masson et al., 2003). The section on egg intake consists of

three questions: number of measures (eggs) per day, and the number of days per week they eat 'Boiled or poached eggs'; 'Fried eggs'; and 'Scrambled eggs or omelette'. Response options were used and converted to monthly egg intake as above.

**Protein intake:** Protein intake was measured using the complete SCG-FFQ, where food intake data were converted to protein intake and protein intake from animal sources, by the Scottish collaborative group (Clark, 2017). Protein intake from animal sources consisted of protein intake from meat, fish, eggs, and dairy foods. Protein intake is known to be related to egg intake (Ruxton, Derbyshire, & Gibson, 2010, questionnaire study), to muscle mass, strength and function (Houston et al., 2008; Loenneke, Loprinzi, Murphy, & Phillips, 2016; Tieland et al., 2012), and to positive health outcomes in older adults (Hannan et al., 2000; Lord, Chaput, Aubertin-Leheudre, Labonté, & Dionne, 2007). Animal based proteins contain all essential amino acids and are considered high quality protein sources (van Vliet, Burd, & van Loon, 2015). Animal protein stimulates a higher net protein synthesis than a high plant protein diet (Pannemans, Wagenmakers, Westerterp, Schaafsma, & Halliday, 1998). These measures also allowed investigation of any replacement of other high protein foods by egg based protein foods.

**Adverse events:** To assess adverse events, participants were asked whether during the previous month they had experienced any of the following effects more of less often than usual: nausea, digestive issues (e.g. constipation or diarrhoea), stomach aches/cramps, hunger, bloating/uncomfortable fullness, thirst, headaches, fatigue/tiredness, restlessness, dizziness, or skin rashes. If they answered yes to any of these questions, they were asked if they knew of any reason for this, or they had experienced anything else that was different than usual.

**Secondary Outcomes:**

**Lean Body Mass:** Fasting resting body composition was assessed by bioelectrical impedance analysis (BIA) with the use of a 50-kHz generator (1500 MDD; Bodystat, Isle of Man, UK). Under standard conditions, BIA measures are validated against MRI measures of skeletal muscle mass (Janssen, 2002; Janssen et al., 2000). The equations have been validated for adults of multiple ethnicities (Janssen et al., 2000), and have been established and recommended for older adults (Cruz-Jentoft et al., 2010; Kyle, Genton, Slosman, & Pichard, 2001; Roubenoff et al., 1997). Assessments were conducted after an overnight fast, following no alcohol or caffeine for 12 hours prior to the measurement. Water consumption, exercise and use of sauna during eight hours prior to the session were recorded and kept constant across sessions.

**Functional measures of lean body mass:** Physical performance was assessed using a short physical performance battery (SPPB) (Guralnik et al., 1994), consisting of three physical functioning tests measuring: lower body strength (chair stands), standing balance, and walking speed (8-foot walk). For the chair stands, participants were asked to stand up from a chair and sit down five times with their arms across the chest and time is measured. For the balance test, participants were instructed to stand in different positions: side-by-side, semi-tandem, and full-tandem position, while being timed for 10 seconds, or until they lost their balance. Walking was measured by asking the participant to walk an 8-foot walking course, at their usual speed, as if they were walking down the street to go to the shops. Times were recorded twice, and the fastest used for analyses. Scores from zero to four are derived for each of the three components and totalled following the instructions by Guralnik et al. (Guralnik et al., 1994). Total SPPB scores range from 0 to 12 with a higher score indicating better physical performance. The measures are relatively quick, include activities that are used in everyday life, and are non-invasive for the participants. SPPB measures have been linked to self-reported disability, predict mortality and nursing home admission (Guralnik et al., 1994), and have been recommended for measuring physical performance in frail old people (Cruz-Jentoft et al., 2010). Statistically significant improvements in SPPB scores have been found in older adults following increased protein intake (Tieland et al., 2012) and the method can measure clinically meaningful changes (Kwon et al., 2009).

Leg strength was measured in a seated position by counting number of times participants could extend their leg wearing ankle weights (of 5kg, 2.5kg, or no weights) during a set amount of time (one minute or 30 seconds). Ankle weight and time was chosen by the researcher during the first test session based on estimated ability of the participant after the participants had performed the other exercises, and held constant for all other test sessions. The 2.5kg weights and one minute duration were chosen for most participants. Handgrip strength was also measured using a hand grip dynamometer (Takei, GRIP-D, T.K.K. 5401) (Watanabe et al., 2005). Participants were asked to take three measures of handgrip strength for each hand, alternating between right and left hands, while standing up (or sitting if standing was not possible). The maximum of the six measures was reported (Roberts et al., 2011). Handgrip strength and leg extension are commonly used non-invasive measures to assess muscle function in older adults (Beasley, Shikany, & Thomson, 2013), and only take a few minutes. These measures have been associated with health related quality of life (Sayer et al., 2006), nutritional status (Norman, Stobäus, Gonzalez, Schulzke, & Pirlich, 2011), and future physical performance and disability (Rantanen et al., 1999). Handgrip strength also relates strongly to lower extremity muscle power and mobility (Lauretani et al., 2003), and has been recommended for a quick and inexpensive sarcopenia screening tool (Lauretani et al., 2003). Sarcopenia cut off points per gender based on measured handgrip strength were used to estimate sarcopenia prevalence (Cruz-Jentoft et al., 2010; Lauretani et al., 2003; Murphy et al., 2017).

**Additional Outcomes:**

**Demographic and lifestyle characteristics:** Assessed demographic and lifestyle characteristics were: date of birth; gender; marital status (married, divorced, widowed, never married); living status (alone, with others); years of education; nationality; most recent level of employment (unemployed, manual worker, non-manual worker, professional / management); frequency of help with food shopping or preparation, eating out or away from the home, and of food delivery (never, sometimes, often), vegetarianism, pescatarianism or veganism (yes or no), and denture wearing (no, partial dentures or full dentures). All variables have previously been shown to be related to eating behaviour in older adults and protein intake specifically (Appleton, 2016; Best & Appleton, 2013; Kremer, Bult, Mojet, & Kroeze, 2007; Steptoe, Pollard, & Wardle, 1995) (Adams et al., 2015; Caraher, Dixon, Lang, & Carr-Hill, 1999). Food neophobia - a measure of willingness to try new foods, was also assessed using a validated questionnaire (Pliner & Hobden, 1992), as increases with age may pose a barrier to trying the recipes in the intervention (Meiselman, King, & Gillette, 2010; Tuorila, Lähteenmäki, Pohjalainen, & Lotti, 2001). Additionally, a validated questionnaire was used to assess symptoms of sarcopenia (Malmstrom & Morley, 2013).

**Energy Intake:** Energy Intake was assessed as part of the SCG-FFQ, by consideration of all foods, by the Scottish collaborative group (Clark, 2017).

**BMI:** Body Mass Index was calculated from measured standing height and body weight (in light clothing, without shoes), measured to the nearest 0.1cm and 0.1kg, using a stadiometer (SECA gmbh & co, Hamburg, Germany) and calibrated scale (The Boots Company PLC, Nottingham, UK) respectively.

**Usual Physical Activity:** Physical activity, as energy expended in physical activity per week, was assessed using the Community Healthy Activities Model Program for Seniors (CHAMPS), a physical activity questionnaire for older adults (Stewart et al., 2001). The CHAMPS lists a variety of light, moderate and vigorous physical activities, and requests weekly frequency of performing each activity in number of hours per week (response options: less than 1 hour, 1-2.5 hours, 3-4.5 hours, 5-6.5 hours, 7-8.5 hours, and 9 or more hours). Responses were converted to four outcomes (Stewart et al., 2001), and estimated caloric expenditure per week on all activities was used for analyses. Physical activity is strongly related to muscle mass and strength (Cruz-Jentoft et al., 2010; Goodpaster et al., 2008), and a recent review showed that experience in exercise made protein supplementation with resistance training more effective (Morton et al., 2017). Studies also show associations between higher egg intake and low physical activity (Hu et al., 1999).

**Health related quality of life:** Health related quality of life (HR QoL) was assessed using the short form-36 questionnaire (SF-36) (Ware Jr & Sherbourne, 1992). Scores for all nine domains were generated (Ware Jr & Sherbourne, 1992) and added up to give a total SF-36 score, where a higher score suggests a greater HR QoL. SF-36 scores have been associated with muscle strength and physical performance (Syddall, Martin, Harwood, Cooper, & Sayer, 2009).

**Reasons for eating or not eating eggs:** Reasons for eating / not eating eggs were assessed by questionnaire, as previously developed [ref], to give insights into other possible barriers to trying the recipes.

**Recipe feedback:** Intervention group participants also received a recipe feedback form in the post after the 12 week intervention and after the following 12 weeks. This form asked for comments on which recipes and herb/spice packets they used or did not use, and why. Recipe feedback forms were sent by post after the test sessions, so that the completion of these does not impact on our primary outcomes, by a researcher unassociated with testing to maintain blinding.

**Procedure**

A schematic overview of the study design can be found in Figure 1. All participants came to Bournemouth University for a test session at baseline (T1), after 12 weeks intervention (T2), and after a further 12 weeks (T3). A week before each test session, participants received a set of questionnaires by post, to complete and bring to the test session. Queries were discussed at the test session, but on a few occasions, participants finished the questionnaires at home within a few days after the test session, and returned them by post. All test sessions were conducted in the morning between 8.00 and 11.00, at a time of the participant's preference. Participants came fasted, and were asked to have had only water to drink since going to sleep the night before the test session. Height and weight were measured first, followed by bioelectrical impedance measures of body composition. After these measures, a breakfast of toast / cereal and coffee / tea was provided. While the participant was eating breakfast, questionnaires were checked for missing values and any queries were discussed. After breakfast, the functional measures of lean muscle mass were undertaken. Questionnaires and tests undertaken were the same for each test session (T1, T2, T3), excepting that demographic and lifestyle characteristics, and reasons for eating / not eating eggs were only assessed at baseline. Test sessions were individual, or if preferred per couple, and lasted approximately an hour per person. After the baseline test session, all participants also received a postcard with a short dietary information message, including the importance of dietary protein for older adults and why it may be beneficial to eat more protein, and to do so for breakfast. This was included to add relevance to the recipes for those in the intervention group, but made no reference to the recipes or intervention, but was identical for all participants.

**Blinding:**

Outcome assessments were undertaken by a researcher who was blind to intervention group (EvdH). Recipes and herb/spice packets were sent to intervention participants by a researcher with no direct contact with participants (KMA). Participants were not blind, although they were blind to the other possible conditions, thus the control group did not know about the intervention, nor the details of the intervention. Participants in the intervention group were asked not to mention the recipes to researchers.

**Ethical Approval and Trial Registration**

Ethical approval for this study was granted by the Research Ethics Committee of Bournemouth University, prior to commencement. Participant recruitment started in May 2016, and test sessions were run from June 2016 to April 2017. The study was registered at ClinicalTrials.gov in May 2016 (NCT02777918).

**Data analyses**

Data were analysed using IBM SPSS Statistics for Windows, version 22, using an Intention-to-treat approach. First, all measures were tested for normality. All baseline measures were then compared

between the intervention and control group using $\chi^2$ tests and Mann-Whitney U tests. Demographic and lifestyle characteristics were used to describe the sample. Correlations were investigated between all baseline measures. Multiple linear regression analyses were then used for the main analyses (Vickers & Altman, 2001). The effect of the intervention on all outcome measures was assessed using multiple linear regression, controlling for age, gender, baseline measures of the dependent variable, previous participation in related studies, and measures of total protein intake, BMI, usual physical activity and HR QoL score as assessed at the same time. Previous participation was included in regression models to allow for possible demand characteristics. Energy intake was not included in regression models following high correlations with BMI. Other demographic and lifestyle characteristics were also not included to maintain statistical power (Howell, 2012]. Separate regression models were used to predict egg intake, total protein intake, protein intake from animal sources, adverse events, lean body mass, physical performance, handgrip strength, and leg extensions as the dependent variables. Similar analyses were conducted once at the end of the 12 week intervention (T2) and once at the end of the following 12 weeks (T3). Egg intakes from the two different measures were compared using correlations and multiple linear regressions were conducted for both measures. Reasons for eating / not eating eggs and details on recipe feedback will be analysed further at a later date. These data and some of the data of participant demographic and lifestyle characteristics will be published elsewhere in exploratory analyses investigating intervention success / failure. Missing data were completed using last observation carried forward. Data are reported using means and standard deviations.

Figure 1: Schematic overview of the study design