Design and Baseline Characteristics of the Biomarkers Of Risk of Colorectal Cancer (BORICC) Follow-Up (BFU) Study: a 12+ years follow-up

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STRUCTURED ABSTRACT

Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Age is the strongest non-modifiable risk factor but it is estimated that over half of CRC cases are linked with lifestyle factors such as diet. The Biomarkers Of RIsk of Colorectal Cancer (BORICC) Study recruited 363 participants in 2005 to investigate the effects of lifestyle factors on biomarkers of CRC risk.

Aim: In the present BORICC Follow-Up (BFU) Study, we are using a longitudinal study design to investigate the effects of ageing (12+ years) and lifestyle factors on biomarkers of CRC risk and on healthy ageing.

Methods: Forty-seven of the original BORICC participants were re-recruited to the BFU Study (mean age 67 years, 51% female). Participants attended a study visit at North Tyneside General Hospital (UK) for collection of biological samples, including blood and rectal biopsies, and information collected included anthropometric measurements, a Health & Medications Questionnaire, physical activity and sedentary behaviour and habitual diet. Furthermore, musculoskeletal function was assessed by heel bone densitometry, timed up and go and hand grip strength as markers of healthy ageing. The BFU Study outcomes will be similar to those measured at baseline in the BORICC Study, such as DNA methylation and mitochondrial function, with additional measurements including the gut microbiome, faecal short-chain fatty acid concentrations and expression of genes associated with CRC.
Summary: Ultimately, identifying lifestyle factors that can reduce CRC risk, and understanding the underlying mechanisms for the effects of lifestyle and ageing on CRC risk which could lead to early prevention strategies.
INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and fourth most common cause of cancer-related death. Older age is the strongest risk factor for CRC and risk increases exponentially after the age of 50. In the UK, between 2013 and 2015, 44% of CRC cases were diagnosed in people aged ≥75 years and the highest incidence rates were in individuals aged 85-89 years. Up to 15% of CRCs are inherited with the majority (>85%) occurring sporadically. CRC risk is also modified by environmental and lifestyle factors, notably diet and physical activity, and it is estimated that 54% of CRC cases in the UK are preventable (Brown et al., 2018). It is therefore important to determine which lifestyle factors have chemoprotective properties or are associated with an increased risk of CRC and to understand the mechanisms by which they modulate CRC risk. For example, obesity, high intake of red and processed meat and high levels of sedentary behaviour increase CRC risk, whereas higher intakes of dietary fibre and high levels of physical activity are protective (WCRF/AICR, 2018). The mechanisms through which lifestyle factors modulate CRC risk include genomic damage caused by inflammation, oxidative stress and metabolic stress which may result in increased cell proliferation and reduced apoptosis. In particular, adverse dietary factors may dysregulate gene expression (increased expression of oncogenes and reduced expression of tumour suppressor genes) through epigenetic mechanisms including DNA methylation, histone modifications, changes in chromatin structure and altered patterns of expression of non-coding RNA e.g. microRNA (miRNA).
The Biomarkers Of Risk of Colorectal Cancer (BORICC) Study recruited 363 patients attending Wansbeck General Hospital, Northumberland, UK for diagnostic endoscopy (flexible sigmoidoscopy or colonoscopy) in 2005. Of these, 262 patients with no detectable large bowel pathology were recruited to the ‘healthy’ arm of the study (BORICC 1) whilst 101 patients with adenomatous polyps were recruited to the ‘polyp’ arm (BORICC 2). The aim of the study was to identify and validate potential biomarkers of CRC risk. The BORICC Study investigated effects of lifestyle factors, particularly diet and adiposity, and age on biomarkers of CRC risk, with a focus on DNA methylation (Tapp et al., 2013).

At baseline, age was a major determinant of gene-specific methylation levels (Tapp et al., 2013). In addition folate status (plasma folate and red cell folate), plasma 25-hydroxyvitamin D and plasma selenium and waist and hip circumferences were associated with both gene-specific, e.g. WIF-1 and SFRP1, and global (LINE-1) DNA methylation levels (Tapp et al., 2013). These findings suggested that these nutrition-related factors modulate methylation patterns in the colorectal mucosa of healthy individuals and that effects on DNA methylation may be a potential mechanism for the modulation of CRC risk by lifestyle factors. In addition, low selenium status was associated with aberrant gene expression detectable at mRNA and protein levels in the colorectal mucosa, including genes implicated in cancer, cell growth and proliferation, cell death and the inflammatory response (Meplan et al., 2016). Using BORICC Study samples, we also observed that clonal expansion of mitochondrial DNA mutations is the main mechanism causing age-related mitochondrial dysfunction in the apparently-healthy colorectal epithelium (Greaves et al., 2014).
The aim of the BFU Study is to investigate the effects of increasing age, as well as lifestyle factors notably diet, adiposity, physical activity and sedentary behaviour, on biomarkers of large bowel health and CRC risk. In addition, we aim to investigate the effects of change in lifestyle factors over 12+ years on markers of healthy ageing, large bowel health and CRC risk.

We hypothesise that i) biomarkers of CRC risk worsen with age and ii) higher risk lifestyles, including unhealthy dietary patterns, higher adiposity and low physical activity, exacerbate this age-related increase in risk. This project aims to test these hypotheses by examining differences in biomarkers of CRC risk cross-sectionally and by determining changes in these biomarkers longitudinally (after 12+ years) in BFU Study participants.

**METHODS**

**Study design**

The BFU Study is a longitudinal 12+ years follow-up of participants in the BORICC Study (recruited in 2005) that investigated the effects of lifestyle on biomarkers of bowel cancer risk. The BFU Study will investigate the effects of ageing (12+ years) on these biomarkers. Longitudinally, we will investigate how lifestyle factors at baseline impact on biomarkers of bowel cancer risk 12+ years later, and how change in lifestyle factors (e.g. increased adiposity or change in diet) influence these biomarkers. Cross-sectionally, we will investigate associations between current lifestyle factors and CRC-related biomarkers.
trial is registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT04005742).

**Study size**
To provide an estimate of the numbers of participants that we would need to re-recruit, we used data for differences in our primary outcome faecal calprotectin (FCP) concentration between participants who differed in mean age by 10 years at baseline for a power calculation. Using an alpha of 0.05 for 2-sided tests, mean FCP concentrations of 11.0 and 16.7 mg/kg for the younger and older groups (differing in mean age by 10 years) respectively and a standard deviation for the whole population of 10.4, showed that we required 53 participants (power of test = 0.8).

**Ethical approval**
Ethical approval for the BFU Study was granted by the West Midlands - Coventry & Warwickshire Research Ethics Committee on 29th November 2016 (REC No. 16/WM/0424). Two amendments were made to the study; the first to include a response card and invite potential participants to Showcase events described below (approved May 2017) and the second to call any participants not returning their response cards to check whether they had received the study invitation letter (approved December 2017). Caldicott approval for the storage of data was provided by the Northumbria NHS Foundation Trust.

**Participant recruitment**
Participants who took part in the BORICC Study at baseline were invited to participate in the BFU Study. Exclusion criteria included not being able to provide informed written consent or not being able to travel to attend the hospital study visit. Participants on anticoagulant medication that may increase their risk of bleeding during rigid sigmoidoscopy were invited to participate in all aspects of the study except the collection of rectal mucosal biopsies. Participant recruitment started in March 2017 and ended in June 2018. The steps taken to recruit participants to the BFU Study are summarised in Figure 1.

**Invitation letter**

An invitation letter, information leaflet explaining the study, a flyer advertising BFU Study Showcase events (please see following section) and a response card with a stamped and addressed envelope were sent to potential participants in batches of 60 invitations. If no response was received within 3 weeks, a second invitation letter was posted. Participants were asked to return their response cards to the research team by ticking one of four possible responses:

- Yes, I am interested in attending the BFU Study Showcase Event on ___
- Yes, I am interested in attending the BFU Study Showcase Event however these dates are not suitable for me
- Yes, I am interested in taking part in the BFU Study but I prefer not to attend a Showcase Event
- No, I am not interested in taking part in the BFU Study and I do not want to be contacted again by the research team
Those interested in taking part in the study were asked to contact the research team by telephone, email or post. If a participant responded saying that they would like to attend a Showcase event, a suitable date and time was arranged and a letter was posted to them with directions to the North Tyneside General Hospital Education Centre.

**Study Pack**

Study packs were posted to participants approximately two weeks before their arranged study visit date. This was to allow participants sufficient time to wear the accelerometer for a week before the visit, to complete the questionnaires and to collect urine and stool samples.

The study packs contained a folder including:

- Study pack instructions
- Consent forms
- Hospital study visit instructions and directions
- Food frequency questionnaire (FFQ)
- Lifestyle questionnaire
- Sunlight exposure questionnaire
- Accelerometer instructions, record sheet and sleep log
- Stool collection instructions
- Urine collection instructions
- At-Home sample and questionnaire collection record sheet

The following were also included in the study pack:

- Accelerometer (GENEActiv™)
- Stool collection pot (Fecotainer®)
- Cool bag and block for transportation of samples
- Urine collection pots x 2
- Urine vacutainers x 8 separated into two zip lock bags

**Food Frequency Questionnaire**

Participants completed a food frequency questionnaire (FFQ) that was adapted from that used in the EPIC Study (Bingham et al., 1997; Kroke et al., 1999). Details of adaptations are provided in Supplementary Material.

**Physical activity and sedentary behaviour**

Physical activity and sedentary behaviour were assessed subjectively via a self-reported lifestyle questionnaire used previously in the EPIC Study (Cust et al., 2008) and used in the BORICC Study at baseline. This was extended to collect information on sedentary behaviour using the LASA (Longitudinal Aging Study Amsterdam) sedentary behaviour questionnaire (Visser and Koster, 2013).

Physical activity and sedentary behaviour were also assessed objectively using the GENEActiv™ accelerometer. Participants were asked to wear the accelerometer on their non-dominant hand, continuously, for 7 days prior to their study visit. Sleep logs were used to determine sleep and non-sleep times for participants and to identify shift workers. An accelerometer record sheet was also completed to record any periods of time when the accelerometer was removed.
**Sunlight exposure questionnaire**

Habitual sunlight exposure including day-to-day sun exposure and sun exposure during holidays was assessed using an adapted questionnaire (Cashman et al., 2008). This sunlight exposure questionnaire has been used previously to determine the effect of sunlight exposure on nutritional requirements for vitamin D with good predictability.

**Urine and stool sample collection**

Equipment for the collection of urine and stool at home were included in the study packs posted to participants. Participants were asked to collect two urine samples, one on a weekend day and one on a weekday. Mid-stream urine specimens were collected in a sample collection container and transferred to 4 vacutainer tubes which were stored in the fridge. Stool samples were collected in a Fecotainer® (AT Medical, The Netherlands). Participants were asked to collect their stool samples on the day of, or the day before, their study visit. Participants recorded the date and time the urine and faeces samples were collected and brought these to their study visit in the cool bags provided. The stool samples were homogenised by mashing in a strong, zip-lock bag and aliquoted using sterile plastic spatulas into five bijou tubes. Urine and stool samples were stored at -80°C.

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**Figure 1: Schematic of participant recruitment to the BFU Study**

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**Study visit**
Study visits, lasting approximately 45 minutes (30 minutes if rectal mucosal biopsies were not collected), were carried out at North Tyneside General Hospital (UK). Consent forms were counter-signed by a member of the research team, questionnaires and paperwork were checked to ensure completion and the accelerometers were collected. Anthropometric measures (described below) and three musculoskeletal function tests (heel bone densitometry, hand grip strength and timed up and go test) were performed. A clinical member of the research team (AJ or KEG) completed the Health & Medications Questionnaire and described the clinical procedures (buccal cell swabs, blood samples and collection of rectal biopsies) to be made. In the clinical sample room, samples were collected in the order blood samples, buccal cell sample and rectal mucosal biopsies.

**Anthropometric Measurements**

Height was measured using a Leicester stadiometer (Seca) in the Frankfurt Plane and Tanita digital scales (Tanita Europe B.V., The Netherlands) were used to measure body mass, body fat percentage and body mass index (BMI). Waist and hip circumferences were measured as described by Marfell-Jones, Olds *et al.* (2006). All measurements were performed twice or repeated until within 0.1cm for height, 0.1kg for weight and 1cm for waist and hip circumferences. The mean of the two closest measurements was used for further analyses.

*Heel bone densitometry*
Heel bone density was measured using the Achilles heel ultrasound device GE AchillesTM EXPII ultrasonometer) in compliance with the product manual guidelines. Participants were asked to remove their shoes and socks and sit on a chair. Participants placed one foot in the footplace of the instrument making sure the heel was as far back as possible and the calf was resting on the calf rest. Participant details were entered onto the machine (participant ID, age, gender). Membranes of the heel ultrasound device and either side of the heel were sprayed with ethanol solution. The membranes were sprayed with 70% ethanol and the measurement was made. The measurement was made twice for both heels and an average calculated for each heel. Calibration and quality control tests were completed prior to each participant using a phantom.

*Hand grip strength*

Hand grip strength was measured with a Jamar dynamometer. The participant’s dominant hand was recorded as well as any problems or pain in their arms or hands. Participants sat on a chair with an armrest and rested one forearm on the armrest, with the elbow bent at a 90° angle. Three measurements were made per arm, following a practice measurement.

*Timed up and go test*

Prior to starting, participants were asked if they suffered from any lower body problems or pain. Participants were asked to sit on a chair and, when instructed, to stand up, without help from arms or leaping, and to walk 3 metres to a floor marker at a comfortable speed without running, turning, walking back to the chair and sitting down, again without jumping or helping with their arms. The
timer was started as soon as the participants were instructed to ‘go’ and stopped as soon as the participant sat down. The test was repeated three times with a 30 second break between tests.

**Biological sample collection:**

**Buccal cell swabs**

Participants were asked to swab up and down the inside of their cheeks firmly with the buccal swab (SK-2S DNA buccal swabs (Isohelix™, UK)) for one minute. Swabs were placed immediately in the 2ml collection tube and 500µl BuccalFix stabilisation buffer (Isohelix™, UK) were added.

**Blood sample collection**

Blood samples were collected in four 5ml BD Vacutainer® SST™ II Advance tubes with gold hemogard closure (Becton Dickinson, UK), two 4ml BD Vacutainer® K$_3$EDTA tubes (Becton Dickinson, UK) and one 5ml fluoride/oxalate tube. An aliquot of whole blood from a K$_3$EDTA tube was separated to be sent for analysis of HbA1c concentration. Vacutainers were centrifuged at 7,000 rpm for 10 minutes immediately after collection. Serum and plasma were aspirated and divided into 500µl aliquots in 1.5ml centrifuge tubes. After aspiration of serum from the BD Vacutainer® SST™ II Advance tube with gold hemogard closure tubes, white blood cells were removed using a Pasteur pipette and aliquoted into four separate 1.5ml centrifuge tubes. Aliquots of whole blood, plasma and serum were sent immediately to North Tyneside General Hospital laboratories for analysis of HbA1c, glucose and lipids,
respectively. Serum aliquots were sent to the Newcastle Laboratories (Royal Victoria Infirmary and Freeman Hospital, Newcastle) for analysis of high-sensitivity C-reactive protein (hsCRP), folate, vitamin B12, vitamin D (total 25(OH)D) and parathyroid hormone (PTH)). The remaining samples were frozen immediately and stored at -80°C for subsequent analyses.

_Human colorectal tissue samples_

Ten biopsies were taken from the mid-rectum (10cm from the ano-rectal verge) from the apparently-normal, healthy mucosa using Sarratt biopsy forceps (Stericom, 2.3mm diameter, smooth without spike REF STE1500). No bowel preparation was used. Following the rigid sigmoidoscopy procedure, participants were given an aftercare advice information sheet. Ten circumferential biopsies were taken from each participant and were processed as detailed in Table 1. Snap-frozen biopsies and those in RNAlater® were immediately placed in liquid nitrogen and then stored at -80°C. Two biopsies were formalin fixed and paraffin-embedded and blocked at Northumbria Healthcare NHS Foundation Trust and stored at room temperature. Biopsies preserved in Carnoy’s solution (70% ethanol, 30% acetic acid) were kept at 4°C for a minimum of 2 hours and maximum of 12 hours and then transferred to 70% ethanol for long-term storage at 4°C.

| Table 1 Biopsy sample collection and storage |
| Colorectal cancer risk outcome measures and statistical analyses |
| In the BFU Study, we will measure similar outcomes to those measured at baseline in the BORICC Study. This will include miRNA expression and |
patterns of DNA methylation in rectal mucosal biopsies and in potential surrogate tissues, e.g. blood and buccal cells. We will quantify gut-specific and systemic inflammatory markers including faecal calprotectin and hsCRP, respectively. We will assess colonic crypt cell proliferative state, including proliferation rates and the distribution of proliferating cells along the crypt, as a marker of CRC risk. We will investigate effects of ageing and of lifestyle factors such as obesity on mitochondrial function, as well as the involvement of the mitochondria in CRC risk. We will characterise the faecal microbiome and quantify short-chain fatty acids.

Relationships between lifestyle factors measured at baseline with markers of CRC risk at follow-up (12+ years later) will be investigated longitudinally, as well as cross-sectionally at follow-up. Relationships between potential predictors measured at baseline e.g. BMI and outcome variables e.g. faecal calprotectin will be examined by regression controlling for potential confounders such as sex, age, smoking, dietary factors and physical activity. We will also investigate relationships between change in lifestyle factors over 12+ years, such as a change in markers of adiposity, on our outcome measures. For example, to test whether change in adiposity over 12+ years is associated with faecal calprotectin concentration, we will divide our participants into those who gained body weight, those who lost body weight and those whose weight was unchanged, with faecal calprotectin as our outcome measure, using the ANOVA General Linear Model.
RESULTS

Baseline participant characteristics

A total of 47 participants were recruited to the BFU Study, comprising 37 initially-healthy participants (originally recruited to the BORICC 1 arm) and 10 polyp participants (originally recruited to BORICC 2) (Table 2). All participants were Caucasian. Fifty-one percent of participants were females, all of whom were post-menopausal. The mean age of participants was 67 years (range 49 - 79 years). The mean BMI was 28.3kg/m² and the means of other markers of adiposity were above recommended cut-offs for healthy body composition (Table 2). Only one participant was a current smoker, with comparable numbers of both male and female ex- and non-smokers.

Table 2 Characteristics of BFU Study participants.

The proportion of BFU participants in each BMI category and comparisons with data from England acquired from the Health Survey for England 2017 are summarised in Table 3. Approximately 38% and 32% of BFU study participants were overweight and obese, respectively, which is similar to data for England for persons aged 65 – 74 years. In the 2017 Health Survey for England, overweight and obesity prevalence increased with age, peaking in the 55 – 64 age range (HSE, 2018). Within England, overweight and obesity rates vary by region and are greatest in the North of England. In the North East of England, 37% and 29% of persons aged 16 and over are overweight or obese, respectively (HSE, 2018) which is similar to data for the BFU Study participants (Table 3).
Table 3 Proportions (%) of participants in each BMI category in the BFU Study, in England and in the North East of England using data from the Health Survey for England 2017 (HSE, 2018).

Blood-based phenotypic markers for BFU Study participants are summarised in Table 4. Values were comparable for male and female participants. Almost half (19 participants) had hsCRP concentrations ≥2mg/L. For the majority of participants, values were within the normal range for the other analytes. All participants had 25(OH)D concentrations classified as vitamin D sufficient (>25nmol/L) using the UK criteria (NICE, 2016). For vitamin B12, two participants (one male and one female) had concentrations below the recommended 145pmol/L and one female participant had a vitamin B12 concentration above the normal range. One female participant had a serum folate concentration suggestive of folate deficiency (<3ug/L). PTH concentrations were above the recommended value of 6.4pmol/L for six participants but none was below 1.1pmol/L. HbA1c concentration was above the diabetes cut-off (48 mmol/mol) for 5 participants all of whom were already diagnosed with type 2 diabetes. A total of 23 participants had total cholesterol concentrations >5 mmol/L and 17 had a total cholesterol to HDL cholesterol ratio greater than 4. Two female participants were below, and four were above, the recommended guidelines (1.2 – 1.8 mmol/L) for HDL cholesterol. Three and eight male participants were below and above, respectively, the reference values for males (1.0 – 1.5mmol/L). Nine participants had high non-HDL concentrations (≥4.9mmol/L).
DISCUSSION

The BFU Study is a 12+ years follow-up of participants in the BORICC Study that has been designed to investigate relationships between ageing and lifestyle factors, such as diet, adiposity and physical activity, and markers of healthy ageing and of CRC risk. We re-recruited 47 of the original 363 BORICC Study participants; approximately half of the BFU Study participants were female and the mean age of all participants was 67 years. Within the BFU Study, 70% of participants were overweight or obese. This is comparable to the proportion of overweight and obese persons aged 16 and over in England (64%) and in the North East (63%) and slightly lower than the national prevalence reported for those aged 65 – 74 (75%) (HSE, 2018).

Our a priori power calculation estimated that we would require 53 participants to detect a statistically significant effect of age on FCP, a local marker of intestinal inflammation. Although we re-recruited just under this number, this power calculation was based on an age difference of 10 years whereas the longitudinal aspect of this study was 12+ years. In addition, the original power calculation was based on cross-sectional data and, since this is a longitudinal study in which we will make within-participant comparisons over time, it is likely that we will have greater power to detect effects.

Study Implications
Samples and data from the BFU Study will be used to investigate the mechanisms through which ageing and lifestyle factors, particularly diet, physical activity and adiposity, influence markers of large bowel health and CRC risk and of healthy ageing (primarily musculoskeletal function). We will focus on mitochondrial function, epigenetic mechanisms, WNT signalling and colonic crypt cell proliferative state in colonocytes and on the intestinal microbiome and their short-chain fatty acid metabolites. We will investigate the effects of lifestyle measured at baseline, particularly diet, adiposity and physical activity, on biomarkers of CRC risk 12+ years later and also examine these cross-sectionally in the BFU Study. Understanding the underlying mechanisms through which protective or detrimental effects of lifestyle factors are achieved could lead to more effective early prevention strategies and lifestyle interventions.

We will also investigate whether these ageing and lifestyle-related markers of CRC risk can be detected in surrogate tissues, such as in blood or buccal cells, and so reduce the need for invasively-collected biopsies from the large bowel, in the future. The ability to make these biomarker measurements in surrogate tissues will i) make it easier for participants in studies, ii) lower the costs for research by avoiding the need for expensive investigations of the large bowel by specialist doctors and iii) speed up the process of biomarker measurement.
Acknowledgements We acknowledge the staff at Northumbria Healthcare NHS Foundation Trust Research and Development. We also thank the BFU Study participants without whom this study would have been impossible.

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Authors’ contributions: JCM, DMB, LCG and FCM designed the study. FCM, SPB, KEG, AJ and RMTKR performed the clinical study, data collection and sample collection and processing. FCM performed data processing and statistical analyses. FCM and JCM wrote the manuscript. SPB, RMTK and TRH edited the manuscript. All authors read and approved the final manuscript.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: Ethical approval for the BFU Study was granted by the West Midlands - Coventry & Warwickshire Research Ethics Committee on 29th November 2016 (REC No. 16/WM/0424).
REFERENCES


Table 1 Biopsy sample collection and storage

<table>
<thead>
<tr>
<th>Sample processing/storage</th>
<th>Number of biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snap-frozen</td>
<td>4</td>
</tr>
<tr>
<td>RNAlater® (Ambion, USA)</td>
<td>3</td>
</tr>
<tr>
<td>Formalin-fixed paraffin-embedded (FFPE)</td>
<td>2</td>
</tr>
<tr>
<td>Carnoy’s solution</td>
<td>1</td>
</tr>
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</table>
Table 2 Characteristics of BFU Study participants.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>47</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>66.7 (49 – 79)</td>
<td>66.3 (49 – 78)</td>
<td>67.2 (52 – 79)</td>
</tr>
<tr>
<td><strong>Menopause status</strong></td>
<td>-</td>
<td>N/A</td>
<td>Post-menopausal</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>28.3 (18.9 – 39.3)</td>
<td>28.8 (18.9 – 39.3)</td>
<td>27.8 (21 – 39)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>81.5 (56.8 – 124.5)</td>
<td>91.4 (60 – 124.5)</td>
<td>72 (56.8 – 107.5)</td>
</tr>
<tr>
<td><strong>Body fat (%)</strong></td>
<td>34.2 (10.9 – 46.2)</td>
<td>29.7 (10.9 – 41.2)</td>
<td>38.6 (28.9 – 46.2)</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>96.5 (72.7 – 137.7)</td>
<td>102.4 (74.8 – 137.7)</td>
<td>80.3 (72.7 – 115.5)</td>
</tr>
<tr>
<td><strong>Hip circumference (cm)</strong></td>
<td>105 (90.3 – 136.8)</td>
<td>106.3 (90.3 – 136.8)</td>
<td>103.7 (92.9 – 128.5)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>1 (2)</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>22 (47)</td>
<td>11 (48)</td>
<td>11 (46)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>24 (51)</td>
<td>11 (48)</td>
<td>13 (54)</td>
</tr>
</tbody>
</table>

Data are presented as means and ranges are in brackets. For smoking status, data are presented as number of participants and proportion in brackets (%).
Table 3 Proportions (%) of participants in each BMI category in the BFU Study, in England and in the North East of England using data from the Health Survey for England 2017 (HSE, 2018).

<table>
<thead>
<tr>
<th>BMI category</th>
<th>BFU Study</th>
<th>England (persons aged 16 and over)</th>
<th>England (persons aged 65 – 74)</th>
<th>North East England (persons aged 16 and over)</th>
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</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>0.0</td>
<td>2.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Normal weight</td>
<td>29.8</td>
<td>34.0</td>
<td>24.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Overweight</td>
<td>38.3</td>
<td>36.0</td>
<td>43.0</td>
<td>37.0</td>
</tr>
<tr>
<td>Obese</td>
<td>31.9</td>
<td>29.0</td>
<td>33.0</td>
<td>29.0</td>
</tr>
</tbody>
</table>
Table 4 Blood phenotype markers: inflammatory, glucose control, lipid profile and nutrient status markers

<table>
<thead>
<tr>
<th></th>
<th>Optimal values</th>
<th>Proportion of participants within optimal range (%)</th>
<th>All</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (mg/L)</td>
<td>&lt;2.00</td>
<td>60</td>
<td>3.41 (0.838)</td>
<td>2.43 (0.524)</td>
<td>4.35 (1.55)</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>&lt;48.0</td>
<td>89</td>
<td>40.5 (1.06)</td>
<td>40.2 (1.40)</td>
<td>40.8 (1.61)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>&lt;5.0</td>
<td>51</td>
<td>5.25 (0.19)</td>
<td>5.03 (0.179)</td>
<td>5.46 (0.328)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.2 – 1.8 (♀)</td>
<td>64</td>
<td>1.50 (0.058)</td>
<td>1.41 (0.097)</td>
<td>1.60 (0.0583)</td>
</tr>
<tr>
<td></td>
<td>1.0 – 1.5 (♂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol to HDL-C ratio</td>
<td>&lt;4.0</td>
<td>64</td>
<td>3.68 (0.155)</td>
<td>3.85 (0.219)</td>
<td>3.51 (0.217)</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td>&lt;4.9</td>
<td>81</td>
<td>3.77 (0.183)</td>
<td>3.62 (0.179)</td>
<td>3.93 (0.321)</td>
</tr>
<tr>
<td>Serum Folate (ug/L)</td>
<td>≥3</td>
<td>98</td>
<td>7.26 (0.422)</td>
<td>8.18 (0.687)</td>
<td>6.33 (0.422)</td>
</tr>
<tr>
<td>Vitamin B12 (pmol/L)</td>
<td>145 - 569</td>
<td>94</td>
<td>284 (15.9)</td>
<td>289 (14.7)</td>
<td>281 (27.6)</td>
</tr>
<tr>
<td></td>
<td>25(OH) Vitamin D (nmol/L)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;25</td>
<td>100</td>
<td>66.3 (4.20)</td>
<td>61.3 (6.20)</td>
</tr>
<tr>
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<td>-------------</td>
</tr>
<tr>
<td></td>
<td>PTH (pmol/L)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10 – 6.40</td>
<td>87</td>
<td>4.50 (0.200)</td>
<td>4.20 (0.300)</td>
</tr>
</tbody>
</table>

Data are presented as means (SEM). <sup>a</sup>n=47. <sup>b</sup>n=46.
Invitation letter, information sheet, showcase event flyer and response card sent

- No contact after 3 weeks
  - No more contact

- Not interested in taking part
  - No more contact

- Wish to attend Showcase Event
  - Attend Showcase Event

- Interested in taking part
  - Arrange study visit date
    - Post study pack
      - Wear accelerometer
        - Attend study visit at NTGH (~45 mins)
Supplementary Material

Modifications made to the FFQ to include foods consumed frequently in the North East of England:

- **Meat and fish category:** the option for ‘fish roe, taramasalata’ was removed. In addition, ‘curry’ was added as an example for the options for ‘beef’, ‘pork’, ‘lamb’, ‘chicken, turkey or other poultry’.

- **Bread and savoury biscuits category:** the option for Crispbread was removed. Three additional options were added: i) ‘Scones, teacakes, crumpets, muffins or croissants’, ii) ‘Pitta bread, naan bread, chapati’ and iii) Garlic bread.

- **Potatoes, rice and pasta category:** Three additional options were added: i) ‘Yorkshire pudding, pancakes, dumplings’, ii) ‘Tinned pasta e.g. spaghetti, ravioli, macaroni’ and iii) ‘Super noodles, pot noodles, pot savouries’. Furthermore, ‘potato waffles’ were added to the ‘chips’ option and ‘cannelloni’ was added to the ‘Lasagne, moussaka’ option.

- **Dairy products and fats:** The following four options were added: i) ‘Low calorie, low fat salad cream’, ii) ‘Salad cream, mayonnaise’, iii) ‘French dressing’ and iv) ‘Other salad dressing’. The option for ‘Very low fat spread (less than 30% fat)’ was removed.

- **Sweets and snacks category:** The two options of ‘home baked’ and ‘ready made’ for ‘Cakes’, ‘Buns, pastries’ and ‘Fruit pies, tarts, crumbles’ were merged to form one option for each food e.g. ‘Cakes e.g. fruit, sponge, sponge pudding’. The option for ‘Sponge puddings’ was removed as a separate entity and added to the ‘Cakes’ option. The options for ‘Chocolates, single or squares’ and ‘Chocolate snack bars’
were merged to produce one option ‘Chocolates (small bar or 0.25lb of chocolates).

- **Soups, sauces and spreads category:** the following options were added to this section i) ‘Tomato based sauces e.g. pasta sauces’, ii) ‘Low calorie, low fat salad cream or mayonnaise’, iii) ‘Salad cream, mayonnaise’, iv) ‘French dressing’, v) ‘Other salad dressing’, vi) ‘Chocolate spread, chocolate nut spread’ and vii) ‘Dips e.g. hummus, cheese and chive’. The option for ‘Pickles, chutney’ was modified to ‘Relishes e.g. pickles, chutney, mustard’. ‘Syrup’ was added as a food example for the option on ‘Jam, marmalade, honey’.

- **Drinks category:** The option for ‘Coffee, decaffeinated’ was removed.

- **Fruit category:** Tangerines and clementines were added as examples to the ‘Oranges, satsumas, mandarins’ option and figs to the ‘Dried fruit’ option.

- **Vegetables category:** ‘Mixed vegetables’ were added as an option to this category. The ‘Beetroot’ option was modified to ‘Beetroot, radishes’. The option for ‘Broccoli, spring greens, kale’ was modified to ‘Broccoli’.

- **Question 5** was modified to include brands for both bread and breakfast cereals

- ‘Olive oil’ was added as an option to question 6 (What kind of fat did you most often use for frying, roasting, grilling, etc?). Further, the option to add the type of vegetable oil used was removed.

- **Questions 2, 7, 10, 11, 12 and 15 from the original EPIC Questionnaire** were removed
- Additional questions added to the modified FFQ included ‘Do you follow a special diet?’ and ‘Over the last year, how often have you eaten organic foods?’