**Protocol: The Effects of Surgery on Plasma Vitamin Levels and Cognitive Function**

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**Background**

Vitamin C has been associated with several health benefits, including the reduction of symptoms relating to colds, shortening the length of illnesses,[1](#_ENREF_1) improving immune function,[2](#_ENREF_2) increasing the synthesis of collagen,[3](#_ENREF_3) and cardiovascular improvements.[4](#_ENREF_4) Its deficiency has been associated with adverse physical effects such as fatigue and muscle weakness. [5](#_ENREF_5)

More recently, increased attention has been devoted to the potential chronic effects of a suboptimal Vitamin C status and its association to impaired brain development[6](#_ENREF_6) and neurodegenerative disease.[7](#_ENREF_7) Vitamin C in the brain is associated with a number of vital neurological functions including modulation of the cholinergic, catecholinergic, and glutamergic systems of the brain, involved in central nervous system signal transduction through neurotransmitters, as well as the general development of neurons through maturation, differentiation and myelin formation.[8](#_ENREF_8) One potential mode of action has been suggested to be through the involvement of Tetrahyrobiopterin: dihydrobiopterin ratio (BH4:BH2) in the metabolism of mono-amine neurotransmitters—dopamine, norepinephrine and serotonin—as Vitamin C maintains reduced biopterin status and may thereby indirectly regulate levels of neurotransmitters, known to be reduced in the memory deficits.[9](#_ENREF_9) It has also been proposed that vitamin C is involved in central nervous system signal transduction through neurotransmitters, as well as the general development of neurons through maturation, differentiation and myelin formation.9

Vitamin C levels as well as other biomarkers like vitamin B12 that have the potential to influence cognitive levels. Normal plasma concentrations of vitamin C are in the range of 50-60μM/L with values below 28 μmol/L representing hypovitaminosis C.21 Studies have also found an association between low vitamin C levels and impairments to cognitive function. A cohort study[10](#_ENREF_10) conducted (n = 117) in a retirement community in Sydney, Australia demonstrated that consumption of vitamin C supplements was associated with a lower prevalence of more severe cognitive impairment. Vitamin C intake was assessed at baseline (1991) with a semi quantitative food frequency questionnaire, and cognitive function was assessed 4 years later (1995).

Lower plasma vitamin C and E have been more likely to be found in demented patients than in healthy controls. This is supported by findings from a prospective cohort study from Department of Health/Medical Research Council Nutritional Programme in which participants (n = 921; ≥65 years) with the lowest Vitamin C status displayed the poorest cognitive function, a finding which persisted when corrected for age, illness, social class, or other dietary variables.[11](#_ENREF_11) Additionally, a recent an epidemiological prospective cohort study showed that high dietary intake of vitamin C and vitamin E may lower the risk of Alzheimer disease.[12](#_ENREF_12) Higher vitamin C blood levels have been found in association with a better memory performance among subjects older than 65 years.[13](#_ENREF_13)

Studies have shown that blood vitamin C concentration falls during acute phase responses such as after surgery, trauma, sepsis, and burns.29 As early as 1940, it was discovered that plasma ascorbic acid measurements from 188 surgical patients depleted following surgery.32 In a recent study, blood concentration of vitamin C decreased postoperatively and the reduction was significant 7 days after coronary heart surgery.30 The decline has also been considered to be the result of redistribution in the body and increased demand.

About one third of patients that undergo anaesthesia experience some kind of cognitive impairment and one-tenth still suffer cognitive impairments three months later. In a 2013 study, doctors at a Hong Kong hospital monitored the brain activity of 462 patients undergoing major surgery, keeping the electrical activity as high as possible while still inducing general anaesthesia. 16 percent of patients who had received light anaesthesia displayed confusion, compared with 24 percent of the routine care group. 15 percent of patients who received typical anaesthesia had postoperative mental setbacks that lingered for at least three months—they performed poorly on word-recall tests. In a study43 that explored the effects of surgery on cognition in a number of age categories (18-39/40-59/ >60) revealed a post-cognitive dysfunction upon discharge in 37% of the younger group, 30.4% of the middle aged and 41.4% of the elderly. The cognitive deficit lingered in 12.7% of the elderly 3 months after surgery. A number of cognitive domains were assessed including word learning, word recall, distractibility and working memory. More recently, a study44 incorporated a computerised cognitive battery to assess cognition post operatively in an elderly sample undergoing elective orthopaedic surgery (hip and knee replacement). Results found early and transient post-operative decline (8 days/4 months post-surgery) in reaction time in a number of cognitive tasks and visuospatial functioning constructional praxis, geometric form association, immediate visual memory, which persisted up to the 13-month follow-up. The incidence is highest in the elderly who lack cognitive reserve or those undergoing major surgery such as cardiopulmonary bypass.

Consensus has been reached that a number of cognitive domains are vulnerable to post-surgical decline, therefore psychometric tests assessing these domains should be explored.45 Domains for assessment should include word learning, short-term memory, working memory, word recall, psychomotor reaction time, information processing, sensorimotor speed and speed of recall.

The mechanisms by which anaesthetics cause persistent memory deficits in adults remain poorly understood. It is been proposed that anaesthetics activate memory-loss receptors in the brain, which may lead to no recollection of traumatic events during the surgery. During anaesthesia, increased activity of γ-aminobutyric acid type A receptors (GABAARs)46 contributes to the desired and profound neurodepressive properties of these drugs, including acute memory blockade.

Additionally, researchers at the University of Adelaide have discovered that a commonly used anaesthetic technique could increase the risk of starving the brain of oxygen with the intention of reducing blood pressure.[14](#_ENREF_14) This drop in blood pressure poses the risk of starving the brain of much needed oxygen and nutrients.

Although a number of studies have confirmed the fact that Vitamin C is depleted as a result of surgery, and the occurrence of post-operative cognitive decline has transient and long-term effects, a study is yet to investigate the effects of depleted vitamin C levels on cognitive function following surgery. A systematic study is yet to explore a link between cognitive decline post-surgery as a cause of vitamin C decline following the surgery itself. The overall project will initially aim to explore the correlation between vitamin C levels and cognitive function, investigate whether anaesthesia reduces vitamin C levels and to assess the effect of vitamin C supplementation post-surgery on cognition. The present study aims to determine whether surgery has an effect on vitamin C levels and cognition with the use of a neuropsychological test battery.

**Aims**

* To determine whether surgery has an effect on plasma vitamin C levels and cognition with the use of a neuropsychological test battery and pen and paper assessments.

**Subjects:** Adults aged between 65-85 yrs., who are scheduled for elective hip surgery or age matched control participants on the waiting list or suffering from hip issues such as osteoarthritis.

**Number:** 30 elective surgery patients and 30 control participants

**Inclusion criteria:**

-Adults 65-85 yrs. scheduled for elective hip replacement orthopaedic surgery or age matched control participants on the waiting list or suffering from hip issues such as osteoarthritis or pain/discomfort.

- Exposed to spinal anaesthetic and heavy sedation during surgery

-Good comprehension of written and spoken English

-Able to give informed consent

-Must not be taking antidepressants, antipsychotics, anxiolytics, illicit drugs or any cognitive enhancing drugs

- Not taking any vitamin C or vitamin B12 supplementation post-operatively for the duration of the study

**Exclusion criteria:**

-Colour blindness or any other severe visual impairment

-Pregnant or lactating

-Cognitive impairment (3MS score < 79)15

-History of neurological or psychiatric disorders

**Study Design:**

A cohort study investigating the effects of surgery on cognition and examining the effect of surgery on cognition and vitamin C levels. Primary outcome will be cognitive function in accuracy and speed using the SUCCAB test battery,18 in relation to plasma vitamin C. The baseline testing will be conducted roughly 1-2 weeks prior to the scheduled surgery and subsequent testing sessions will be performed within 1 week, 4-6 weeks, 3 months and 6 months after surgery. The first post-operative testing session will be dependent on length of stay for the surgery type and follow-up. For those undergoing the same type of surgery (hip replacement replacement), the first post-operative cognitive assessment will be performed roughly on the same post-operative day (i.e., 4/5 days for hip replacements). This assessment will be conducted in hospital while participants are still in-patients or coming back for follow-ups. Secondary outcome measures will include additional cognitive assessments through paper and pen testing including the Hopkins verbal learning test revised (HVLT-R)17 and symbol digits modalities test (SDMT).16 Additional secondary measures will include serum vitamin B12, pulse wave velocity (PWV)/Pulse wave analysis (PWA), wound healing, sleep , pain and mood questionnaires, cytokines involved in inflammation (Tumor necrosis factor (TNF-α), interleukin‐1 (IL‐1), and interleukin‐6 (IL‐6), and dietary patterns. A control group on the waiting list for orthopaedic surgery will have the same primary and secondary measures assessed the same number of times at the same time points as the surgery patients. This group will be used to control for practice effects on the cognitive assessments due to the repeated testing sessions.

**Recruitment and Enrolment:**

Recruitment will include any individuals who are planning to undergo orthopaedic hip replacement surgery. These participants will be recruited from a hospital with elective surgery patients (The Avenue Hospital- 40 The Avenue, Windsor, VIC 3181, Dr. David Young). Recruitment will involve the student investigator being present at the surgeon’s consultation rooms where he will approach the patients, explain the study to them, give them an opportunity to ask questions and obtain written informed consent from those agreeing to participate in the trial.

If they decide they would like to participate in the study, the student investigator will advise them to bring along the signed informed consent form to the initial testing session.

Participants will be consenting to:

* Contact details and date of surgery distributed to researcher
* Undergoing cognitive assessment and having blood tests taken 1-2 weeks before surgery, and again within 1 week, 4-6 weeks, 3 months and 6 months after surgery
* Surgery details (i.e. length and type of surgery, type of anaesthetic used, and any complications) to be made available to the researcher.

The control group will consist of participants on the waiting list or suffering from hip issues such as osteoarthritis. These participants will be potentially recruited from two sites. The first site will include patients attending The National Institute of Integrative Medicine (NIIM) who are suffering from hip issues such as osteoarthritis. A large number of these patients present to NIIM. The second site will include the surgeon’s consultation rooms, where patients who will post-pone their surgeries for over 6 months, or decide not to go ahead with their surgeries, will be recruited for the study. All of these patients will have the study explained to them by the student investigator (Nikolaj Travica).

Participants agreeing to participate will need to consent to have their contact details distributed to the researcher (including date and type of surgery of surgery, patient contact details) prior to surgery. This will enable the researcher to work around a schedule and book participants for their testing sessions at NIIM. Following surgery, details such as surgery type, duration of surgery, type and amount of anaesthetic, amount of transfusions, and any complications will be made available to the researcher for analysis.

A series of questionnaires and cognitive tests will be undertaken during each of the testing sessions:

|  |  |  |
| --- | --- | --- |
| **Test** | **Duration** | **Time points 1,2,3** |
| Demographic, history questionnaire | 5 minutes | 1,2,3,4,5 |
| Modified version of the DQES v2 (focus on vitamin C and vitamin B12) | 10 minutes | 1,2,3,4,5 |
| Nicholas Pain Self-Efficacy Questionnaire (PSEQ)34  The Verbal Numerical Rating Scale (VNRS)35 | 10 minutes  5 minutes | 1,2,3,4,5 |
| Pittsburgh sleep questionnaire assessment (PSQI)37 | 5 minutes | 1,3,4,5 |
| Modified Cardiff wound impact questionnaire (CWIS)36 | 5 minutes | 2,3,4 |
| Bond Lader Mood questionnaire38 | 5 minutes | 1,2,3,4.5 |
| Modified mini-mental state (3MS)15 | 15 minutes | 1,2 |
| Hopkins verbal learning test revised (HVLT-R)22 (Immediate) | 20 minutes | 1,2,3,4,5 |
| Swinburne University Cognitive Aging Battery (SUCCAB)18 | 20-30 minutes | 1,3,4,5 |
| HVLT-R (Delayed) | 5 minutes | 1,2,3,4,5 |
| Symbol Digits Modalities Test (SDMT)16 | 5 minutes | 1,2,3,4,5 |
| Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2)23 | 15 minutes | 1,4,5 |
| Pulse wave velocity /Pulse wave analysis (PWV/PWA) assessment 39 | 10 minutes | 1,3 |
| Blood test (vitamin C/Vitamin B12)  Cytokines | 5-10 minutes | 1,2,3,4,5  1,3,5 |
| Time point: |  |  |
| 1 | 120-130 minutes |  |
| 2 | 70-90 minutes |  |
| 3/4 | 110-120 minutes |  |

**1.** 1-2 weeks before surgery **2.** Within 1 week after surgery (Subject to surgery type) **3.** 4-6 weeks after surgery **4.** 3 months after surgery **5.** 6 months after surgery

***Study Flow***

**1-2 weeks pre-surgery**

Cognitive Assessment

Vitamin C

Vitamin B12

Note: Control group has the same assessments undertaken at the same time points, without exposure to surgery

**Immediately before surgery**

Vitamin C

Vitamin B12

*Surgery*

**Within 1 week post-surgery**

Cognitive Assessment

Vitamin C

Vitamin B12

**4-6 weeks post-surgery**

Cognitive Assessment

Vitamin C

Vitamin B12

**3 months after surgery**

Cognitive assessment

Vitamin C

Vitamin B12

**6 months after surgery**

Cognitive assessment

Vitamin C

Vitamin B12

**Demographic, History questionnaire** (15 minutes)

Assessment will include potential confounding variables, dietary intake of vitamin C, vitamin B12, medications and any life events that may play a part in cognitive functioning. In order to control for further confounding variables, a modified food frequency questionnaire based on the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2)23 (Cancer Council’s questionnaire) will be administered, with general questions regarding physical activity being administered. The questionnaire will assess the consumption of foods containing high vitamin C and the frequency at which these foods are consumed. Results will be compared with the plasma levels.

**Cognitive function testing procedure** (60-70 minutes)

Firstly, participants will complete the 3MS,15 implemented as a valid and reliable screening test for the purpose of evaluating cognitive impairment. Administration takes between 15-20 minutes, consisting of a number of different simple tasks equating to a total of 100 points. Secondly, if 3MS scores are over 79 points, participants undertake the HVLT-R22 which examines verbal learning and memory. Participants are required to recall and recognise two lists of words over immediate and delayed (20 minute) trials. This will be incorporated prior to the SUCCAB and the delayed trial (30-40 min) will be completed after the SUCCAB.

Participants will be required to complete eight computer-based tasks from the SUCCAB18 taking about 30 minutes to assess various aspects of cognitive performance. A 4-button response box was used to complete the tasks, with each button representing a colour (red, blue, green or yellow), yes or no, or the spatial location of objects on the screen (top, bottom, left or right). Following the SUCCAB and the delayed HVLT-R, participants will complete the SDMT.16 Participants are presented with a key consisting of number 1-9 with a corresponding symbol under each number. Below are rows of random numbers with blank squares below each number, and participants are given 90 seconds to fill in the blank squares. The score is the number of correctly coded items from 0-110 in 90 seconds. For time point 2 (first cognitive assessment after surgery), while participants are still in-patients, only the paper and pen cognitive tests will be administered. These will include the 3MS, HVLT-R and SDMT.

**Pain Scale and self-efficacy questionnaire** (10 minutes)

Studies have indicated that pain may play a major role in the performance on cognitive tests, especially post-surgical associated pain. Pain will also be related to wound healing and vitamin C levels. A scale assessing self-reported pain levels34 and a self-efficacy questionnaire35 will be utilised pre and post-surgery. The Nicholas Pain Self-Efficacy Questionnaire (PSEQ) is a 10-item questionnaire, developed to assess the confidence people with ongoing pain have in performing activities while in pain.

The Verbal Numerical Rating Scale (VNRS) is a reliable measure of postoperative pain. The VNRS consists of a list of adjectives describing different levels of pain intensity from a scale between 0 and 10. The VNRS of pain intensity includes the adjectives that reflect the extremes of this dimension; from 'no pain' to 'extremely intense pain' and sufficient additional points to capture gradations of pain intensity that may be experienced between these two extremes. Both of these questionnaires will be administered before each testing session.

**Sleep Quality Assessment (PSQI)** (5 minutes)

The Pittsburgh Sleep Quality Index (PSQI)37 is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates “poor” from “good” sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

**Wound healing questionnaire** (5 minutes)

Vitamin C deficiencies have been characterised by haemorrhage, impaired secretory functions, vasomotor instability, hematologic alterations, impaired wound healing, and depressed immune response. The Cardiff wound impact questionnaire (CWIS)36 is a validated self-report measure designed to assess the impact of wounds on activities of daily living (ADL) and health-related quality of life. It also has an item evaluating global subjective well-being. It also has an item evaluating global subjective well-being. Four domains: Quality of Life – 2 items; Well-Being – 7 items, graded on a 5 point scale; Physical Symptoms and Daily Living (Experienced) – 12 items, graded on a 5 point scale (1 = ‘not at all’ to 5 = ‘always’); Physical Symptoms and Daily Living (Stressfulness of Experience) – 12 items, graded on a 5 point scale (1 = ‘not at all’ to 5 = ‘always’).

**Bond-Lader Mood questionnaire** (5mins)

The Bond-Ladder consists of a 10 centimetre line anchored at each end by words descriptive of opposing statements (bipolar). On this linear scale, the person indicates their mood by placing a mark between the 2 opposite words on either side. It consists of 16 bipolar scales that measure four different concepts of mood: Mental Sedation or intellectual impairment, Physical Sedation or bodily impairment, tranquillization or calming effects, other types of feelings or attitudes.38 This will be undertaken both before and after each SUCCAB assessment at each testing session.

**Food frequency questionnaire** (15 minutes)

Additionally, the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2)23 is a validated modification of a food frequency questionnaire (FFQ) that was developed by Cancer Council Victoria in the late 1980s to measure dietary intake of people taking part in the Melbourne Collaborative Cohort Study (MCCS). The DQES v2 covers five types of dietary intake based on the previous 12 months, incorporating 80 items: cereal foods, sweets and snacks, dairy products, meats and fish, fruit and vegetables and alcoholic beverages. This will be incorporated in order to determine nutritional intake prior to surgery and determine which other possible foods/vitamins may contribute to the outcome of surgery. The DQES v2 will be completed online, following cognitive testing, 1-2 weeks prior to scheduled surgery.

**Pulse wave velocity (PWV)** (10 minutes)

**PWV** is a measure of arterial stiffness, or the rate at which pressure waves move down aterterial vessels. It has been established as a highly reliable prognostic parameter for cardiovascular morbidity and mortality in a variety of adult populations including older adults.39 Central PWV can be collected by placing a pressure catheter around the femoral artery and measuring the distance to the carotid artery. The time it takes the pressure wave to go from the upstream pressure catheter to the downstream pressure catheter provides the Pulse Transit Time (PTT).  PWV can then be calculated by dividing the distance by the transit time providing a measure of cardiovascular health. A link between PWV and cognitive function has been well established recently.41

**Vitamin C level testing – pathology testing** (30-40 minutes)

Participants will be required to fast for 8-12 hours prior to their blood test (not during hospital stay). Blood tests will be performed prior to breakfast at the hospital on the day of the cognitive testing session. Vitamin C levels will be analysed by an external lab through a pathology company. While patients are in hospital, the student investigator will take the bloods. On other testing sessions, blood tests will be performed by the researcher and sent to the pathology company.

1. Sullivan Nicolaides Pathology utilise a high performance liquid chromatographic (HPLC) method for the quantitation of vitamin C in plasma samples. HPLC is a form of column chromatography that pumps a sample mixture in a solvent at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion.

**Vitamin B12 testing – pathology testing**

Serum Vitamin B12 testing will also be conducted through Melbourne Pathology. A vitamin B12 immuno assay (Roche E602) is used to measured total vitamin B12 concentrations. This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to vitamin B12 has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labelled vitamin B12 and unlabelled vitamin B12 (Standards or samples). Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of vitamin B12 in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of vitamin B12 in the sample.

**Cytokine Testing**

In addition, blood Serum will be stored which will be used to measure a number of cytokines involved in inflammation. These cytokines will include Tumor necrosis factor (TNF-α), interleukin‐1 (IL‐1), and IL‐6, all of which have been shown to be affected during trauma such a surgery. This will be NutriPATH, a credible research lab capable of performing reliable cytokine analysis. <https://nutripath.com.au/wp-content/uploads/2013/12/4004-CYTOKINES-Serum.pdf>

**Study Procedure. Conducted in the NIIM cognitive lab, level 3.**

**Measure**

**25-30 minutes**

**75-80 minutes**

**90-100 minutes**

**DQES v2**

**Blood test**

**100-110 minutes**

**85-95 minutes**

**Time elapsed**

**Baseline questionnaire inc food frequency**

**5-10 minutes**

**3MS**

**HVLT-R test**

**(Immediate)**

**35-40 minutes**

**SUCCAB test**

**65-70 minutes**

**HVLT-R test**

**(Delayed)**

**SDMT test**

**Sample size calculation**

No study has explored the effect of surgery/anaesthesia exposure on cognition using the SUCCAB test battery. However, based on previous studies assessing POCD using a range of cognitive assessments, the expected trend would be a reduction in speed and accuracy on a number of SUCCAB tasks post-surgery. The cognitive domains expected to be affected include working memory, visuospatial performance, immediate visual memory, distractibility and reaction time.

 The study conducted by Pipingas18 explored the mean SUCCAB values in a healthy sample. It is assumed there will be a 20-50% reduction (1-2 Standard deviations respectively) in performance on SUCCAB tasks post-surgery.

For example, based on Pipingas et al., (2010) (n=120, mean age= 53, SD= 16 years):

|  |  |  |
| --- | --- | --- |
| **Cognitive domain** | **Mean (m/s) baseline score (SD)** | ***Predicted* mean (m/s) percentage score reduction Post-surgery** |
| Spatial working memory | 963 (211) | 22% (1SD)- 752  40% (2SD)- 541 |
| Stroop incongruent | 894 (184) | 21% (1SD)- 710  42% (2SD)- 526 |
| Stroop congruent | 888 (186) | 21% (1 SD)- 702  42% (2SD)-  516 |
| Immediate recognition | 1064 (134) | 25% (2SD)- 796 |
| Choice reaction time | 448 (68) | 30% (2SD)- 312 |
| Simple reaction time | 256 (45) | 35% (2SD)- 166 |

A McNemar sample size calculation47 revealed that after applying continuity correction, the study would require a sample size of 30 participants to achieve a power of 80% and two sided significances of 5% for detecting differences between -0.20 and -0.50 between testing sessions (pre and post-surgery) and controlling for a 10% drop out. A control group of matching size consisting of participants on the waiting list for orthopaedic surgery will be used to control for potential confounders such as physiological condition, cognitive practice affects, mood, blood levels and pain. The overall sample size would be 60 participants, tested at various time points.

**Analysis**

Analyses will be performed using SPSS version 22.0 and SAS version 9.2. Statistical significance will be set at p < 0.05. Regression analyses will be undertaken in order to compare vitamin C concentrations with cognitive performance before and after surgery. A repeated measures t-test will be used to determine any vitamin concentration and cognitive changes between testing sessions. Descriptive analysis will be conducted on all study variables collected at each testing session.

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