The Effects of Surgery on Leukocyte Vitamin C Concentrations: A Systematic Review and Meta-Analysis

Nikolaj Travica, Karin Ried, Irene Hudson, Andrew Scholey, Andrew Pipingas, Avni Sali

This is a PDF file of an article which was accepted for publication in Pharmaceutical Sciences. It is assigned to an issue after technical editing, formatting for publication and author proofing

The Effects of Surgery on Leukocyte Vitamin C Concentrations: A Systematic Review and Meta-Analysis

Nikolaj Travica*[1,2,3], Karin Ried[3,4,5], Irene Hudson [2,6,7], Andrew Scholey[2], Andrew Pipingas[2], Avni Sali[3]

[1] Food & Mood Centre, School of Medicine, Barwon Health, Deakin University, the Institute for Mental and Physical Health and Clinical Translation (IMPACT), Geelong, Australia

[2] Centre for Human Psychopharmacology, Swinburne University of Technology, Melbourne, VIC, Australia

[3] The National Institute of Integrative Medicine, Melbourne, VIC, Australia

[4] Honorary Associate Professor, Discipline of General Practice, University of Adelaide, South Australia, Australia

[5] Adjunct Associate Professor, Torrens University, Melbourne, VIC, Australia

[6] Digital Health, CRC, College of STEM, Mathematical Sciences, Royal Melbourne Institute of Technology (RMIT), Melbourne, Australia

[7] Conjoint Professor, School of Mathematical and Physical Science, University of Newcastle, Newcastle, Australia

Running Title: Surgery and Leukocyte Vitamin C

* Corresponding author:
Nikolaj Travica
Address: 1 Gheringhap St, Geelong, VIC, 3220
Tel: +03-5227 8288
Email: nikolaj.traivca@deakin.edu.au

Abstract

Background

Vitamin C (ascorbic acid) is a micronutrient imperative for many bodily functions, with research revealing its functional support of leukocytes. The purpose of this meta-analysis was to determine the effects of surgery on leukocyte vitamin C concentrations by assessing the amount and longevity of post-operative leukocyte vitamin C change.

Data Sources

We searched the PUBMED, SCOPUS, SciSearch and the Cochrane Library databases for relevant research papers. Studies were included until January 2022, with no time limits. Studies that reported means and standard deviations of leukocyte/buffer layer vitamin C concentrations before and after surgery were included into the meta-analysis. The meta-analysis comprised of eight studies.

Results

Seven studies (which included eight individual studies) were included into our meta-analysis. Combined data from the meta-analysis displayed a significant depletion in mean leukocyte vitamin C concentrations during the first 24 hours following the surgery by a mean of 5.37 $\mu g/10^8$ (32.3%) (CI = -6.35, -4.40) (n = 6, p < 0.001) and during the first post-operative week by 4.43 $\mu g/10^8$ (23%) (-7.27, -1.58) (n = 7, p < 0.001). However, this depletion was followed by an uprise in mean ascorbic acid leukocyte concentrations of 0.93 $\mu g/10^8$ (4.8%) (-0.79, 2.66) (n = 6, p = 0.29) at 7 days (or beyond) post-operatively.

Conclusion

Significant post-operative leukocyte vitamin C depletions were particularly observed during the first post-operative week. Further research is required to validate the observed results and to determine whether the observed depletions may be linked to compromised immunological function and pathophysiologies that arise during the post-operative period.

Keywords: ascorbic acid, leukocyte, post-operative, surgery, vitamin C

1. Introduction

Vitamin C, otherwise known as ascorbic acid, is an essential micronutrient for a range of biological processes.[1] The vitamin's functions have been studied extensively, with both animal and human studies highlighting its pivotal involvement in immune function.[2] Indeed, vitamin C can stimulate the production[3] and support the optimal function[2, 4] of leukocytes (white blood cells), particularly neutrophils, lymphocytes, and phagocytes.

Specific leukocyte vitamin C functions include cellular motility,[2] chemotaxis[5] and phagocytosis. These functions are mediated by the vitamin's ability to enhance neutrophil movement in response to chemoattractants (chemotaxis), enhance the phagocytosis of microbes and stimulate the production of reactive oxygen species (ROS) for the destruction of microbes.[2] Phagocytic leukocytes also produce and release antiviral interferons, which are

augmented in response to vitamin C exposure (in vitro).[2] Moreover, it has been proposed that vitamin C is involved in the potential proliferation and differentiation of B and T-lymphocytes.[3] The protective antioxidant properties of vitamin C largely support leukocytes.[2] The accumulation of large concentrations of vitamin C into neutrophils, particularly following an oxidative burst, is thought to protect neutrophils.[6] Based on animal models, vitamin C can diminish both oxidant generation and pro-inflammatory transcription factor nuclear factor κB (NFκB) activation in neutrophils.[7] Vitamin C has also shown the ability to attenuate IL-4 secretion and augment IFN-γ secretion.[8] Moreover, vitamin C may attenuate the activation of microglia, consequently reducing the synthesis of pro-inflammatory cytokines.[9]

Additionally, lymphocyte ascorbic acid concentrations have demonstrated a strong association with lymphocyte glutathione,[10] a potent antioxidant with central roles in the defence against free radicals and oxidants. A synergistic relationship has also been observed between ascorbic acid and lymphocyte glutathione, with ascorbic acid demonstrating the capacity to convert the oxidized free radical of glutathione back into its reduced form within lymphocytes.[10]

States of vitamin C deficiency may compromise the ability of leukocytes to exert their functions optimally. This has been exemplified with impaired phagocytosis and/or ROS production in neutrophils amongst vitamin C deficient species.[11] It has been postulated that the vitamin C deficiency observed in patients with severe infections may contribute to compromised neutrophil chemotactic ability,[12] decreased ability to destroy microbes and generate ROS.[11] Possible links have also been established between vitamin C deficiency and neutrophil necrotic cell death and attenuated neutrophil apoptosis.[13] Further studies have found possible links between vitamin C deficiency and elevated production of pro-inflammatory cytokines (TNF-α and IL-1α/β), and decreased production of an anti-viral cytokine (IFN-α/β).[14]

The pharmacokinetics of leukocyte vitamin C are indicative of the vitamin's importance in leukocyte functionality. Leukocytes, namely neutrophils and monocytes, accumulate vitamin C concentrations that are 50 - 100 fold of those found in plasma.[15] As such, neutrophils contain intracellular levels of at least 1 mM.[16] Neutrophils can further increase their intracellular concentration of vitamin C by absorbing the vitamin's oxidized form (dehydroascorbate-DHAA).[6] DHAA is rapidly reduced to ascorbate intracellularly, increasing levels to as much as 10 mM.[17] Levels below 20 μg/10^8 leukocyte[18, 19] have been identified to represent deficient concentrations.

Given the inability of humans to biosynthesise vitamin C, and the molecules water-soluble nature, a number of situations and conditions can influence its deficiency. Additionally, lower mean vitamin C status is present amongst free-living or institutionalized elderly people, who demonstrate reductions in leukocyte vitamin C concentrations.[20] Hospitalized patients are particularly vulnerable to deficient vitamin C concentrations that are significantly lower from the general population.[21, 22]

Surgical trauma has been demonstrated to have an immense impact on immunological function. During the surgical acute phase period, the peripheral white blood cell count can double within hours after trauma as a result of the large bone marrow storage and intravascular marginated pools of neutrophils.[23] Leukocytosis occurs with the purpose of initiating an inflammatory response that ultimately clears the injured cells/debris or kills invading pathogens and stabilises

tissue function.[24] Conversely, a compensatory anti-inflammatory immune response is an adaptive mechanism involving acquired immunity, which may lead to eventual post-surgical immunosuppression.[25] These changes may be due to the activation and partial degranulation of circulating neutrophils,[26] lymphocyte depression, and monocyte deactivation.[27]

Due to multiple mechanisms, surgery has been shown to systematically initiate a significant reduction in plasma vitamin C concentrations.[28, 29] However, given the importance of vitamin C in the optimal function of leukocytes, it is imperative to determine the effects of surgery on leukocyte vitamin C concentrations. A review has not systematically assessed post-operative vitamin C leukocyte concentrations and compared them with pre-operative concentrations. The aim of this review was to systematically assess the amount of post-operative leukocyte vitamin C change based on previous investigations.

2. Methods

2.1 Literature search

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) were used in this review. We searched the PUBMED, SCOPUS, SciSearch and the Cochrane Library for publications from inception until January 2022. Keywords related to vitamin C ("vitamin C" OR "ascorbate" OR "ascorbic acid" OR "antioxidant") and white blood cells ("buffy layer" OR "buffy coat" OR "leukocyte" OR "white blood cell") and surgery ("surgery" OR "surgical trauma" OR "post-operative"). We also searched for references published in relevant papers. Reference lists of previously published systematic reviews and meta-analyses were examined for additional primary studies. Table 1 outlines the PICOS criteria utilised for the literature search.

2.2 Study selection

In the systematic review and meta-analysis, we included studies that incorporated prospective pre-post designs or single group pre-post designs. In addition, placebo controlled groups derived from placebo controlled trials who were not exposed to vitamin C interventions. These studies were required to assess leukocyte/buffy layer vitamin C concentrations pre and post operatively, with at least 1 post-operative measurement. Studies were not excluded based on the timing of the post-operative vitamin C assessment and were only considered if pre-operative assessments were taken within 1 week of surgery commencement. Studies only performed on adults and published in English were included. Case-reports and grey literature (i.e. conference proceedings, dissertations) were not considered for inclusion.

Studies in which the placebo intervention contained a vitamin C source, or a source that could influence leukocyte vitamin C concentrations directly were avoided from analysis. Additionally, given the established effects of blood transfusions on leukocyte vitamin C concentrations,[30] study groups which were reportedly subjected to receiving blood transfusions during the surgical procedures were not included into the meta analysis. This was done to

ensure no interference from pre and post-operative interventions or transfusions on leukocyte concentrations, and to evaluate the influence of surgery itself on leukocyte concentrations. Only studies reporting mean leukocyte/buffy layer vitamin C concentrations and standard deviations were appropriate for meta-analysis. If considered potentially eligible, the full-text publication was retrieved and reviewed by two authors independently.

2.3 Data extraction

The number of participants, mean leukocyte vitamin C concentrations along with their variance (SD) prior to and following surgery were collated from text, tables or figures. Data extraction was done for the following parameters: study design, sample size, mean/median age, timing of perioperative vitamin C measurement, mean leukocyte vitamin C concentrations, percentage of change (relative to baseline) in leukocyte vitamin C concentrations and type of surgery.

2.4 Quality assessment/Risk of bias

Methodological quality was assessed independently by two investigators (NT and KR) using The Risk of Bias Assessment tool for Non-Randomized Studies (RoBANS).[31] This tool was used to assess study bias in a recent review that assessed post-operative plasma vitamin C following surgery.[28] This tool was selected due to leukocyte concentrations being prospectively assessed without participants being randomised to surgical exposure. The RoBANS assessed a number of domains, which included: selection of participants, confounding variables, incomplete outcome data, selective outcome reporting and the addition of other bias. One additional assessment domain relating to blinding was not applicable to the included studies. The tool categorised a possible risk of bias into "Low," "Unclear," or "High" measures, not relying on numerical scores.

2.5 Statistical analysis

Mean leukocyte vitamin C concentrations before and after surgery, standard deviation and n (number of participants) formed the meta-analysis using Review Manager version 5.3.[32] To calculate differences between pre and post-operative concentration, mean leukocyte concentrations were extracted. In order to achieve standardised concentrations for comparison between studies, vitamin C concentrations were converted into micrograms per 10^8 leukocytes ($\mu g/10^8$). Standard error of the mean was converted into standard deviation values. A number of studies reported buffy layer/coat vitamin C concentrations. Given the main buffy coat constituents include both leukocytes and platelets, the leukocyte ascorbic acid concentration from the buffy layer was estimated by adopting a method devised by Gibson et al. [33]

Studies were sub grouped into those that assessed leukocyte vitamin C concentrations within the first post-operative day (immediate 24-hour assessment), those that assessed leukocyte vitamin C within the first post-operative week (days 2-6) and those that assessed leukocyte vitamin C concentrations at or beyond the first post-operative week ($\geq$7 days). Studies were grouped into these separate post-operative time frames as a means of identifying any patterns in concentration changes within the post-operative period. Furthermore, the first post-operative week is indicative of the most severe acute phase stress response,[29, 34] while concentrations

measured 7 days following surgery are more indicative of long term surgical consequences. A number of studies had data points that fell into multiple sub groups.

A random model, which allows for differences in the treatment effect from study to study, sampling variability and high heterogeneity between studies (I^2 statistic >50%) was applied for each meta-analysis.[35] The overall effect sizes examined the significance of the differences between means. A p-value of < 0.05 was deemed statistically significant. Potential publication bias was investigated by use of Egger's regression asymmetry test,[36] which was conducted using STATA software, version 16.0 (STATA corp.). A non-significant Egger's test (>0.05) was indicative of no publication bias.

3. Results

In total, seven studies (that included eight studies) that investigated changes to leukocyte vitamin C concentrations following surgery met the inclusion criteria for meta-analysis.[20, 30, 37-41] The study screening and selection process is summarized in Figure 1. Major characteristics of the studies included into the meta-analysis are presented in Table 2.

From the included studies, 263 patients were recruited and had their leukocyte vitamin C concentrations assessed pre and post operatively, with no reports of attrition rates. From the included studies, ages ranged from 18-80yrs, with five studies reporting a mean age below 60 years. Mean pre-operative vitamin C concentrations varied between studies, with a majority of studies [20, 30, 37, 40] reporting deficient (20 μg/10^8 leukocyte)[18] ascorbic acid concentrations prior to surgery. All studies reported on the biochemical protocol utilised for assessing leukocyte concentrations. According to the hierarchy of evidence,[42] the pre-post nature of the included studies corresponds to level 4 evidence.

Six studies assessed leukocyte vitamin C concentrations within the first 24 hours post-surgery, six assessed concentrations within 2 and 6 post-operative days and seven assessed concentrations post-operatively at 7 days or beyond. One study separated mean pre and post-operative leukocyte concentrations between gender groups.[37]

Surgery type varied between studies, ranging from bariatric and gastrointestinal surgery, to surgery for varicose veins, groin hernia, orthopaedic surgery, and elective benign peptic ulceration. A number of studies included multiple surgery types that were categorized broadly (i.e. major surgery, general surgery). One study[39] separated surgery types into either minor or moderate surgery based on the degree of surgical trauma.

Based on the Cochrane Collaboration Risk of Bias Assessment Tool (Figure 2), the quality of the included studies ranged from medium to high. A majority of studies had an unclear risk of bias for the confounding variable domain. This was due to these studies not reporting on a number of potential confounding variables that may influence leukocyte vitamin C concentrations prior to surgery and during the post-operative period. These include variables such as dietary intake (particularly vitamin C containing foods), medication intake, supplementation, and smoking/alcohol consumption. Similarly, the domain measuring

exposure was unclear for a majority of included studies due to lack of information regarding the anaesthetic type and duration, and specific surgical details such as surgery length.

Findings from meta-analyses assessing mean leukocyte vitamin C concentrations throughout the first post-operative day are displayed in Figure 3. Overall, there was no evidence of publication bias in either of the meta-analyses (Egger's test p >0.05).

The meta-analysis of six trial arms involving 184 patients assessed the effect of surgery on leukocyte vitamin C concentrations during the initial post-operative 24 hours (subgroup 1.1.1, Figure 3). Mean difference (95% CI) between pre and post-operative leukocyte vitamin C concentration was -5.37 µg/10^8 (32.3%) (-6.35, -4.40) (p < 0.001). Low heterogeneity was observed between these trials ($I^2 = 0\%$, p = 0.70).

The meta-analysis of eight trial arms involving 128 patients assessed the effect of surgery on leukocyte vitamin C concentrations between post-operative days two and six (subgroup 1.1.2). Surgery also significantly decreased leukocyte vitamin C concentrations during the first post-operative week (2-6 days post-operatively). Mean difference (95% CI) between pre-operative and post-operative leukocyte concentration was -4.43 µg/10^8 (23%) (-7.27, -1.58) (p < 0.001). Higher heterogeneity was displayed between these trials ($I^2 = 89\%$, p < 0.001).

The meta-analysis of eight trial arms involving 150 patients assessed the effect of surgery on leukocyte vitamin C concentrations at 7 days (or beyond) post-operatively (subgroup 1.1.3). The pooled data from studies assessing mean leukocyte vitamin C concentrations at 7 days or beyond post-surgery demonstrate a marginal, non-significant increase in mean leukocyte vitamin C concentrations. The mean difference between pre-operative and post-operative leukocyte vitamin C concentration was 0.93 µg/10^8 (4.8%) (-0.79, 2.66) (p = 0.29). High heterogeneity was observed between these trials ($I^2 = 60\%$, p = 0.01).

4. Discussion

This meta-analysis was performed to pool the data from previous investigations assessing the effects of surgery on leukocyte vitamin C concentrations. Our meta-analysis revealed a significant depletion of 5.37 µg/10^8 (36.3%) (CI = -6.35, -4.40) (p < 0.001) in leukocyte vitamin C concentrations during the first 24 hours following surgery. A significant depletion of 4.43 µg/10^8 (23%) (-7.27, -1.58) (p < 0.001) was also observed during 2-6 days post-operatively. However, this depletion may be predominantly confined to the first post-operative week, with results demonstrating a marginal uprise in mean vitamin C leukocyte concentrations of 0.93 µg/10^8 (4.8%) (-0.79, 2.66) (p = 0.29) at 7 days or beyond post-operatively.

This is the first meta-analysis to examine the effects of surgery on mean leukocyte vitamin C concentrations. Cellular immunity can be compromised for 3-10 days post-operatively

following major surgery.[43] This timeframe is consistent with the compromised vitamin C leukocyte concentrations post-operatively.

A number of multifactorial explanations may account for our observed results. One proposed explanation for the depletions in leukocyte vitamin C concentrations is the redistribution of plasma vitamin C into leukocytes. Given the proliferation in leukocyte numbers following surgery, the plasma concentrations are distributed into a larger number of newly secreted cells, which may result in a depletion in both plasma and leukocyte concentrations during the leukocytosis phase.[44] Immature neutrophils have decreased functionality compared to differentiated neutrophils.[45] As a consequence, leukocytes may display a higher affinity for the absorption of circulating ascorbic acid to support leukocyte functionality, and the recycling of intracellular vitamin E and glutathione.[46, 47]

An uprise in oxidative stress is common following surgical trauma.[48] Consequently, the generation of post-operative ROS may further utilise ascorbic acid, particularly during the immediate post-operative phase. Moreover, it has been shown that the adrenal and pituitary glands utilize ascorbic acid in response to a major stressor,[49] which may affect leukocyte concentrations.

Post-operatively, there is a change in the types of leukocytes present, which may affect measured leukocyte ascorbic acid concentrations. The post-operative leukocytosis includes a predominant increase in polymorphonuclear cells such as neutrophils which contain less than double the amount of ascorbic acid found within mononuclear cells such as lymphocytes.[40, 50] One of our reviewed studies [20] found increases in polymorphonuclear cell counts and decreases in mononuclear counts during the immediate post-operative phase, with a rise in mononuclear cell counts by the 7th post-operative day.

An alternative explanation for the observed results may be due to alterations in absorption. Depending on the level of oxidative stress and inflammation, the absorption of ascorbic acid into leukocytes may be compromised. Research has speculated a compromised sodium dependant vitamin C transporter (SVCT2) plasma membrane translocation and consequential ascorbic acid cellular uptake during severe oxidative stress.[51]

A number of limitations can be derived from the present meta-analysis. The protocol of the present review was not pre-registered, preventing initial feedback of the review. However, we have adhered to a review method that has enabled the successful identification of the relevant studies that match the review's inclusion criteria, and have avoided the potential duplication of this review. A large number of the reviewed studies were based on single group pre-post designs. A common flaw of these designs is the absence of a control group and the potential influence of extraneous factors on outcomes.[52] Therefore, only an association can be conferred between surgery and leukocyte vitamin C concentrations in this case. This study design and level of evidence can be strengthened with future studies including multiple post-operative data collection points and a control group.

A majority of included studies failed to assess a number of variables known to influence vitamin C status, such as vitamin C intake, supplementation, smoking status, alcohol consumption and medication intake.[53] Additionally, surgical details such as the length of surgery, or surgical complications were not reported. One of our included studies suggested

that post-operative changes in leukocyte concentrations were unrelated to the extent of surgical trauma.[39] The type of administered anaesthetic may also effect leukocyte concentrations. Total intravenous anaesthesia with propofol and remifentanil has shown to affect blood neutrophil/leukocyte ratios diversely to inhalational anaesthesia with sevoflurane.[54] Moreover, commonly prescribed post-operative medications such as blood thinners and anti-inflammatories may affect leukocyte concentrations and their vitamin C concentrations.[55]

High heterogeneity was observed between studies assessing post-operative concentrations within 2-6 days, and 7 days (or beyond) following surgery. This may be attributed to a series of factors such as varying surgery types and administered anaesthetics, and differences in pre-operative patient health status, perioperative complications or length of hospital stay. Additional investigations should also take patient age into consideration in an effort to determine whether the degree of post-operative vitamin C change may be influenced by age.

Future research should consider adopting cell separation techniques and assessing vitamin C concentrations in separate leukocyte cell fractions including mononuclear and polymorphonuclear cells during the post-operative period, a phase during which the numbers of these cells may vary greatly. In order to gauge a sense of severity and longevity of the post-operative inflammatory response, future studies should assess changes in inflammatory cytokine markers, hepatic complement proteins such as C reactive protein, albumin, fibrinogen and markers of oxidative stress.[56] Vital patient details such as dietary intake, supplementation, surgical details, and patient age will allow for subgroup analyses to be performed. Finally, more up to date, larger scale studies are required for more generalizable results to be generated.

Conclusion

This preliminary meta-analysis examined the effects of surgery on mean leukocyte vitamin C concentrations. According to the pooled data, a significant mean leukocyte vitamin C depletion following surgery both immediately (within 24 hours) and during the first post-operative week (2-6 days from surgery) was observed. Results further revealed an increase in mean leukocyte concentrations, once the 7th post-operative day was reached. Further research is required to validate the observed results and to determine the mechanisms of action responsible for the depletions in leukocyte concentrations. This could incentivise future studies to explore whether compromises in immune function or commonly observed post-operative pathophysiologies may be associated with the significant leukocyte vitamin C depletion witnessed during the initial post-operative period.

Acknowledgements

None declared

Conflict of Interest

Authors Contributions

NT and AS conceptualised the review. NT, KR and IH were involved in the acquisition, analysis, and interpretation of data for the work. NT drafted the work. All authors revised the work critically for important intellectual content.

References

1. Walingo K. Role of vitamin C (ascorbic acid) on human health-a review. *African J Food, Agric, Nutr, Dev.* 2005; 5(1)

2. Carr AC, Maggini S. Vitamin C and Immune Function. *Nutrients.* 2017; 9(11): DOI:10.3390/nu9111211

3. Van Gorkom GN, Klein Wolterink RG, Van Elssen CH, Wieten L, Germeraad WT, Bos GM. Influence of vitamin C on lymphocytes: an overview. *Antioxid.* 2018; 7(3): 41. DOI: 10.3390/antiox7030041

4. Selvamary VN, Brundha M, Girija A. Role of Vitamin C in Immune Function of Human Body. *Indian J Forensic Med Toxicol.* 2020; 14(4): DOI: 10.37506/ijfmt.v14i4.12429

5. Bozonet SM, Carr AC, Pullar JM, Vissers M. Enhanced human neutrophil vitamin C status, chemotaxis and oxidant generation following dietary supplementation with vitamin C-rich SunGold kiwifruit. *Nutrients.* 2015; 7(4): 2574-88. DOI:10.3390/nu7042574

6. Padayatty SJ, Levine M. New insights into the physiology and pharmacology of vitamin C. *Cmaj.* 2001; 164(3): 353-5

7. Mohammed BM, Fisher BJ, Kraskauskas D, Farkas D, Brophy DF, Fowler AA, 3rd, *et al.* Vitamin C: a novel regulator of neutrophil extracellular trap formation. *Nutrients.* 2013; 5(8): 3131-51. DOI:10.3390/nu5083131

8. Gao YL, Lu B. The Parenteral Vitamin C Improves Sepsis and Sepsis-Induced Multiple Organ Dysfunction Syndrome via Preventing Cellular Immunosuppression. 2017; 2017: 4024672. DOI:10.1155/2017/4024672

9. Portugal CC, Socodato R. Caveolin-1-mediated internalization of the vitamin C transporter SVCT2 in microglia triggers an inflammatory phenotype. *Sci Sig.* 2017; 10(472): DOI:10.1126/scisignal.aal2005

10. Lenton KJ, Sane AT, Therriault H, Cantin AM, Payette H, Wagner JR. Vitamin C augments lymphocyte glutathione in subjects with ascorbate deficiency. *Am J Clin Nutr.* 2003; 77(1): 189-95. DOI:10.1093/ajcn/77.1.189

11. Muggli R. Vitamin C and phagocytes. *Nutrient modulation of the immune response*: CRC Press; 2020. p. 75-90.

12. Carr AC, Shaw GM, Natarajan R. Ascorbate-dependent vasopressor synthesis: a rationale for vitamin C administration in severe sepsis and septic shock? *Crit Care.* 2015; 19(1): 418. DOI: 10.1186/s13054-015-1131-2

13.   Vissers MC, Wilkie RP. Ascorbate deficiency results in impaired neutrophil apoptosis and clearance and is associated with up- regulation of hypoxia- inducible factor 1α. *J Leukoc Biol.* 2007; 81(5): 1236-44. DOI: 10.1189/jlb.0806541

14.   Kim Y, Kim H, Bae S, Choi J, Lim SY, Lee N*, et al.* Vitamin C is an essential factor on the anti-viral immune responses through the production of interferon-α/β at the initial stage of influenza A virus (H3N2) infection. *Immune Netw.* 2013; 13(2): 70-4. DOI: 10.4110/in.2013.13.2.70

15.   Szőcs A, Vancea S, Kiss I, Donáth-Nagy G. Quantification of plasma and leukocyte vitamin C by high performance liquid chromatography with mass spectrometric detection. *J Anal Chem.* 2020; 75(9): 1168-76

16.   Wang Y, Russo TA, Kwon O, Chanock S, Rumsey SC, Levine M. Ascorbate recycling in human neutrophils: induction by bacteria. *Proceed of the Nat Acad of Sci.* 1997; 94(25): 13816-9. DOI: 10.1073/pnas.94.25.13816

17.   Washko PW, Wang Y, Levine M. Ascorbic acid recycling in human neutrophils. *J Biol Chem.* 1993; 268(21): 15531-5

18.   Shidfar F, Baghai N, Keshavarz A, Ameri A, Shidfar S. Comparison of plasma and leukocyte vitamin C status between asthmatic and healthy subjects. *East Mediterr Health J.* 2005; 11(1-2): 87-95

19.   Hill G, Pickford I, Young G, Schorah CJ, Blackett R, Burkinshaw L*, et al.* Malnutrition in surgical patients: an unrecognised problem. *Lancet.* 1977; 309(8013): 689-92. DOI: 10.1016/s0140-6736(77)92127-4

20.   Schorah CJ, Tormey WP, Brooks GH, Robertshaw AM, Young GA, Talukder R*, et al.* The effect of vitamin C supplements on body weight, serum proteins, and general health of an elderly population. *Am J Clin Nutri.* 1981; 34(5): 871-6. DOI: 10.1093/ajcn/34.5.871

21.   Sharma Y, Miller M, Shahi R, Doyle A, Horwood C, Hakendorf P*, et al.* Vitamin C deficiency in Australian hospitalised patients: an observational study. *Intern Med J.* 2019; 49(2): 189-96. DOI:10.1111/imj.14030

22.   Evans-Olders R, Eintracht S, Hoffer LJ. Metabolic origin of hypovitaminosis C in acutely hospitalized patients. *Nutrients.* 2010; 26(11-12): 1070-4. DOI: 10.1016/j.nut.2009.08.015

23.   Deirmengian GK, Zmistowski B, Jacovides C, O'Neil J, Parvizi J. Leukocytosis is common after total hip and knee arthroplasty. *Clin Orthop Relat Res.* 2011; 469(11): 3031-6. DOI:10.1007/s11999-011-1887-x

24.   Dobson GP. Addressing the global burden of trauma in major surgery. *Front Surg.* 2015; 2: 43. DOI: 10.3389/fsurg.2015.00043

25.   Munford RS, Pugin J. Normal responses to injury prevent systemic inflammation and can be immunosuppressive. *Am J Respir Crit Care Med.* 2001; 163(2): 316-21. DOI: 10.1164/ajrccm.163.2.2007102

26.   Rosales C. Neutrophil: a cell with many roles in inflammation or several cell types? *Front Physiol.* 2018: 113

27.   Angele MK, Faist E. Clinical review: immunodepression in the surgical patient and increased susceptibility to infection. *Crit care.* 2002; 6(4): 298. DOI: 10.1186/cc1514

28.   Travica N, Ried K, Hudson I, Scholey A, Pipingas A, Sali A. The effects of surgery on plasma/serum vitamin C concentrations: A systematic review and meta-analysis. *Br J Nutr.* 2020: 1-39. DOI: 10.1017/S0007114520004353

29.   Travica N, Ried K, Hudson I, Scholey A, Pipingas A, Sali A. The effects of cardiovascular and orthopaedic surgery on vitamin concentrations: a narrative review of the

literature and mechanisms of action. *Crit Rev Food Sci Nutr.* 2021: 1-31. DOI: 10.1080/10408398.2021.1983762

30. McGinn F, Hamilton JC. Ascorbic acid levels in stored blood and in patients undergoing surgery after blood transfusion. *Br J Sur.* 1976; 63(7): 505-7. DOI: 10.1002/bjs.1800630702

31. Park J, Lee Y, Seo H, Jang B, Son H, Kim S*, et al.*, editors. Risk of bias assessment tool for non-randomized studies (RoBANS): development and validation of a new instrument. *19th Cochrane Colloquium*; 2011.

32. RevMan. Computer program]. Version 5.3. The Nordic Cochrane CentreThe Cochrane Collaboration Copenhagen; 2014.

33. Gibson S, Moore F, Goldberg A. Measurement of leucocyte ascorbic acid. *Br Med J.* 1966; 1(5496): 1152

34. Santonocito C, De Loecker I, Donadello K, Moussa MD, Markowicz S, Gullo A*, et al.* C-reactive protein kinetics after major surgery. *Anesth Analg.* 2014; 119(3): 624-9. DOI:10.1213/ane.0000000000000263

35. Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. *Bmj.* 2011; 342: d549

36. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Bmj.* 1997; 315(7109): 629-34. DOI: 10.1136/bmj.315.7109.629

37. Louw JA, Werbeck A, Louw M, Kotze T, Cooper R, Labadarios D. Blood vitamin concentrations during the acute-phase response. *Crit Care Med.* 1992; 20(7): 934-41. DOI:

38. Taylor T, Raftery A, Elder J, Loveday C, Dymock I, Gibbs A*, et al.* Leucocyte ascorbate levels and postoperative deep venous thrombosis. *Br J Surg.* 1979; 66(8): 583-5

39. Irvin T. Vitamin C requirements in postoperative patients. *Int J Vit Nutr Res Supp.* 1982; 23: 277-86. DOI: 10.1002/bjs.1800660821

40. Vallance S. Changes in plasma and buffy layer vitamin C following surgery. *Br J Surg.* 1988; 75(4): 366-70. DOI: 10.1002/bjs.1800750424

41. Crandon JH, Landau B, Mikal S, Balmanno J, Jefferson M, Mahoney N. Ascorbic acid economy in surgical patients as indicated by blood ascorbic acid levels. *New Engl J Med.* 1958; 258(3): 105-13. DOI: 10.1056/NEJM195801162580301

42. Burns PB, Rohrich RJ, Chung KC. The levels of evidence and their role in evidence-based medicine. *Plast Reconstr Surg.* 2011; 128(1): 305. DOI: 10.1097/PRS.0b013e318219c171

43. Gutierrez T, Hornigold R, Pearce A. The systemic response to surgery. *Surg (Oxford).* 2011; 29(2): 93-6

44. Hughes SF, Hendricks BD, Edwards DR, Maclean KM, Bastawrous SS, Middleton JF. Total hip and knee replacement surgery results in changes in leukocyte and endothelial markers. *J Inflamm (Lond).* 2010; 7: 2. DOI:10.1186/1476-9255-7-2

45. Drifte G, Dunn-Siegrist I, Tissières P, Pugin J. Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. *Crit Care Med.* 2013; 41(3): 820-32. DOI: 10.1097/CCM.0b013e318274647d

46. Luo JL, Hammarqvist F, Andersson K, Wernerman J. Surgical trauma decreases glutathione synthetic capacity in human skeletal muscle tissue. *Am J Physiol.* 1998; 275(2): E359-65. DOI:10.1152/ajpendo.1998.275.2.E359

47. Luo M, Fernandez-Estivariz C, Jones DP, Accardi CR, Alteheld B, Bazargan N*, et al.* Depletion of plasma antioxidants in surgical intensive care unit patients requiring parenteral feeding: effects of parenteral nutrition with or without alanyl-glutamine dipeptide supplementation. *Nutrition.* 2008; 24(1): 37-44. DOI:10.1016/j.nut.2007.10.004

48. Kotzampassi K, Kolios G, Manousou P, Kazamias P, Paramythiotis D, Papavramidis TS*, et al.* Oxidative stress due to anesthesia and surgical trauma: importance of early enteral nutrition. *Mol Nutr Food Res.* 2009; 53(6): 770-9. DOI:10.1002/mnfr.200800166

49. Padayatty SJ, Levine M. Vitamin C: the known and the unknown and Goldilocks. *Oral Dis.* 2016; 22(6): 463-93. DOI: 10.1111/odi.12446

50. Castelli A, Martorana G, Meucci E, Bonetti G. Vitamin C in normal human mononuclear and polymorphonuclear leukocytes. *Acta vitaminol Enzymol.* 1982; 4(3): 189-96.

51. Acuña AI, Esparza M, Kramm C, Beltrán FA, Parra AV, Cepeda C*, et al.* A failure in energy metabolism and antioxidant uptake precede symptoms of Huntington's disease in mice. *Nat Commun.* 2013; 4: 2917. DOI: 10.1038/ncomms3917

52. Marsden E, Torgerson CJ. Single group, pre-and post-test research designs: Some methodological concerns. *Oxf Rev Educ.* 2012; 38(5): 583-616.

53. Carr AC, Rowe S. Factors affecting vitamin C status and prevalence of deficiency: A global health perspective. *Nutrients.* 2020; 12(7): 1963. DOI:10.3390/nu12071963

54. Kim WH, Jin HS, Ko JS, Hahm TS, Lee SM, Cho HS*, et al.* The effect of anesthetic techniques on neutrophil-to-lymphocyte ratio after laparoscopy-assisted vaginal hysterectomy. *Acta Anaesthesiol Taiwan.* 2011; 49(3): 83-7. DOI:10.1016/j.aat.2011.08.004

55. Merchant M, Modi DN. Acute and chronic effects of aspirin on hematological parameters and hepatic ferritin expression in mice. *Indian J pharmacol.* 2004; 36(4): 226

56. Marrocco I, Altieri F, Peluso I. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxid Med Cell Longev.* 2017; 2017: 6501046. DOI:10.1155/2017/6501046

Table 1. PICOS criteria for inclusion and exclusion of studies

PICOS	Description
Population	Adult humans (healthy or chronic disease populations)
Intervention	Surgery exposure
Comparator	Pre and post-operative leukocyte/buffy layer vitamin C concentrations
Outcome Measure	Leukocyte/buffy layer vitamin C concentrations
Setting	Hospital while in-patient

Table 2. Characteristics of studies included into meta-analysis

NA = Not reported, SD = Standard Deviation, post op = post-operative, μg/10^8 = microgram per 10^8

Study	Year	Sample Size Enrolled	Mean Age ± SD	Surgery type	Study Deign	Longest post-op measure	Pre-op mean ± SD (ug/10^8)	Post-op mean ± SD (ug/10^8)	Percentage change (%)
Louw et al., (males)[37]	1992	12	31.5 ± 3.0	Uncomplicated orthopaedic surgery	Single group pre-post	7 days	19.5 ± 2.4	Day 2: 11.6 ± 1.4	40.5
								Day 7: 22.4 ± 3.4	14.9
Louw et al., (females)[37]	1992	14	38.8 ± 3.2	Uncomplicated orthopaedic surgery	Single group pre-post	7 days	18.7 ± 2.3	Day 2: 12.8 ± 1.1	31.6
								Day 7: 20.4 ± 1.8	9.09
Irvin (study 1) minor surgery[39]	1982	30	49.5 ± 3	Minor surgery (operations for varicose veins/groin hernia)	Single group pre-post	1 day	29 ± 16.4	Day 1: 19 ± 10.9	34.5
Irvin (study 1) moderate surgery[39]	1982	8	53.5 ± 5.6	Moderate surgery (surgery for gall stone or vagotomy)	Single group pre-post	10 days	28.5 ± 12.7	Day 1: 17 ± 11.3	40.4
								Day 4: 21 ± 8.5	26.3
								Day 10: 23 ± 8.5	19.3
Irvin (study 2)[39]	1982	10	62.2 ± 5.1	Major surgery	Single group pre-post	10 days	15 ± 7.9	Day 1: 10 ± 6.3	33.3
								Day 4: 8 ± 6.3	46.7

								Day 10: 9.5 ± 6.3	36.7
Taylor et al.[38]	1979	23	>70	General surgery	Prospective randomised controlled trial	9 days	31 ± 7	Day 6: 26 ± 6.5	16.1
								Day 9: 36 ± 11	16.1
McGinn et al.[30]	1976	5	20-34	Elective benign peptic ulceration	Single group pre-post	7 days	14.5 ± 4.5	Day 1: 8 ± 0.5	44.8
								Day 3: 9.5 ± 1.1	34.5
								Day 7: 19 ± 3	31.0
Vallance et al.[40]	1988	37	NA	Wide range of surgical procedures	Randomised controlled trial	10 days	13.8 ± 3.9	Day 1: 8.8 ± 1.3	36.2
								Day 4: 14.5 ± 4	5.1
								Day 10: 14.1 ± 3	2.2
Schorah et al.[20]	1986	19	37-68 (mean = 52)	gastro-intestinal resection, cholecystectomy, selective vagotomy	Single group pre-post	7 days	12 ± 3	Day 1: 6.5 ± 2	45.8
								Day 4:12 ± 8	0
								Day 7: 10.7 ± 5.2	10.8
Crandon et al.[41]	1958	105	NA	Major surgery	Single group pre-post	Immediately	54 ± 35	43 ± 35.8	20.4
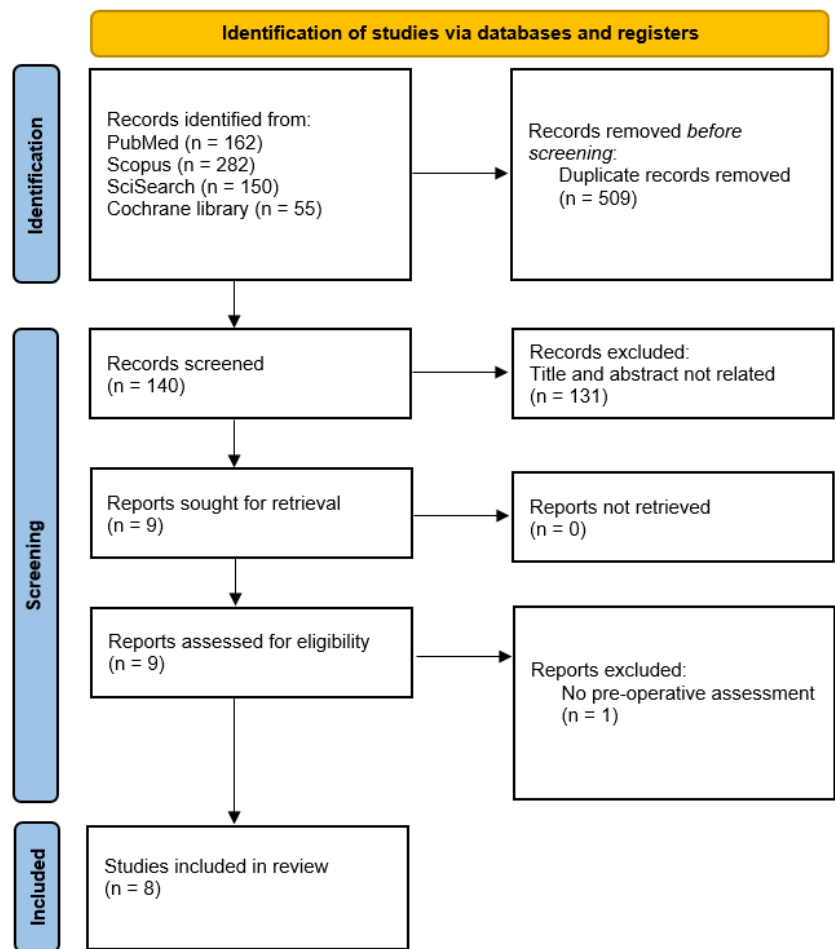
Figure 1. PRISMA flow diagram of the literature search process

	Selection of participants	Confounding variables	Measurement of exposure	Incomplete outcome data	Selective reporting	Other bias
Louw et al., 1992	+	+	?	+	+	+
Irvin, 1982 (Study 1)	+	?	?	+	+	+
Irvin, 1982 (Study 2)	+	?	?	+	+	+
Taylor et al., 1979	+	?	?	+	+	+
McGinn et al., 1976	+	?	?	+	+	+
Vallance et al., 1988	+	?	?	+	+	+
Schorah et al., 1986	+	?	?	+	+	+
Crandon et al., 1958	+	?	?	+	+	+

Figure 2. Risk of bias assessment of studies included in meta-analysis, yellow circle (question mark = unclear bias, green circle (plus sign) = low risk of bias.

Study or Subgroup	post operative Mean	SD	Total	Pre operative Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Year	Mean Difference IV, Random, 95% CI
1.1.1 Leukocyte vitamin C within 24 hours of surgery										
Crandon 1958	43	35.8	105	54	35	105	2.3%	-11.00 [-20.58, -1.42]	1958	
McGinn 1976	8	0.5	5	14.5	4.5	5	4.8%	-6.50 [-10.47, -2.53]	1976	
Irvin 1982 (Study 2)	10	6.3	10	15	7.9	10	3.6%	-5.00 [-11.26, 1.26]	1982	
Irvin 1982- Moderate surgery (Study 1)	17	11.3	8	28.5	12.7	8	1.7%	-11.50 [-23.28, 0.28]	1982	
Schorah 1986	6.51	2	19	12	3	19	5.9%	-5.49 [-7.11, -3.87]	1986	
Vallance 1988	8.8	1.31	37	13.8	3.9	37	6.0%	-5.00 [-6.33, -3.67]	1988	
Subtotal (95% CI)			184			184	24.2%	-5.37 [-6.35, -4.40]		
Heterogeneity: Tau² = 0.00; Chi² = 3.01, df = 5 (P = 0.70); I² = 0%										
Test for overall effect: Z = 10.82 (P < 0.00001)										
1.1.2 Leukocyte vitamin C 2-6 days following surgery										
McGinn 1976	9.5	1.1	5	14.9	4.5	5	4.7%	-5.40 [-9.46, -1.34]	1976	
Taylor 1979	26	6.5	23	31	7	23	4.8%	-5.00 [-8.90, -1.10]	1979	
Irvin 1982- Moderate surgery (Study 1)	21	8.5	8	28.5	12.7	8	2.0%	-7.50 [-18.09, 3.09]	1982	
Irvin 1982 (Study 2)	8	6.3	10	15	7.9	10	3.6%	-7.00 [-13.26, -0.74]	1982	
Schorah 1986	12	8	19	12	3	19	4.8%	0.00 [-3.84, 3.84]	1986	
Vallance 1988	14.55	4	37	13.8	3.9	37	5.8%	0.75 [-1.05, 2.55]	1988	
Louw 1992 (Male)	11.6	1.4	12	19.5	2.4	12	5.9%	-7.90 [-9.47, -6.33]	1992	
Louw 1992 (Female)	12.8	1.1	14	18.7	2.3	14	6.0%	-5.90 [-7.24, -4.56]	1992	
Subtotal (95% CI)			128			128	37.6%	-4.43 [-7.27, -1.58]		
Heterogeneity: Tau² = 12.61; Chi² = 60.87, df = 7 (P < 0.00001); I² = 89%										
Test for overall effect: Z = 3.05 (P = 0.002)										
1.1.3 Leukocyte vitamin C 7 days or beyond following surgery										
McGinn 1976	19	3	5	14.9	4.5	5	4.3%	4.10 [-0.64, 8.84]	1976	
Taylor 1979	36	11	23	31	7	23	4.0%	5.00 [-0.33, 10.33]	1979	
Irvin 1982 (Study 2)	9.5	6.32	10	15	7.9	10	3.6%	-5.50 [-11.77, 0.77]	1982	
Irvin 1982- Moderate surgery (Study 1)	23	8.5	30	28.5	12.7	30	4.0%	-5.50 [-10.97, -0.03]	1982	
Schorah 1986	12	8	19	12	3	19	4.8%	0.00 [-3.84, 3.84]	1986	
Vallance 1988	14.08	2.64	37	13.8	3.9	37	5.9%	0.28 [-1.24, 1.80]	1988	
Louw 1992 (Female)	20.4	1.8	14	18.7	2.3	14	5.9%	1.70 [0.17, 3.23]	1992	
Louw 1992 (Male)	22.4	3.4	12	19.5	2.4	12	5.6%	2.90 [0.55, 5.25]	1992	
Subtotal (95% CI)			150			150	38.2%	0.93 [-0.79, 2.66]		
Heterogeneity: Tau² = 3.04; Chi² = 17.72, df = 7 (P = 0.01); I² = 60%										
Test for overall effect: Z = 1.06 (P = 0.29)										
Total (95% CI)			462			462	100.0%	-2.98 [-4.80, -1.16]		
Heterogeneity: Tau² = 13.95; Chi² = 204.28, df = 21 (P < 0.00001); I² = 90%										
Test for overall effect: Z = 3.21 (P = 0.001)										
Test for subgroup differences: Chi² = 39.14, df = 2 (P < 0.00001), I² = 94.9%										

-20 -10 0 10 20
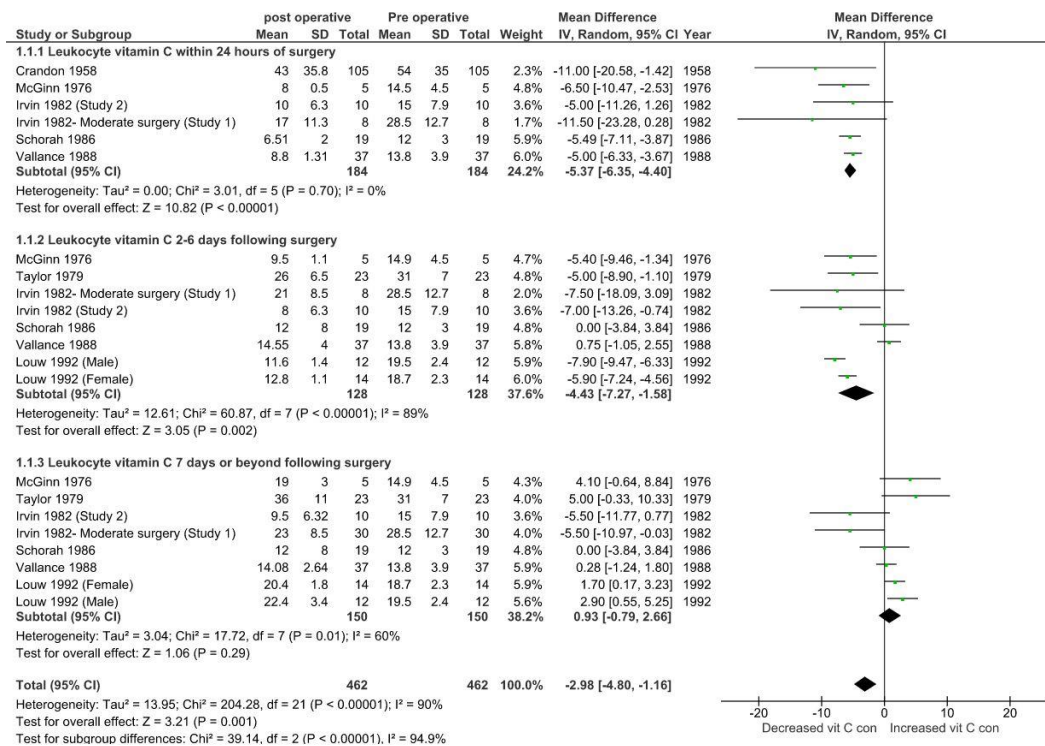Decreased vit C con   Increased vit C con

Figure 3. Meta-analyses comparing pre-operative leukocyte vitamin C concentrations with post-operative concentrations at time points. Diamond represents overall effect size of the meta-analysis. Boxes represent mean differences in concentrations and lines across the boxes represent respective 95% CI, Vit = vitamin, Con = concentration