Title

Protein Recommendation to Increase Muscle (PRIMe): Study protocol for a randomized controlled pilot trial investigating the feasibility of a high protein diet to halt loss of muscle mass in patients with colorectal cancer

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Abstract

Background: Severe muscle mass (MM) loss is a defining feature of cancer observed across all types and stages of disease and is an independent predictor of poor clinical outcomes including higher incidences of chemotherapy toxicity and decreased survival. Protein is essential to build MM, yet the optimal amount for preventing or treating muscle loss in patients with cancer remains undefined.

Methods: The Protein Recommendation to Increase Muscle (PRIMe) study is a single-center, two-armed, parallel, randomized, controlled pilot trial that assesses the feasibility of utilizing a high protein (HP) diet to positively impact clinical outcomes in people undergoing chemotherapy to treat colorectal cancer. Forty patients with newly diagnosed stage II-IV colorectal cancer who are scheduled to receive chemotherapy will be included. Participants are randomly assigned to a HP or normal protein (NP) diet for twelve weeks. The HP and NP groups receive nutrition recommendations to achieve 2.0 grams of protein per kilogram of body weight per day ($g \cdot kg^{-1} \cdot d^{-1}$) and 1.0 $g \cdot kg^{-1} \cdot d^{-1}$, respectively. These values refer to the upper and lower recommended range of protein intake for people with cancer. Energy recommendations are based on measured energy expenditure. Assessments are completed within two weeks of starting chemotherapy (baseline), at week 6, and at week 12. Changes to skeletal MM, physical function, anthropometrics, body composition, muscle strength, physical activity, energy metabolism, metabolic markers, nutritional status, quality of life, readiness to change and psychosocial determinants of behavioural change are assessed between the HP and NP groups. Feasibility of the nutritional intervention is assessed by change in MM as a surrogate marker.

Conclusions: This evidence-based study investigates the feasibility of increasing protein intake following a diagnosis of cancer on clinical outcomes during treatment for colorectal cancer. This study will inform larger trials assessing the impact of increasing protein intake in cancer to determine their importance and integration into standard clinical care for people with cancer.

Keywords

Protein, Colorectal cancer, Muscle, Physical function, Oncology nutrition, Sarcopenia

Introduction

Malnutrition is prevalent among people with cancer. Unfortunately, limited improvements to this problem have been observed over the past several decades [1]. Few cases of cancer-related malnutrition present visually in the form of low body mass index (<18.5 kg·m^{-2}) [1]. A more common but hidden condition is loss of muscle mass (MM), which is widespread across cancer types and stages at the time of diagnosis [2–7]. A review of the literature found prevalence of low MM to vary significantly among tumor topography, ranging from 5% in cancers of the respiratory tract to 89% in advanced pancreatic cancers [2,8,9]. Low MM in cancer is more pervasive than in healthy older adults aged 60-70 years [10], and is a defining feature of malnutrition in cancer, occurring with or without losses of fat mass [11].

Metabolic alterations (e.g. systemic inflammation, hypercatabolism) induced by cancer and anti-cancer treatments have compounding effects on muscle catabolism [2,3,12,13]. In addition to the high prevalence of low MM at the time of cancer diagnosis, these patients are at risk for losing a significant amount of MM during chemotherapy [14,15]. Low MM in cancer patients is a concern due to its association with diverse negative health outcomes including decreased physical function and mobility, higher incidences of chemotherapy toxicity and surgical complications, increased length of hospital stay, and decreased survival [2,16–22]. Fortunately, awareness of malnutrition in cancer has been heightened in recent years, implications of MM loss are being recognized, and maintenance of MM is emerging as an important health outcome in this population [7,8,19,23–26].

While loss of MM is a hallmark of cancer, muscle anabolism remains possible despite the detrimental influences of age and physical deconditioning. An observational longitudinal study of patients with mixed cancer types found that 15% exhibited spontaneous increases in MM earlier in the disease trajectory [27]. Importantly, as reviewed by Engelen *et al*., anabolic potential is normal

but driven by the amount and quality of nutrients (with the exception of refractory cachexia) [28]. Thus, targeted therapies are warranted to mitigate the impact of low MM in cancer [26].

Amino acids are essential for muscle health and a primary stimulator of muscle protein synthesis [29,30]. Negative changes in MM are accentuated when protein consumption is insufficient to support anabolism; thus, an adequate supply of exogenous protein and energy is required [10,26,31,32]. The link between protein intake and MM is such that other anabolic promoters may not succeed without sufficient protein intake, which is known to be variable in people with cancer [26,33]. The literature depicts a wide range of protein intake levels in this population, ranging from 0.2–2.7 grams of protein per kilogram of bodyweight per day ($g \cdot kg^{-1} \cdot d^{-1}$) [34,35]. One study suggested that 35% of people living with cancer did not meet the minimum protein recommendation of 1.0 $g \cdot kg^{-1} \cdot d^{-1}$ [35]. International oncology nutrition guidelines recommend 1.0-1.5 $g \cdot kg^{-1} \cdot d^{-1}$ but specify 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ as a target. These standards are higher than those for healthy adults (0.8 $g \cdot kg^{-1} \cdot d^{-1}$) but nonetheless do not account for MM loss caused by cancer and its treatment [36–39].

Poor nutritional practices are commonly linked to cancers of the gastrointestinal tract, including colorectal cancer [40,41]. Colorectal cancer (CRC) was the third most common cancer diagnosis and the second most common cause of cancer-related mortality worldwide in 2018 [42]. In North America, CRC is ranked fourth in terms of new cases but is the second most common cause of cancer-related death [42]. Although the prevalence of low MM varies across tumor groups, cancers of the gastrointestinal tract are associated with a high risk of malnutrition [43]. Thus, we developed the Protein Recommendation to Increase Muscle (PRIMe) study to inform the feasibility of a 12-week high protein (HP) diet (2.0 $g \cdot kg^{-1} \cdot d^{-1}$) versus a normal protein (NP) diet (1.0 $g \cdot kg^{-1} \cdot d^{-1}$), the extent to which nutrition therapy can halt MM loss during treatment, and the corresponding impacts on patient outcomes in this population [36]. A HP diet is safe for people with normal kidney function and the NP diet attains the minimum standard of care in oncology nutrition

guidelines [34,36,44,45]. This study is the first of its kind and will provide insight into the feasibility of conducting future large-scale studies exploring the impact of a higher protein intake on preventing loss of MM in cancer.

Study Objectives

The primary objective of the PRIMe study is to inform the feasibility of utilizing a HP diet to halt MM loss during cancer treatment. The secondary objective is to assess potential effects of a HP compared to NP diet on maintaining physical function over the course of cancer treatment. Exploratory objectives are to assess the feasibility of a HP diet during cancer treatment and compare effects of a HP to NP diet on anthropometrics, body composition, muscle strength, physical activity, energy metabolism, metabolic markers, nutritional status, quality of life (QoL), readiness to change and psychosocial determinants of behavioural change.

Methods/design

Trial design

The PRIMe study is a single-center, two-arm, randomized, controlled pilot trial that is currently recruiting participants [46]. This study takes place at the Human Nutrition Research Unit (HNRU) at the University of Alberta in Edmonton, Alberta, Canada. Patients are recruited at the Cross Cancer Institute, which provides cancer care to the largest catchment area in Alberta, Canada. A visual depiction of participant flow through the study is provided in **Figure 1**. Participation in this study takes place over the period of 12 weeks with outcome assessments conducted at 0, 6, and 12 weeks as shown in The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Figure for the PRIMe study (**Figure 2**).

Eligibility criteria

Ambulatory men and women between the ages of 18-85 years with a recent diagnosis of CRC (stage II-IV) who are able to provide written informed consent in English are eligible to

participate in the PRIMe study if they are able to complete all baseline study assessments within two weeks of starting chemotherapy. Those with stage I disease are not eligible as these patients are considered cured after tumour resection [47]. Additionally, eligible participants have an estimated life expectancy of at least one year. Participants must have adequate hepatic and renal function and women of childbearing potential must agree to use an effective form of contraception for the duration of the study. Reasons for exclusion include: (1) acute inflammation (assessed by neutrophil/lymphocyte ratio greater than five [48]), (2) ongoing (non-treatment related) nutritional impact symptoms, (3) severe dietary restrictions, (4) a medical condition that impacts ability to increase muscle (e.g. cachexia [49]), (5) a pacemaker *in situ*, (6) active treatment for another cancer site, (7) body weight >450 lbs, (8) uncontrolled diabetes, (9) or a recent diagnosis of thyroid disease.

Recruitment

Patients attending their initial medical oncology consultation appointment are screened for eligibility by clinic nurses. A study coordinator obtains final eligibility confirmation from the treating medical oncologist before approaching the potential participant. The study coordinator follows up with interested individuals by telephone to schedule their screening/orientation study visit at the HNRU. Once a participant has provided written informed consent, their medical record is checked for final eligibility. We more recently developed an educational video to assist with recruitment; the video can be shown to participants live or through a link sent by email. The video addresses the importance of low muscle mass and how nutrition can help [50].

Randomization and blinding

After baseline assessments are complete, participants are randomly assigned to the HP or NP arm of the study in a 1:1 allocation ratio using block randomization. The random allocation sequence is concealed by blocked cells in an Excel spreadsheet that was created by a member of the study team who does not have any interaction with the study participants or any role in study arm

allocation. A research coordinator consecutively unveils blocks for each new participant to be randomized.

Due to the nature of the intervention, neither the study team nor the participants are blinded to group allocation. The registered dietitian and members of the study team must know the group allocation to create individualized nutritional plans for participants and monitor adherence throughout the study. Being as the intervention is based on body weight and participants are asked to achieve a specified protein intake, it is possible that participants know which study arm they have been allocated to. The main outcome measure is assessed and quantified by technicians not associated with the PRIMe study who are blinded to group allocation. Secondary and exploratory outcomes are assessed by research personnel who are trained to follow a strict study protocol to avoid measurement bias.

Nutrition intervention

Participants complete a readiness to change questionnaire and a one-hour resting energy expenditure (REE) test at their screening/orientation visit. Participants are provided a paper-based 3-day food record and a food scale to record their dietary intake prior to their baseline visit. Resting energy expenditure is measured at orientation to provide the registered dietitian with information needed to create a eucaloric (promote energy balance) diet plan unique to that participant, using Food Processor Nutrition Analysis Software (version 11.0.124, ESHA Research, Salem, OR, USA). Specifically, REE is multiplied by a physical activity factor and a coefficient of 1.075 that represents the metabolizable energy content of the diet to obtain estimated energy expenditure [51].

Within two weeks of starting chemotherapy, but at least three days after chemotherapy infusion, participants return to the HNRU to complete all baseline outcome measures prior to study randomization. Once completed, the registered dietitian meets the participant to provide medical nutrition therapy. This involves a complete dietary assessment and providing nutrition counselling on the study diet unique to that participant. The unique study diet is based on the participants'

energy expenditure and study arm allocation (HP or NP diet based on body weight). Participants

assigned to the NP diet receive instructions from the registered dietitian to achieve protein intake in

line with the minimum standard of care (1 g·kg^{-1}·d^{-1}) while those assigned to the HP diet are

instructed to follow a diet containing 2 g·kg^{-1}·d^{-1} of protein [36]. Prescribed diets are translated into

a daily meal pattern that are individualized and adapted for the participant's typical dietary pattern

and preferences based on their reported usual intake, mimicking an approach described elsewhere

[32]. An example of three meals and two snacks for a 53 kg person randomized to the HP group is

depicted in **Figure 3**. The meal pattern specifies the number of 'choices' from each food group that

is recommended per day. Participants are provided with an adapted version of the *Choose Your*

Foods for Weight Management book developed by the Academy of Nutrition and Dietetics [52].

The book contains a list of foods and their respective serving size that represents one 'choice'.

Items are stratified by food groups and provide an estimated breakdown for the macronutrient

content of one 'choice'. For example, one 'choice' from the protein group contains seven grams of

protein while one 'choice' from the milk group contains eight grams of protein [52]. The reference

amount of protein from each group counts towards total protein intake. To increase accuracy of

estimated intake, participants are encouraged to use the food scale provided to them at the

beginning of the study to weigh their food portions. Participants are strongly encouraged to weigh

meat, poultry, fish, and seafood products as portion sizes are often difficult to estimate based on

volume. Changes to individual nutritional plans can be made by the dietitian as needed. Examples

of reasons for change could include significant change in body weight or energy expenditure (the

latter captured at week 6). Changes made to the intervention would not include a change in study

arm allocation.

Analogous to how pre-intervention protein intake is evaluated, participants' ability to

achieve their recommended level during the intervention is assessed by 3-day food records

completed prior to week 6 and week 12 study visits. For those that are struggling to attain their

recommended intake or anticipating protein intake to be challenging, an oral whey powder supplement made from high-quality whey protein is provided for the duration of the study. In this case, participants are encouraged to intake most of their allotted protein from whole foods and only use the protein powder provided to supplement their diet. Additional approaches to overcoming dietary challenges include information/resources on symptom management, high-protein recipes, and/or availability of pre-cooked frozen meat products.

Regardless of study group allocation, a member of the research team contacts participants by telephone on a weekly basis throughout the study to address any questions about the study diet, assess adherence (level of protein intake by 24-hour recall), inquire about any potential chemotherapy-related nutrition-impact symptoms, and monitor self-reported body weight. An in-person follow-up visit with the study team, including the registered dietitian, occurs at week 6 and at week 12 for a final round of outcome assessments.

To improve study adherence, the research team contacts participants by telephone on a weekly basis throughout the study. The midpoint study visit (week 6) is also expected to improve adherence to the study intervention as it provides the opportunity for in-person interaction with the study team and sustain motivation for dietary changes [53]. Participants are encouraged to reach out to the study team with any questions throughout the study.

During the 12-week intervention, participants are required to take a daily multivitamin that is provided to them (natural product number [NPN]: 80050882 or 80024313). They are also asked to avoid intentional weight changes and maintain baseline levels of physical activity if possible, in attempt to avoid cofounders. All other forms of concomitant standard of cancer care are permitted throughout the PRIMe study.

Although highly unlikely due to our inclusion criteria, nutritional care of the participants is transferred to oncology dietitians as part of standard of care if serious nutritional impact symptoms occur, including substantial weight loss. The study registered dietitian uses clinical judgement to

assess which participants require transfer of care at study completion.

Outcomes

As previously mentioned, participant flow is depicted in **Figure 1** and a detailed list of study outcome assessments and timeline is found in **Figure 2**. Outcome measures are assessed at baseline, week 6, and week 12.

Primary outcome

The feasibility of a HP compared to NP diet to halt MM loss is assessed by change in absolute MM as measured by appendicular skeletal muscle (ASM in kg) from baseline to week 12, as described below. We will also explore changes in ASM as a percent change from baseline to week 12.

Secondary outcome

The ability of a HP compared to NP diet to maintain physical function is assessed by Short Physical Performance Battery (SPPB) test score. Change in integral test score is assessed from baseline to week 12.

Exploratory outcomes

Feasibility of a HP diet during cancer treatment is assessed by change in ASM as a surrogate marker of increased protein intake and study attrition rate. The ability of a HP compared to NP diet to effect anthropometrics, body composition, muscle strength, physical activity, energy metabolism, metabolic markers, nutritional status, QoL, readiness to change and psychosocial determinants of behavioural change from baseline to week 12 is assessed as described in the section below.

Data collection and management

Anthropometry, muscle mass, and body composition

Anthropometric measurements including weight, height, and waist and calf circumferences are assessed. These measurements are taken with participants wearing thin, light clothing or a hospital gown. Mean weight is measured to the nearest 0.1 kg by taking three repeated measures per

assessment using a calibrated digital scale (Health o meter® Professional Remote Display, Sunbeam Products Inc., Fla., USA). Height is measured to the nearest 0.1 cm using a 235 Heightronic Digital Stadiometer (Quick Medical, Issaquah, Wash., USA). Waist and calf circumference are measured to the nearest 0.1 cm three and two times, respectively, using a measuring tape and mean value is recorded.

Appendicular skeletal MM is assessed by DXA using a General Electric Lunar Prodigy High Speed Digital Fan Beam Densiometer with encore 9.20 software (General Electric Company, Madison, WI, USA). Dual-energy X-ray absorptiometry is a safe and non-invasive measure of body composition that has minimal radiation exposure and provides compartmentalized and whole-body data on fat, lean, and bone content of the body.

Additional tools to evaluate body composition are used for future exploratory analysis of multicompartment modelling and validation of tools against more sophisticated measure in this population. Bioelectrical impedance analysis (BIA) is measured using a portable device (BODYSTAT® QuaScan 4000, BODYSTAT [Isle of Man] Ltd., Douglas, Isle of Man, British Isles) that can be used in the clinical setting to measure total body water, phase angle and impedance ratio [54]. Air-displacement plethysmography (ADP) (BOD POD Gold Standard Body Composition Tracking System, COSMED USA, Inc., Concord, CA, USA) is used to measure body volume and hence, density. When available, computed tomography (CT) scans originally used for diagnostic purposes are accessed from the patient's medical record for analyses of muscle radiodensity—the extent of lipid infiltration within the muscle [55,56]. We expect these images to be available at baseline.

Physical function, muscle strength, and physical activity

Physical function is assessed by the SPPB test, a validated measurement that includes a sit-to-stand test (five repetitions), balance testing (three variations: feet side-by-side, semi-tandem, and tandem), and a timed 2.44 meter walking test, as described elsewhere [57]. Each activity can score

up to four points, for a total of twelve points. Clinically, the SPPB is used as a measure to assess physical performance, with validated cut-points established [58].

Handgrip strength is a validated and commonly used measure of muscle strength [7]. Change in muscle strength is assessed using a Jamar® Hydraulic Hand Dynamometer (Sammons Preston Rolyan, Bolingbrook, IL, USA). The highest score from three consecutive measures of strength in the non-dominant hand is used.

Free-living physical activity levels are measured for seven consecutive days following baseline-week 12 study visits by an ActiCal accelerometer (Philips Respironics, Murrysville, PA, USA) worn on the hip. Participants are asked to keep a written log throughout the seven days, indicating use of the accelerometer and times they wake up and go to bed. Daily step count and time spent in sedentary, light, or moderate/vigorous levels of physical activity are assessed. The International Physical Activity Questionnaire (IPAQ)—Short Form is a measure of self-reported physical activity that is used to complement the accelerometer data [59]. The IPAQ inquires about time spent sitting and time spent doing physical activity (walking, moderate-intensity activities, and vigorous-intensity activities) over the past seven consecutive days [60]. A continuous total physical activity score is obtained, expressed in metabolic equivalencies of tasks minutes per week, and used to categorize self-reported physical activity as low, moderate, or high [60].

Energy metabolism

Energy metabolism is assessed by indirect calorimetry. The volume of oxygen (VO_2) and carbon dioxide (VCO_2) is measured using an open-circuit whole-body calorimetry unit (WBCU) using the Oxymat 6 O_2 analyzer (Siemens AG, Munich, Germany) and the Advance Optima AO2000 Series CO_2 analyzer (ABB Automation GmbH, Frankfurt, Germany). Participants complete a one-hour REE test in the WBCU. Differences in VCO_2 and VO_2 concentrations of air are calculated every minute during the WBCU test by the Advance Optima AO2000 Series CO_2 analyzer (ABB Automation GmbH, Frankfurt, Germany) and the Oxymat 6 O_2 analyzer (Siemens

AG, Munich, Germany). This information is transferred from the gas analyzers to a computer using the National Instruments NI USB-6221 device (National Instruments Corporation, Austin, Tex., USA) and PMCSS Software version 1.8 (Pennington Metabolic Chamber Software Suite, Pennington Biomedical Research Center, La., USA). Pre-WBCU testing preparation includes fasting for ten hours and refraining from physical activity for 24 hours. Water, medication, and minimal physical activity (e.g., morning activities of daily living and commuting to the research unit) are allowed prior to study visit. Once in the WBCU, participants are instructed to lie on their back and rest for one hour without significant movement or falling asleep.

Total energy expenditure (TEE) is assessed in a sub-group of the study population due to the increased time-commitment from participants. At baseline and week 12, participants are offered the opportunity to complete an optional 24-hour WBCU stay to measure TEE in addition to REE. Preparation for TEE measurement is the same as for REE. A standard schedule is followed for all participants who choose to complete a 24-hour WBCU stay. Since fatigue is often associated with cancer treatment, participants can nap during their stay if they feel this is representative of their typical daily activities. Scheduled physical activity is not conducted while inside the WBCU, however the participants are able to move freely within the unit. A standardized menu (3 meals, 2 snacks) is prepared on-site in the HNRU metabolic kitchen based on their estimated energy requirements (eucaloric diet). Appetite sensations are completed immediately before a meal or snack and thirty minutes after finished eating using a validated 100-mm vertical visual analogue scale to assess sensations of hunger, satiety, and desire to eat [61]. Urine is collected throughout the 24-hour WBCU stay. Participants are asked to collect their urine in a sterile plastic jug throughout the 24-hour stay and keep the jug refrigerated in the WBCU when not in use. Urine collected is analyzed for urinary nitrogen (N) to assess N balance. Total urine volume is measured then aliquoted and banked in a -80°C freezer at the HNRU for future analysis. Twenty-four hour urinary N will be assessed by chemiluminescence using a Total Organic Carbon Analyzer High-Sensitivity

model (TOC-L$_{CPH}$) with an ASI-L autosampler and TNM-L Total Nitrogen unit (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan).

Metabolic markers

Approximately 25 mL of blood is sampled from participants by venipuncture after a ten hour overnight fast. The sample is collected into BD Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) containing spray-coated silica and a polymer gel for serum separation or K$_2$-ethylenediaminetetraacetic acid (K$_2$EDTA) for plasma separation. A protease inhibitor 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (Sigma-Aldrich, Oakville, ON, Canada) is added to the K$_2$EDTA tubes and all samples are centrifuged at a relative centrifugal force of 1176 times gravity (x g) for ten minutes. Samples are aliquoted, stored at -80°C, and banked for future analysis. Hydrochloric acid (1 N, 100 µL) is added to the ghrelin aliquot prior to freezing. Plasma samples will be analyzed for ghrelin (active) using enzyme-linked immunosorbent assay (ELISA) kits from EMD Millipore Co. (Billerica, Mass., USA). Serum samples will be analyzed for leptin, insulin-like growth factor 1, adiponectin, interleukin 6, and C-reactive protein.

Nutritional status

Dietary intake is assessed by 3-day food records that include two weekdays and one weekend day. Blank records are provided, and participants are asked to record the details of the food/beverages (brand name, preparation method, etc.), time, place, and weight of what was consumed. Information on supplement and meal replacement use is also captured in the dietary records as participants are encouraged to include recipes, packaging, and labels to increase the accuracy of their dietary record. Food records are reviewed by the study team for missing information, and clarifications are discussed with participants as needed. Dietary intake is also monitored on a weekly basis by 24-hour recall that is administered over the phone by a trained member of the study team using the multiple-pass method [62]. A total of ten 24-hour recalls are collected throughout the study. Weekly assessment of protein intake allows the researchers to tailor

their nutrition advice based on each participant's ability to meet the protein quantity prescribed to them. All dietary data is entered into Food Processor Nutrition Analysis Software (version 11.0.124, ESHA Research, Salem, OR, USA), checked by a different member of the study team and then analyzed for total caloric and macronutrient content.

The Patient-Generated Subjective Global Assessment (PG-SGA) Short Form© is commonly used to assess nutritional status in the clinical and nutritional trial intervention settings [63]. In completing the PG-SGA, participants report weight change over the past one and six months; changes to food intake over the past month; nutritional impact symptoms; and functional capacity over the past month. The PG-SGA is then scored and associated with a nutritional stage (well nourished; moderately, or suspected of being, malnourished; or severely malnourished) whereby a lower score indicates a better nutritional status [63].

Quality of Life

The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) version 3 is used to assess health-related QoL in cancer [64]. Scales are used to measure function, symptoms, and global health status/QoL where a high score indicates a greater response to the measure [65]. Additionally, the Functional Assessment of Anorexia/Cachexia Treatment (FAACT) is used to measure challenges related to anorexia and cachexia [66]. A total score and subscale scores for anorexia/cachexia and physical, social/family, emotional and functional well-being are calculated as described elsewhere [67]. Quality of life can also be affected by taste and smell, which are often altered in cancer [68]. A validated questionnaire is used to assess self-reported changes to taste and smell and a chemosensory complaint score is calculated [68,69].

Psychosocial Determinants of Behavioral Change

As the intervention (in both study arms) involves dietary change from the participant, their readiness to make behavioral change is assessed using a questionnaire adapted from Marcus *et al*.

prior to the intervention [70]. Resulting scores from the questionnaire are associated with one of four stages of change (precontemplation, contemplation, action, or maintenance) [70]. Determinants of behavioral change is an optional assessment conducted through a semi-structured interview that explores gendered experiences of nutritional preferences, perceived association between diet and disease, and adherence to the study diet. Interviews are recorded and transcribed verbatim. Coding is done by hand and analyzed using thematic analysis by two members of the research team [71]. Data analysis is ongoing and data collection from the semi-structured interviews will cease once data saturation is attained.

Feasibility and safety

Feasibility of the nutritional intervention is assessed based on attrition rates and change in ASM as a surrogate marker of increased protein intake. The use of a clinical outcome in addition to traditional markers of feasibility allows for evaluation of the potential effectiveness of the intervention and provides insight into the suitability of MM as a surrogate outcome in a larger trial design. Safety is monitored by renal function using the same parameters adopted by patient's medical oncologists in which an estimated glomerular filtration rate greater than sixty millilitres per minute is considered as normal. Participants are also asked to report their weight during the weekly phone calls for close monitoring of significant weight changes that require immediate intervention. Safety is also assessed by monitoring adverse events and documenting them as they are presented.

Data management

Study data is managed using Research Electronic Data Capture (REDCap®) electronic data capture tools hosted at the University of Alberta [72,73]. REDCap® is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources [72,73]. All data is stored

in a secure location for five years. All manually entered data will be checked by a different member of the research team for accuracy. Results of this study will be disseminated to researchers, health professionals, and the public using peer-reviewed manuscripts and poster and/or oral presentations at national and international nutrition and cancer conferences or meetings.

Sample size

As this is a pilot study, a sample size calculation was not performed [74]. Instead, for a medium (0.5) effect size, 90% power, and two-sided 5% significance, a sample size of 16 per arm was chosen [75]. To account for an estimated 20% attrition rate, we are recruiting n=20 per arm for a total sample size of 40. The effect size and estimates obtained from this pilot study will be used to design future studies and conduct further statistical testing.

Statistical methods

Statistical analysis will be conducted using IBM SPSS® Statistics version 25 (IBM Corp. Released 2017. IBM Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Analysis of primary and secondary outcome variables will be assessed using the intention-to-treat principal meaning that data will be assessed based on study arm randomization, regardless of adherence to the intervention. Due to the nature of this study (pilot) all outcome variables (including primary and secondary) will also be investigated using the per-protocol method of analysis meaning that data will be analyzed based on the intervention received (level of protein intake) rather than study arm allocation. All participants with complete data on primary and secondary outcomes at baseline and week 12 will be included in the analysis. Where data on a variable is missing in over 5% of cases, multiple imputation will be used. Sensitivity analysis will be conducted between complete data and incomplete data to minimize bias.

Descriptive univariate statistics will be performed on all variables. Counts and percentages, means and standard deviations, or medians and inner quartile ranges will be used, as appropriate. All data comparisons will be carried out with an alpha-level of 5% but caution will be used when

interpreting analysis as this study was not powered for drawing statistical inference on the outcomes assessed but rather, inform the feasibility of the study intervention and a larger scale trial. Data will be examined for outliers and distribution. Normality will be assessed through graphical visualization and Shapiro-Wilk tests. Non-normally distributed data will be transformed (logarithm, square root, etc.), and if data normalization is not possible, non-parametric tests will be used for analysis. Comparisons between individuals in the HP and NP arms will be performed using the Student's t-test or the Mann–Whitney U test. Chi-squared test will be used to compare frequencies of categorical and ordinal outcome variables. We will use statistical modelling (regression analysis and generalized estimating equations) to investigate the relationship between secondary variables (e.g. tumour topography, stage, sex, and age) and changes in MM. Change over time will be explored using generalized estimating equations, a statistical technique that accounts for between-subject and within-subject correlation that is seen in repeated measures studies. Confounders (e.g. age, sex) known to affect the outcome variable will be included if collinearity is not present after verification by multiple linear regression at each time point is assessed.

Subgroup analysis of participants lost to follow up will take place to assess baseline characteristics in comparison to those who completed the trial to assess whether lost to follow up occurred at random. Total energy expenditure, urinary nitrogen, and appetite assessments will be assessed cross sectionally at baseline and separately as change over time for tests completed at both baseline and week 12.

In addition to a frequentist approach to analysis, Bayesian estimation will be used to explore evidence for intervention success as primary and secondary outcome data is gathered [76,77]. The Bayesian method allows for model parameters to be estimated in addition to testing the hypotheses of intervention effect on our primary and secondary outcomes by utilizing prior information (evidence and/or expert belief) [76–78].

Lastly, multicompartment modelling will be explored based on the simultaneous collection

of various body compartment data. Bone mineral mass is collected by DXA, total body water by BIA, body density by ADP, and body mass by scale [79]; data which will be utilized to foster the construction of a four-compartment model to improve assessment of the impact of the intervention on body composition.

Discussion

Loss of MM is prevalent across different types and stages of cancer at the time of diagnosis and is accentuated with cancer treatment [2–7,13,25]. Uni- and multi-modal therapies from various sectors of health research (e.g. exercise, pharmaceutical, and nutrition) have been investigated in the context of MM loss and cancer but significant advances in this field remain necessary [1,26]. In addition to nutrition, muscle anabolic potential can be enhanced by a combination of therapies in a multi-modal approach (e.g. exercise, anti-inflammatory therapy, optimal oncological management, etc. [26]). Exploring the synergistic effect of different concurrent therapies on muscle anabolism is needed. Despite scepticism, various nutritional therapies alone can positively impact the nutritional status of people with cancer and present as a promising ally in the fight again muscle depletion [26]. Protein is a fundamental component of muscle and thus, exogenous protein presents as a viable therapy to halt MM loss in cancer and must first be characterized in isolation before exploring the effects of a multi-modal approach.

Exploring the feasibility of utilizing a HP diet to positively impact clinical outcomes in people undergoing chemotherapy to treat colorectal cancer allows for a deeper understanding of the willingness and ability of people in this circumstance to consume a HP diet and the resulting effect that this has on MM, physical function, and other clinically-important outcomes. These findings can be used to guide a phase III clinical trial to investigate the effectiveness of dietary protein as a nutritional intervention to halt MM loss in various types of cancer, in addition to CRC. Further, oncology nutrition guidelines are based on body weight and do not consider the quantity of target tissue—muscle [26,54]. The new era of nutritional interventions should consider nutrition as a

therapy with the goal of individualized recommendations to halt MM loss in cancer. To our knowledge, this is the first study to use a whole-body calorimetry unit to assess total energy expenditure in cancer. Our exploration of multicompartment modelling could lead to more accurate predictive equations in the future for people with cancer. Ultimately, this cumulative work can help guide future oncology nutrition guidelines and begin to have a positive impact on the detrimental effects of muscle depletion in cancer.

Trial status

Enrollment started in April 2016 and will continue until desired sample size is reached. Recruitment is expected to be completed by April 2021.

Abbreviations

ADP: Air-displacement plethysmography; ASM: appendicular skeletal muscle; BIA: bioelectrical impedance analysis; CRC: colorectal cancer; CT: computed tomography; DXA: dual-energy X-ray absorptiometry; ELISA: enzyme-linked immunosorbent assay; EORTC QLQ-C30: European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30; FFM: fat-free mass; FM: fat mass; FAACT: Functional Assessment of Anorexia/Cachexia Treatment; $g \cdot kg^{-1} \cdot d^{-1}$: grams of protein per kilogram of body weight per day; HNRU: Human Nutrition Research Unit; HP: high protein; K_2EDTA: K2-ethylenediaminetetraacetic acid; IPAQ: International Physical Activity Questionnaire; MM: muscle mass; NPN: natural product number; N: nitrogen; NP: normal protein; PG-SGA: Patient-Generated Subjective Global Assessment; PRIMe: Protein Recommendation to Increase Muscle study; QoL: quality of life; REDCap®: Research Electronic Data Capture; REE: resting energy expenditure; SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials; SPPB: short physical performance battery; TEE: total energy expenditure; VCO_2: volume of carbon dioxide; VO_2: volume of oxygen; WBCU: whole-body calorimetry unit; x g: times gravity;

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Authors' contributions

CMP conceptualized the study and all authors were involved in the design of the study. All authors revised the manuscript for critically important intellectual content. All authors have read and approved the final manuscript.

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Availability of data and materials

Data sharing is not applicable to this article because no datasets were generated or analysed during the current study.

Ethics approval

This study is approved by the Health Research Ethics Board of Alberta - Cancer Committee (HREBA.CC-15-0193) and complies with the standards on the use of human participants in research. A trained member of the study team obtains written informed consent from participants. This process includes the option for blood banking and for biological specimens to be used in ancillary studies. This is a separate informed consent; the participant is not excluded if they decline to give the above-mentioned permission.

Competing interests

KNPS has received compensation from Abbott Nutrition Health Institute for speaking and consultation services provided.

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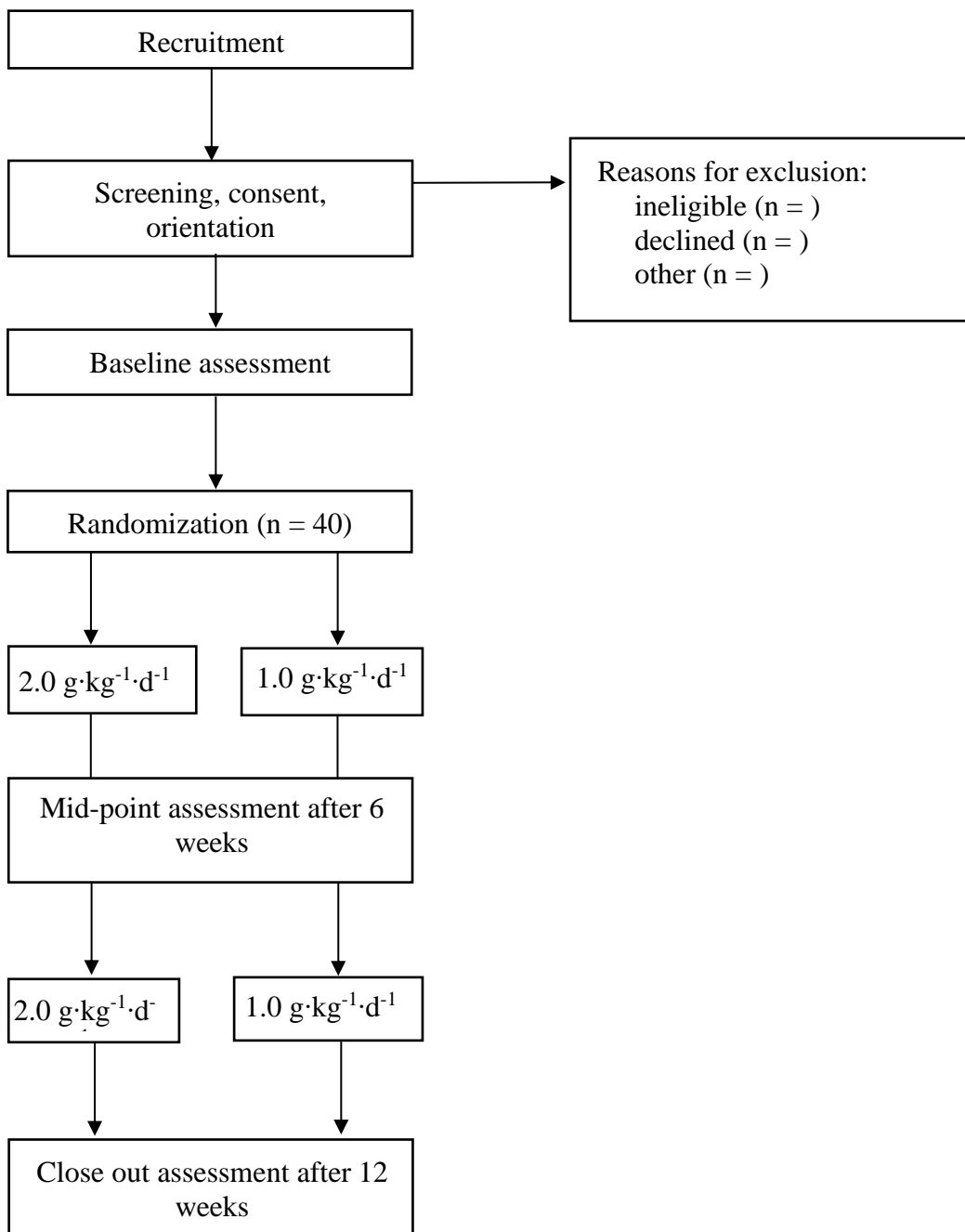
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Figure 1 Diagram of participant flow through the Protein Recommendation to Increase Muscle

(PRIMe) study.

g·kg^{-1}·d^{-1} grams of protein per kilogram of body weight per day.

	STUDY PERIOD						
	Enrollment	**Pre-allocation**	**Allocation**	**Post-allocation**			**Close-out**
TIMEPOINT	*Week -2 or Week -1*	*Baseline*	**Week 0**	*Week 1*	*Week 6*	*Week 12*	*Week 12*
ENROLLMENT:							
Eligibility screen	X						
Informed consent	X						
Allocation			X				
INTERVENTIONS:							
1.0 g protein/kg body weight/day				●━━━━━━━━━●			
2.0 g protein/kg body weight/day				●━━━━━●			
ASSESSMENTS:							
Primary outcome: Muscle mass		X			X		X
Secondary outcome: Physical function		X			X		X
Exploratory outcomes: Anthropometry body composition muscle strength physical activity energy metabolism metabolic markers nutritional status quality of life behavior change*	X (Energy metabolism only)	X			X		X
Feasibility and safety outcomes:	X	X		X	X	X	X

Figure 2 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Figure for Protein Recommendation to Increase Muscle (PRIMe) study. *Completed in a sub-set of participants.

Figure 3 Example of one-day worth of food consumed by approximately a 53 kg person randomized to the 2.0 g/kg study arm. From top left to bottom right: breakfast, lunch, supper, morning snack, evening snack.