Principal Investigator

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Study Title:

Influence of **N**utrition and **S**arco**P**enia on **E**sophageal **C**ancer ou**T**comes (INSPECT study)

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A. SPECIFIC AIMS

Hypothesis: Esophageal cancer is associated with changes in patient's body composition that are correlated with biochemical markers of cell metabolism (acetone) and systemic inflammation. Knowledge of these changes can influence patients' care through modification of treatments and outcome prediction.

Purpose: An observational, pilot study to assess longitudinal variation in exhaled acetone, body composition and other markers of nutritional status and inflammation in patients undergoing multimodal therapy for esophageal cancer. Where possible variations in these markers will be correlated with changes to early and late treatment outcomes as well as nutritional interventions that form part of patients routine care.

B. STUDY OUTCOMES

Primary outcome

i. Longitudinal assessment of breath acetone in patients undergoing multimodal therapy for esophageal cancer

Secondary outcomes

i. Correlation of breath acetone with alternate measures of nutritional status including anthropometric measurements, body composition, resting energy expenditure and blood parameters
ii. Correlation of breath acetone and alternate measures of nutritional status with patient reported nutritional intake and quality of life
iii. Assessment of the repeatability and accuracy of point of care testing of breath acetone using the Ketonix device

C. BACKGROUND AND SIGNIFICANCE

Rates of cachexia and malnutrition in patients with esophageal cancer are amongst the highest of all cancers (1-3) and are a negative prognostic indicator. Explanation for this is multifactorial but principally reflects changes in energy metabolism, tumor burden and the effects of aggressive multimodal therapy. Current methods of assessing cachexia and nutritional status however rely on measures that are often subjective and dependent on effective patient recall and co-operation. Assessment of body composition (skeletal muscle and adiposity), has found success in a number of other areas of medicine and oncology (4-7), supporting the argument that such measures should become part of routine clinical practice. The association between body composition and biochemical markers of nutritional status, including acetone, has yet to be studied in patients with esophageal cancer.

Critical knowledge gap 1: Despite its clinical promise, there remains inconsistency and a lack of consensus in regard to the adoption of methods of body composition assessment in patients with esophageal cancer. Following a systematic review of the current literature, we identified 29 studies that reported methods of body composition assessment in esophageal cancer patients. Meta-analysis of outcomes presented within these studies indicated that sarcopenia was associated with significantly lower overall survival (HR 1.70, 95% CI 1.33 to 2.17, P<0.0001) and higher rates of postoperative pneumonia (OR 2.03, 95% CI 1.32 to 3.11, P=0.001) (**APPENDIX I**). It was also noted that the reported incidence of sarcopenia in preoperative esophageal cancer patients varied between 16% and 75% and was not associated with global region. The strength of conclusions drawn from this review were however tempered by a lack of clear guidance regarding optimal methodology and reporting standards.

Critical knowledge gap 2: the association between cachexia and biochemical markers of nutritional status and inflammation in patients with esophageal cancer remains incompletely understood. Characterization of the relationship between changes in body composition and concurrently measured biochemical markers may contribute to our understanding and support the development of future assessment tools.

The measurement of acetone (a ketone body) within exhaled breath may offer a novel approach to the assessment of cancer cachexia. Ketosis occurs in all humans and is a critical part of energy metabolism, influenced by dietary intake, body composition and the presence of disease states including cancer. Ketosis relies on the breakdown of adipose tissue to produce free fatty acids that are metabolized by the liver to acetyl-CoA and subsequently to ketone bodies (**APPENDIX II**). Due to its specific chemical properties, acetone is capable of diffusing into alveolar airspaces permitting detection within exhaled breath. Several authors have demonstrated exhaled acetones potential

as a marker of fat loss (8). Our own recent studies, in patients undergoing treatment for obesity, identified that exhaled acetone increased significantly in patients following both a low carbohydrate diet and bariatric surgery compared to patients prior to these interventions (**APPENDIX III**). Exhaled acetone levels were also found to be inversely proportional to BMI and excess body weight.

C-reactive protein (CRP) is the most widely accepted index of systemic inflammation and is recognized as a robust biochemical marker for inflammation in consensus statements for malnutrition (9, 10). Accordingly CRP has been incorporated in to several clinical indices, including the Glasgow Prognostic Score (GPS), owning to its powerful association with mortality (11). The GPS has been validated as a prognostic tool in multiple cancers, predicting tumor progression, survival and symptom burden (11, 12). Other inflammatory mediators have also been linked to the development of cancer cachexia and associated poor outcomes. Macrophage inhibitory cytokine 1 (MIC-1) is elevated in cachectic patients with levels correlating to weight loss (13). Cytokines including IL-1, IL-6, IL-10 and TNFα are also believed to increase catabolism in cancer patients (14).

Significance: The accurate assessment and prevention of cachexia and malnutrition in patients with esophageal cancer continues to present a major unmet clinical need.

It is intended that this study should provide pilot data showing how variation in breath acetone is correlated to changes in body composition (particularly adipose tissue) and other markers of nutritional status and inflammation. It is hoped that based on the findings of this study we will be well placed to apply for future funding for a prospective interventional trial to assess the influence of supplementary nutrition in patients with esophageal cancer. Ultimately we hope to change clinical practice within the field through validation of the routine assessment of body composition and point of care testing of breath acetone.

D. PRELIMINARY STUDIES

Expertise, Experience and Resources: Based on his leadership and established collaborations Dr Low is internationally recognized for his ongoing contribution to the field of esophageal surgery. He has established the international Esophagectomy Complications Consensus Group (ECCG) that serves as a foundation for ongoing research within the field. Professor Baracos is a world leader in the field of cancer cachexia and is recognized for her role in developing CT methods of body composition assessment and in establishing consensus definitions for cancer-associated malnutrition. Professor Hanna is an esophageal surgeon with expertise in translational research including exhaled breath analysis. Mr Boshier is a surgical resident and 2017 Ryan Hill Research Fellow with experience in translational and outcomes research. The applicant and co-applicants have extensive experience in clinical research that is evidenced by their publications and ability to secure competitive funding. The University of Alberta and Imperial College London are world renowned academic institutions with the capacity to support the proposed research including an established methodology for body composition assessment and one of the world's largest research laboratory for clinical breath research.

Patient recruitment: Virginia Mason Medical Center and St Mary's Hospital are both high volume expert centers for the surgical treatment of esophageal cancer. Both centers have the required patient numbers and infrastructure to support prospective patient accrual.

CT body composition (University of Alberta): a leading research group in the field of cancer cachexia and malnutrition led by one of the sub-investigators (VB) will support prospective CT assessment of body composition in a consulting capacity. The group have published extensively on standardized methods of body composition assessment. The group has a proven track record in this field as evidenced by peer reviewed publications and successful funding applications. Actual CT analysis will be performed on site at Virginia Mason Medical Center, with instruction and guidance provided by University of Alberta's sub-investigator (VB). No images or subject data will be sent to University of Alberta.

Volatile organic compound (VOC) lab (Imperial College London): an established laboratory led by one of the sub-investigators (GH) will support investigations in to the role of acetone as potentially non-invasive markers of nutritional status. Over the last 10 years the group has developed reliable methods for the high throughput analysis of VOCs utilizing a combination of complementary mass-spectrometry based platforms for gas phase VOC analysis. The group has a proven track record in this field as evidence by peer reviewed publications and successful funding applications.

Immunology lab (Benaroya Research Institute at Virginia Mason): an established laboratory led by Dr Alice Long PhD will support the analysis of inflammatory markers in collected blood samples using enzyme-linked immunosorbent assays (ELISA). This group has extensive experience in performing and interpreting the results of this analysis.

E. RESEARCH DESIGN AND METHODS

Design: prospective multicenter longitudinal (observational) study recruiting from tertiary centers for the surgical management of esophageal cancer; Virginia Mason Medical Center (Seattle, USA) and St Mary's Hospital (Imperial College Healthcare NHS Trust, London, UK). This is intended to be a pilot study.

Sample size: 35 patients (15 from St Mary's Hospital Imperial College Healthcare NHS Trust, London, UK recruitment center and 20 from Virginia Mason Medical center, Seattle, USA). Sample size has been determined pragmatically based on the predicted number of patients undergoing treatment for esophageal cancer in recruitment centers during the study period. It is noted that even in high volume centers such as Virginia Mason Medical Center and St Mary's Hospital the longitudinal accrual of patients at the time of diagnosis and definitive surgery seldom exceeds 20-30 patients per year. Accordingly number of patients decided from each site during the intended 8 month study period is a conservative estimate of study recruitment. It is intended that data obtained from this pilot study will support formal power calculations for future studies.

Aspects of recruitment, consenting, data collection, and data storage will be the responsibility of each of the recruiting center. Separate ethical approval for this study will be sought at both Virginia Mason Medical Center and St Mary's Hospital where the conduct of the research will be required to meet local standards. Responsibility for the conduct of research at each site will be assigned to the local lead (Dr Low at Virginia Mason Medical Center and Professor G Hanna at St Mary's Hospital).

Inclusion criteria:
Patients with the following characteristics will be eligible for inclusion in this study:

i. aged 18-90 years
ii. newly diagnosed (prior to treatment) with esophageal and/or gastroesophageal junctional cancer (adeno- or squamous cell carcinoma)
iii. planning to undergo curative treatment, including surgical resection with neoadjuvant therapy

Exclusion criteria:
Patients with the following characteristics will not be eligible for inclusion in this study:

i. pregnant females
ii. without malignant esophageal disease
iii. inability or unwillingness to provided informed written consent

Methodology: patients undergoing curative treatment for esophageal cancer will be recruited at the time of routine clinical assessment shortly following initial diagnosis

and will undergo clinical evaluation at two study time points: (1) staging investigation (e.g. laparoscopy or endoscopy) and (2) definitive surgical resection. Study time points have been judiciously chosen to coincide with interventions that form part of patient's routine clinical care. It is intended that sampling will occur on the day of each intervention/surgery following a routine period of fasting.

Breath sample collection:
Analysis of breath acetone from samples collected from Virginia Mason Medical Center and St Mary's Hospital will be performed using a KETONIX® point of care device. Patients will be asked to exhale directly into this handheld device, which provides an on-line and real-time measurement of breath acetone concentration in parts per million.

At St Mary's Hospital breath acetone analysis will also be performed using the Proton Transfer Reaction Mass Spectrometry (PTR-MS) in order to provide cross platform validation of results.

Blood sampling:
At each study time point a single sample of venous blood (15ml) will be collected for the purpose of determining serum concentrations of a number of metabolic and inflammatory markers, e.g. C-reactive protein (CRP), albumin, lipid profile, glucose, insulin, acetone, insulin-like growth factor (IGF), macrophage inhibitory cytokine-1 (MIC-1), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10), and; tumor necrosis factor alpha (TNFα). Once collected, samples will be centrifuged and the supernatant (serum) will be aliquoted for storage at -80^{o}C.

Analysis of serum CRP, albumin, lipid profile, glucose and insulin will be performed within the biochemical pathology laboratories of the individual recruitment centers. The remaining biochemical parameters will be analyzed using commercial available enzyme-linked immunosorbent assays (ELISA) kits. An agreement has been reached with the Benaroya Research Institute at Virginia Mason to conduct the ELISA analysis for all samples.

Indirect calorimetry:
At the time of sample collection, patients will also undergo assessment of resting energy expenditure by indirect calorimetry (Fitmate, COSMED Inc, Chicago, USA). This non-invasive measurement is made by asking patients to exhale at rest into a sterile facemask that is placed over their mouth and nose. Subjects will be asked to exhale into this facemask at a normal rate and tidal volume for approximately 5 minutes while measurement of their resting energy expenditure is determined. Prior to taking measurements patients will be required to remain seated at rest for 20 minutes (in the majority of cases patients will have already been waiting for longer than this duration in department wherein they are undergoing either their surgery or other intervention). Patient will be assessed on the day of surgery or other intervention and hence will be

fasting as part of their routine care. Patients will be requested to abstain from smoking and exercise for four hours prior to testing. We will also record their food consumption in the preceding 3 days.

Assessment of body composition:
CT images that are routinely obtained at the time of diagnosis and following neoadjuvant therapy will be used to determine body composition parameters. Pseudonymized images at the anatomical level of the midpoint of the body of the third lumbar vertebra will be obtained and exported to a Digital Imaging and Communications in Medicine (DICOM) format. Images are pseudonymized at the time of retrieval from the hospital picture archiving and communication system (PACS, which removes all patient related information from the saved DICOM image. Images will be subject to segmentation to determine cross sectional muscle and adipose area using commercially available software (SlicOmatic Ver 5.0, Tomovision, Magog, Canada). Analysis of CT images will be performed at each recruiting institution. Definitions of skeletal muscle index, sarcopenia and visceral obesity have been previously (**APPENDIX IV**)(15, 16).

Data collection
The following data will be retrieved from the patient's electronic medical record (EMR) for inclusion into the database: medical record number; date of birth; sex; ethnic origin; comorbidities; details of neoadjuvant and adjuvant therapy; details of pre-operative nutritional supplementation; pathological stage and tumor characteristics. Additional data capture at the time of sampling will include: height; weight; any recent antibiotics (≤4 weeks); time of last oral intake; three day dietary report (to be completed retrospectively on the day of sampling); health-related quality of life questionnaires (QLQ-OES18, QLQ-C30, QLQ-CAX24); Patient generated subjective global assessment (PG-SGA) food intake category; performance status (ECOG, determined by either Dr Low or Professor Hanna); GPS, and; patient reported weight loss in last 6 months at study time point 1. The CTCAE (17) and ECCG (18) definitions will be used when reporting outcomes of chemotherapy and surgery respectively.

Sample storage
Blood samples will be stored in a -80^{o}C freezer within the Benaroya Research Institute. Samples will be labeled using a unique study identifier composed of the patient's study ID and the date the sample was collected.

Data storage
Virginia Mason Medical Center subject data will be stored in a pseudonymized form within a suitable platform (e.g Microsoft Office Excel or REDCap). Data will be stored on a password protected institutional (Virginia Mason Medical) computer. Paper records (including completed questionnaires) will be stored in locked filing cabinet with the department of Thoracic Surgery Virginia Mason Medical Center. Access to the data will

be limited to the principal investigator and nominated members of the research team only. Any data from samples shared with external collaborators in the United Kingdom or Canada will be in a linked anonymized form.

Data collected at St Mary's Hospital will be stored and protected as per local IRB requirements and sent to Virginia Mason Medical for inclusion in the data analysis via a secure system (e.g., Box).

Data analysis

Univariable and linear regression analysis will be used to compare exhaled acetone concentrations with body composition parameters. The coefficient of variation will be determined for repeated measurements recorded using the Ketonix device. Acetone concentrations measures using the Ketonix device and PTR-MS (St Mary's Hospital only) will be compared using Bland Altman plots. Comparisons between continuous data will be made using either the Mann-Whitney U test or Student t test, whilst categorical variables will be compared by χ^2 or Fischer exact test. Statistical significance will be assigned to 2-sided P values <0.05.

F. PROTECTION OF HUMAN SUBJECTS

Risks to human subjects:

Human Subject involvement and characteristics

Human subjects will be enrolled into this study. The purpose of recruiting human subjects is to determine the influence of esophageal cancer and neoadjuvant therapy on nutritional status and inflammatory markers. Recruitment of human subjects is critical to achieving the intended outcomes of this study.

Patients with newly diagnosed esophageal and/or gastroesophageal junctional cancer (adeno- or squamous cell carcinoma will be recruited from Virginia Mason Medical Center (n=20) and St Mary's Hospital (n=15). Patients will be asked to donate an exhaled breath sample and blood sample (15ml) at two study time points. Patients' resting energy expenditure will also be determined and patients will be asked to complete a three day dietary diary, PG-SGA food intake and quality of life questionnaires at each study time point. Any other data that are utilised as part of this study originates from patients' routine clinical care and EMR.

We do not plan to enroll vulnerable subject groups (children, persons with mental disability, prisoners) where ability to give voluntary informed consent maybe questioned or compromised.

Enrollment:

Collaborating sites:
This work will be conducted as part of a collaboration between Virginia Mason Medical Centre (Seattle, USA) and St Mary's Hospital (Imperial College Healthcare NHS Trust, London, UK).

Patient recruitment has been divided equally between the two sites and each site will be responsible for local recruitment, consenting, and data collection. All biological samples will be analyzed at the local recruiting hospital, with the exception of the analysis of specific mediators (i.e. acetone, IL-1, IL-6, IL-10, TNFα, MIC-1, IGF) which will be conducted within the Benaroya Research Institute for samples collected from both study sites.

Sources of Materials:

Patients will be required to provide written informed consent prior to enrollment in this study. Participants will be provided with an approved informed consent form and will receive careful explanation by the investigator of what is involved by taking part in the study, including potential risks and benefits.

i. Patient demographics and meta-data will be recorded for all patients (please see data collection section above). There is a small risk of breach of confidentiality.
ii. Breath samples: breath sampling is entirely non-invasive and poses negligible risk of harm to patients. Patients will be asked to provide a breath sample by blowing directly into a disposable (single use) mouthpiece attached to a hand-held breath acetone analyzer. This test is entirely non-invasive and no sample is retained. At St Mary's Hospital, an additional breath sample will be collected for analysis by PTR-MS. This sample is stored temporarily (typically <24hrs) on a Thermal Desorption (TD) tube and destroyed during the analysis process.
iii. Blood samples: blood samples (15ml) will be collected at each study time point. Where possible blood sampling will be combined with routine blood tests in order to avoid the requirement for repeated needle sticks. It is recognized that blood sampling can lead to temporary discomfort and a bruise where the needle enters the vein. Patients will be counselled about these risks as part of the process of giving informed consent.
iv. Resting energy expenditure measurement by indirect calorimetry: this is an entirely non-invasive technique, however patients will be counselled regarding the possibility of feeling discomfort when the sample mask is applied over their mouth and nose.
v. Quality of life questionnaires, PG-SGA food intake and three day food diary: patients will complete these at each study time point. There is a small risk of patients feeling uncomfortable with some of the questions; they will be counselled they may skip any questions they do not wish to answer.

Potential Risks:

Risks and benefits:
The principal risks of this study relate to the preservation of patient confidentiality in a multicenter study and the requirement for minimally invasive blood sampling.

Whilst the collection of patient data at collaborative sites will be governed by local policies, St Mary's Hospital's sharing of linked anonymized date with Virginia Mason will be subject to prior regulatory approval in the UK.

All study related documents and patient information for subjects enrolled at Virginia Mason will be stored securely within a password protected networked computer within the department of Thoracic Surgery at Virginia Mason. Hard copies of consent forms and paper questionnaires will be stored in a locked filing cabinet within a locked office in the department of Thoracic Surgery. The same standards regarding data storage are practiced at St Mary's Hospital.

Where possible blood sampling will be performed at the time of a routine blood draw so as to prevent patients having to undergo a further needle stick. Blood sampling will be performed by an appropriately trained individual. Blood sampling has the potential to cause temporary discomfort and bruising at the site of needle entry. Risk of serious harm from this intervention is considered to be low.

The risk of additional non-invasive interventions including breath sampling and measurement of resting energy expenditure, quality of life questionnaires and food diaries are considered to be negligible.

It is intended that the current study should have minimal impact on patient's routine clinical care. No extra financial costs will be passed to patients or their insurance provider.

Whilst no direct benefit is intended for individuals who agree to take part in this study, some patients may receive altruistic reward through participation in medical research that seeks to help other patients in the future.

Benefit is intended for future patients by establishing a greater understanding of methods of assessing and monitoring nutritional status in the setting of esophageal cancer and surgery.

Adequacy Of Protection Against Risks:

Recruitment and Informed Consent:

Informed written consent will be sought from all patients recruited to this study.

Patient identification: patients eligible for inclusion will be identified from the surgical practice of the local Principal Investigator (Dr Low Virginia Mason Medical Center, Professor Hanna St Mary's Hospital). Members of the research team will meet regularly with the multidisciplinary clinical team to ensure suitable identification and recruitment of patients.

Patients will be provided with details of the study in an Informed Consent Form. They will be allowed adequate time to decide whether they wish to take part in this study. Patients will be approached either at the time of a routine outpatient appointment or by telephone prior to surgery. At the time when patients are initially approached the details and requirement of the study will be explained to them. If initial contact is in-person patients will be provided with relevant study documents including the consent form. If initial contact is via telephone patients will be offered the opportunity to have these study documents sent to them either in hard copy or electronically. Written consent will be obtained in person prior to enrollment at the time of either routine outpatient appointment or on the day of sample collection.

When informed written consent is being sought within the hospital setting it will be obtained in a place of privacy by a trained member of the research team. In all cases the patient will be helped to understand the purpose of the intended research project as well as the potential benefits and risks of involvement in the study.

Where a patient does not adequately understand verbal explanations or written information in English then interpreters will be used to translate this information, in line with local codes of practice/regulations.

Patients will be made aware that any personal and confidential data related to their participation in this study will not be shared with anyone outside of their routine clinical care team or designated members of the research team. Similarly it will be explained to patients that data from collected questionnaires will be pseudonymized before being analyzed.

It will be explained to the patient that a decision not to take part will have no influence on the standard of care they receive. Likewise patients will be informed that their consent for participation can be withdrawn at any time without need for explanation and without affecting the standard of care they receive.

All of this information shall be included in the Informed Consent Form. We will not seek informed consent from vulnerable groups including: children, prisoners or patients lacking mental capacity to provide informed consent for this study.

Protection against Risk:

Risk of breach of confidentially:
This study will involve the handling, storage and transportation of sensitive patient data including: consent forms; demographic data; meta-data; questionnaire responses, and; results of physiological and biochemical analysis.

With the exception of consent forms, all data will be stored in a pseudonymized form using patients' unique study ID. Electronic data will be stored within password protected institutional computers. Paper records and clinical samples will be stored within appropriate institutional facilities protected by mechanical and/or electronic security controls. The principal investigator at each site will have access to the code linking patient identities to pseudonymized data.

Patient demographics, meta-data, food diary and questionnaire responses will be collected either in paper or electronic (e.g. Microsoft Office Excel or REDCap platform) form. Where data are collected in paper form they will be subsequently transcribed to electronic form.

Blood samples will be labelled using a unique study identifier composed of patient's study ID and date sample was collected.

Any data transferred from St Mary's Hospital to Virginia Mason Medical Center will done so in a linked anonymized from, such that patients cannot be identified by outside parties. No data from Virginia Mason Medical Center will be passed to St Mary's Hospital.

Study data will be securely stored for a period of 10 years following study conclusion.

Risk of physical harm:
It is considered that the risk of physical harm associated with this study is low. With the exception of blood testing, all other interventions are non-invasive. To minimize the risks of blood sampling, which include discomfort and bruising, where possible blood samples will be collected at the time of routine venipuncture.

Identifying and reporting adverse events:

Reporting Procedures
All adverse events (serious or non-serious) that are directly related to study activities will be reported. Adverse events (serious or non-serious) that occur as a result of patient's disease process or routine care, and any AEs that are not directly related to study procedures, will not be reported.

Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the local Principal Investigator.

Non serious adverse events
All unexpected non-serious adverse events which are related to study procedures should be recorded.

Serious adverse events
All serious adverse events related to a study procedure will be considered unexpected and thus will be reported. A report including all pertinent details related to a serious adverse event (including but not limited to: start date, stop date, thorough description of the event, treatment, and outcome) should be emailed to the local Principal Investigator (Virginia Mason Medical Center, Dr Low; St Mary's Hospital, Professor Hanna) within 24 hours. Additionally, all such serious adverse events will be reported to the IRB.

Contact details for reporting SAEs
USA
Dr Donald Low MD
Donald.Low@virginiamason.org
Tel: (206) 223-6164 (Mon to Fri 09.00 – 17.00)

UK
Professor George Hanna
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Tel: 07980323539 (Mon to Fri 09.00 – 17.00)

Potential Benefits Of The Proposed Research To The Subjects and Others.

There are no immediate benefits for the research participants recruited in to this study. Benefit is intended for future patients through aiding in surgical research. Specifically this will be through an improved understanding of the assessment of nutritional status in patients with esophageal cancer. Patients may nevertheless gain altruistic reward through participation.

It is considered that the proposed study does not pose unreasonable risk to patients. Participation in this study is intended to have no adverse impact of patient's routine standard of care and quality of life. Enrollment to the study will be conducted in accordance with Good Clinical Practice and patients will be requested to provide informed written consent.

G. REFERENCES

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H. CONSULTANTS

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Head of Division of Surgery
Imperial College London, St Mary's Hospital Campus, London, UK
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Area of expertise: surgical management of esophagogastric cancer surgery and breath analysis.
Intended role: local principal investigator St Mary's Hospital (UK)

Professor V. E. Baracos
Professor, Department of Oncology
Division of Palliative Care Medicine
University of Alberta
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Area of expertise: expert in the field of cancer-associated wasting and assessment of body composition
Intended role: assistance with body composition assessment and interpretation of study findings in a consulting and instructional capacity

Mr P. R. Boshier
Honorary Clinical research Fellow,
Imperial College London, St Mary's Hospital, London, UK
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Area of expertise: surgical outcomes of esophagogastric surgery
Intended role: link person between Virginia Mason Medical Center and St Mary's Hospital with responsibilities for daily running of the study

Dr A. Long PhD
Research Associate Member; Manager, Human Immunophenotyping Core Lab
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along@benaroyaresearch.org
Area of expertise: translational immunology
Intended role: ELISA analysis of selected inflammatory markers in blood samples collected as part of this research study

I. APPENDIX

APPENDIX I

Results of meta-analysis:

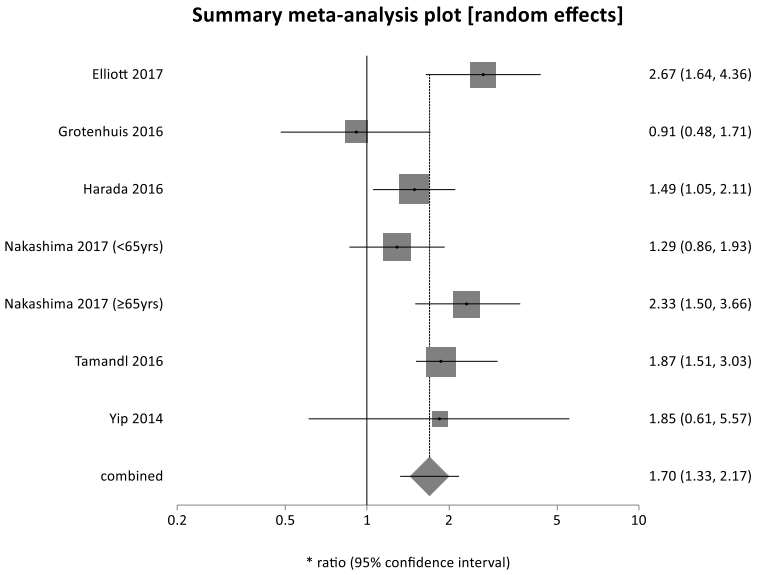

Figure IA. Summary meta-analysis of studies reporting the effect of sarcopenia on the survival of patients undergoing esophagectomy for esophageal cancer (HR 1.70, 95% CI 1.33 to 2.17, P<0.0001)

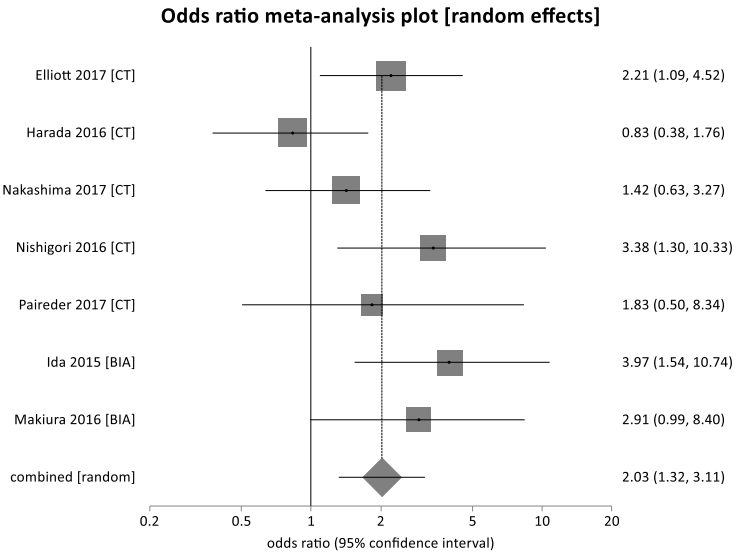

Figure IB. Summary meta-analysis of studies reporting the effect of sarcopenia on the occurrence of postoperative respiratory complications in patients undergoing esophagectomy for esophageal cancer (OR 2.03, 95% CI 1.32 to 3.11, P=0.001)

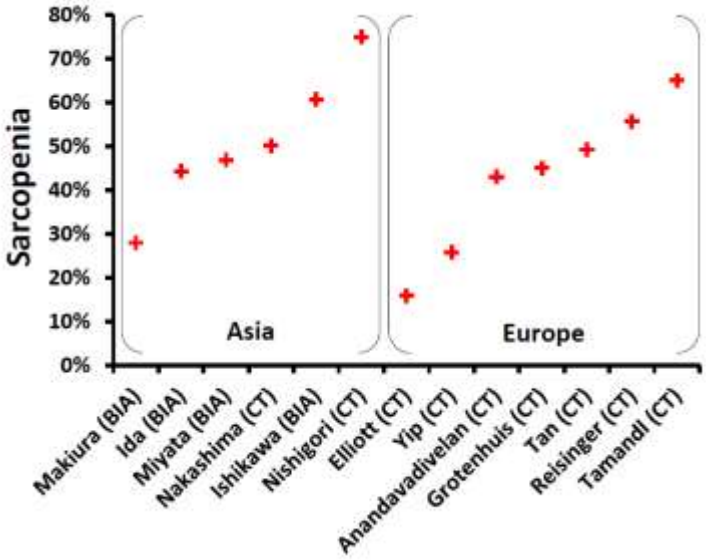

Figure IC. Reported incidence of sarcopenia in esophageal cancer patients determined prior to esophagectomy

A draft of the manuscript of this meta-analysis is available from Dr Donald Low upon request
Donald.Low@viginiamason.org

APPENDIX II

Synthetic pathway if endogenous acetone synthesis:

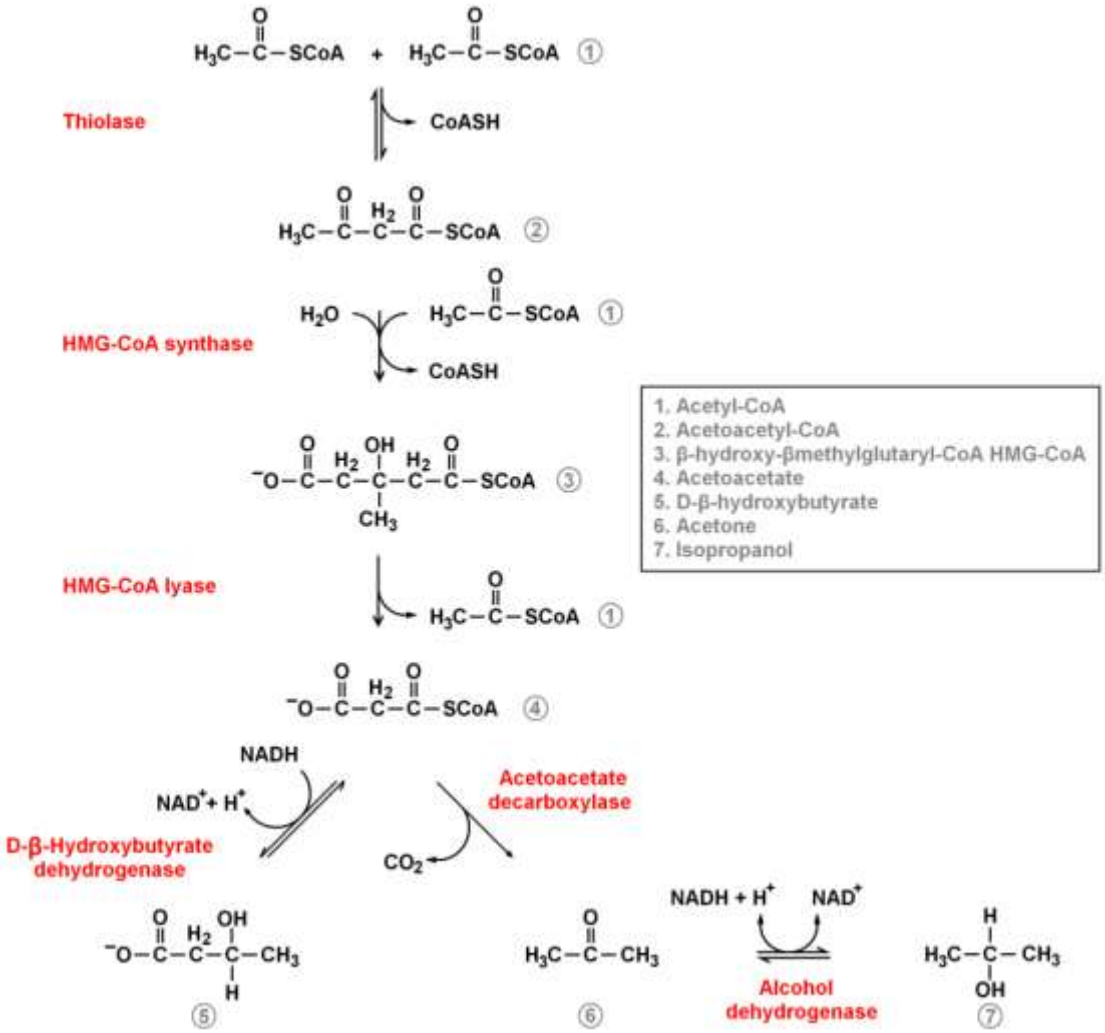

Figure IIA. Synthetic pathway of endogenous acetone synthesis

APPENDIX III

Influence of pre-operative low carbohydrate diet and bariatric surgery on exhaled acetone levels:

Methodology: Patients at different stages of treatment for obesity were recruited to this single center cross-sectional study. Sample time points were: (i) at the time of initial attendance prior to dietary or surgical interventions; (ii) on the day of surgery following low carbohydrate diet, and; (iii) >3 months after either Roux-en Y gastric bypass or sleeve gastrectomy. The concentration of acetone within breath samples, collected into steel bags, was analyzed by selected ion flow tube mass spectrometry (SIFT-MS). Exhaled acetone was correlated with measures of weight loss and biochemical markers of nutrition.

Summary of key findings relevant to current application: Exhaled acetone was observed to vary significantly in patients receiving treatment for obesity. Higher levels of acetone were observed in patients following pre-operative diet and bariatric surgery: pre-diet 410 ppb (IQR 161-796); post-diet 1396 ppb (357-2990), and; postoperative 1693 ppb (1149-2547)($P < 0.0001$)(Figure IIIA).

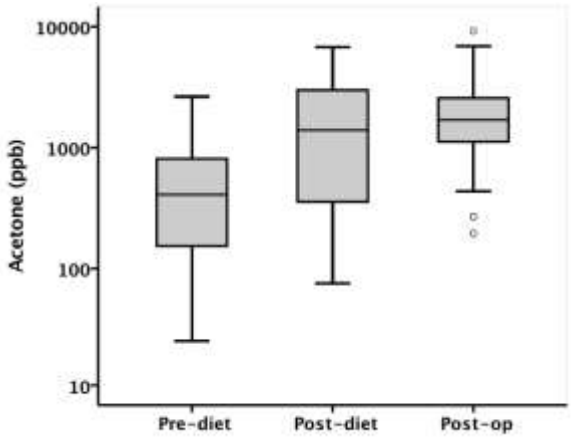

Figure IIIA. Variation in exhaled acetone concentration (Kruskal Wallis test, $P<0.001$)

Exhaled acetone levels were inversely correlated to patient BMI (ρ -0.262, P=0.005), excess body weight (EBW, ρ -0.264, P=0.005) and percentage loss of EBW (for post-diet and postoperative patients; ρ -0.375, $P<0.0001$) determined at the time of sampling. Exhaled acetone did not however correlate with rate of weight change after either dietary modification or surgical intervention ($P>0.05$).

For EBW, the results of multivariate linear regression demonstrated that neutrophil count, glucose, acetone, and low-density lipoprotein concentration explained a significant amount of variation in patients BMI (F(df 3, 43) = 7.4, P = 0.0001, R^2 = 0.41, R^2 adjusted = 0.36). With the exception of glucose (Beta = 0.24 , t(46) = 2.00, P = 0.053) each of these variables significantly predicted EBW within the model: neutrophil count (Beta = 0.41 , t(46) = 3.39, P = 0.002), acetone (Beta = -0.34 , t(46) = -2.78, P = 0.008), and low-density lipoprotein (Beta = -0.30 , t(46) = 2.42, P = 0.020).

APPENDIX IV

Methodology for calculating specific parameters of body composition:

CT or positron emission tomography with computed tomography scan acquired as part of patient's routine care will be acquired. Images at the anatomical level of L3 or the L3-L4 intervertebral disc space will be obtained and exported to a Digital Imaging and Communications in Medicine (DICOM) format. Images will be subject to automatic segmentation to determine cross sectional muscle and adipose area. Definitions of skeletal muscle index, sarcopenia and visceral obesity are provided below (15, 16).

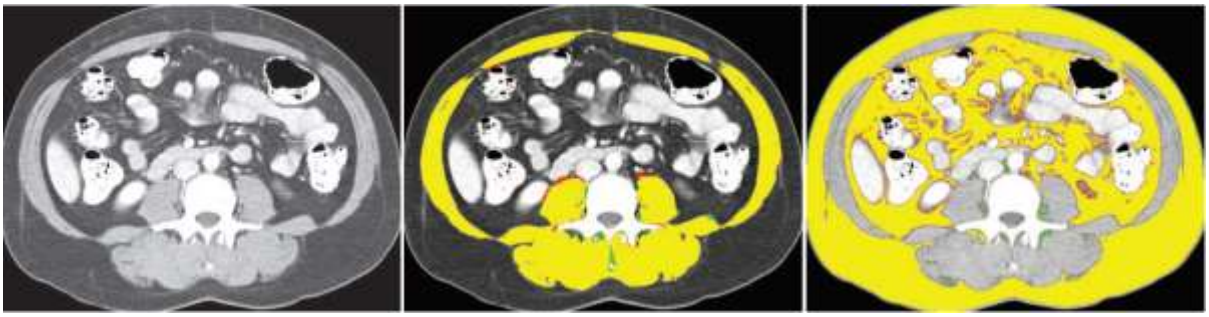

Figure IVA. Example of automatic skeletal muscle (central panel) and adipose tissue (right panel) segmentation

Skeletal muscle index (SMI) will be calculated as the ratio of lean tissue area to height:

$$SMI\ (cm^2/m^2) = \frac{Lean\ tissue\ area\ (cm^2)}{Height\ (cm^2)}$$

Sarcopenia will initially be defined as SMI <52.4 cm^2/m^2 for men and <38.5 cm^2/m^2 for women (15). The following formulae will be used to determine lean body mass and fat mass:

$$Lean\ body\ mass\ (Kg) = 0.30\ \times\ [Lean\ tissue\ area\ (cm^2)] + 6.06$$

$$Fat\ mass\ (Kg) = 0.042\ \times\ [Total\ fat\ area\ (cm^2)] + 11.2$$

Visceral obesity is defined as a fat area >163.8 cm^2 for men and >80.1 cm^2 for women.