**Citrate Metabolism in critically ill patients receiving continuous renal replacement therapy using regional citrate anticoagulation**

**(The CiMet Study)**

**Study Protocol**

Fig.6: Schematic diagram representing the possible mechanism of fatty acid synthesis by supplementation of citric acid in Drosophila melanogaster. Supplementation of citric acid increases intracellular citric acid level and activates ATP:Citratelyase (ACLY) enzyme. Activation of ACLY will produce Acetyl-CoA and oxaloacetate by utilizing citrate molecule. Acetyl-CoA will be converted into manonyl Co-A (FAs building block) by Acetyl-CoA carboxylase (ACC) enzyme. ACC will be also allosterically regulated by citrate. Manonyl Co-A will be converted into fatty acids and triglycerides by Fatty acid synthase (FAS) enzyme which will be ultimately stored in fat body tissue of Drosophila.  
                

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**3. List of abbreviations**

*Abbreviation Term*

SCHHS Sunshine Coast Hospital and Health Service

SCUH Sunshine Coast University Hospital

SCHI Sunshine Coast Health Institute

PQ Pathology Queensland

RBWH Royal Brisbane and Womens Hospital

ICU Intensive Care Unit

SCICCR Sunshine Coast Institute for Critical Care Research

(SCUH and SCHI share a common campus at Birtinya)

HREC Human research and ethics committee

HREA Human research ethics application

RCA Regional citrate anticoagulation

CRRT Continuous renal replacement therapy

CVVHDF Continuous veno-venous haemodiafiltration

HF Haemofiltration

**4. Synopsis**

Study title Citrate metabolism in critically ill patients receiving continuous renal replacement therapy with regional citrate anticoagulation

Clinical phase Clinical

Trial design Single centre, prospective study

Aim The primary aim of this study is to investigate the potential metabolic products of infused trisodium citrate.

Hypothesis Infused citrate is metabolised to triglyceride, free fatty acids and sterols and not to bicarbonate as is the current belief.

Number of subjects This study is investigating the potential rise in the concentration of plasma metabolites, postulated to be triglycerides, over time. It is designed as a paired (before/after) study. To calculate the sample size, an estimate of the degree of that rise and its standard deviation (SD) is required. It is known that the plasma citrate concentration rises by as much as 6 fold or more during RCA for CRRT therefore it is not unreasonable to assume a conservative 50% increase in plasma triglyceride concentration from say a normal level of less than 1.50mmol/L (SD 0.50mmol/L) to greater than 2.25mmol/L with the same standard deviation (1). Using a one-sided (superiority) design with a level of significance of 0.025, a power of 0.80 and factoring a 10% dropout/refusal rate, a sample size of 20 is required.

Study duration The study will likely require a six to twelve month recruitment and experimental phase followed by a two month analytical phase.

Endpoints The study will be closed when a total of 20 patients have been recruited.

Inclusion criteria Clinical requirement for RCA CRRT in patients admitted to the SCUH ICU.

Exclusion criteria Patients under the age of 18 years

Patients who are pregnant

Patients with a haemoglobin concentration of less than 80 grams per litre (2).

Patients with advanced hepatic disease (Child’s C)

Patients likely to die within 24 hours of admission to the ICU

Patients with known hyperlipidaemic states (eg: pancreatitis)

Patients taking lipid lowering drugs (eg: statins)

Known hypersensitivity to citrate compounds

Centres Sunshine Coast University Hospital

Ethical approval Applications for ethical approval for the performance of the study will be submitted to the recommended HREC via the HREA as appropriate and in accordance with the national guidelines.

**5. Administrative structure**

Study co-ordination and data collection will be based in the SCUH ICU as a single centre. This centre will be responsible for all administrative aspects of the study including, HREC applications, protocol design, study performance, protocol training, data collection, organisation of investigator meetings as required, data analysis and ultimately, publication of results.

General specimen analysis will occur in the PQ laboratory at SCUH with specialised analyses occurring at the PQ laboratory at RBWH. Measurement of citrate concentration will occur in batches in the designated laboratory space in SCHI. Specimen logging, analysis, quality control and recording of results will be supervised at SCUH.

Where offsite analysis is required, suitable storage, transportation and tracking will be arranged.

**6. Funding**

The study will be funded through either an external grant or from the SCICCR Research Fund. The total cost is expected to be approximately AUD50,000

**7. Background information**

Adult patients admitted to general ICUs with organ failures from any cause frequently require some form of renal support. In Australian ICUs, the most common method of supplying that support is using one of the modalities of CRRT.

As a routine, the SCUH ICU conducts CVVHDF as a modality of CRRT using regional anticoagulation with a proprietary citrate based haemofiltration solution containing trisodium citrate in a concentration of 15.0mmo/L (NamSol 0K).

Sodium citrate chelates ionised calcium in the plasma. Because calcium is an essential co-factor in the coagulation cascade, a state of reversible anticoagulation will exist when ionised calcium levels fall far enough (i[Ca2+] < 0.40 mmol/L). Ultimately the aim is to anticoagulate only the extracorporeal circuit (regional anticoagulation) and to ensure a normal state of coagulation in the intravascular compartment by infusing sufficient calcium, as calcium chloride, to restore normal levels of ionised calcium in the blood (1.15 < iCa2+ < 1.32 mmol/L).

The citrate containing anticoagulant solution is presented in 5.0 litre bags and infused directly into the afferent limb of the extracorporeal circuit at a rate of approximately 2.0 litres per hour. A proportion of the citrate is expectedly lost across the haemofilter membrane whilst the remainder enters the patients’ venous circulation. It is ultimately metabolised mainly in the liver to currently unknown metabolites.

Citrate is normally present in trivial amounts in the plasma as a by-product of normal metabolism with a mean plasma concentration in adults of approximately 0.10 mmol/L (95% CI 0.08, 0.18 mmol/L) (3). Whilst citrate is not toxic per se, a significant rise in plasma concentration is known to occur in patients receiving RCA CRRT (4).

In 2017, the SCUH ICU used approximately 20,000 litres of citrate containing HF fluid and whilst the safety profile is well documented, the metabolites of plasma citrate remain unknown. Documentation of the major metabolites will facilitate a broader understanding of the role of citrate in the critically ill patient population and will lead to further research.

In general, patients requiring CRRT, in whatever form, will need to be supported until their endogenous renal function recovers. This may take days in some cases to weeks to occur. It is anticipated that both the concentrations of plasma citrate and its metabolites will be tracked over time with levels taken once a day at the same time as the routine daily bloods.

Whilst in the ICU, all patients are intensively monitored looking for progress of their disease or recovery as well as early indicators of complications using standard protocols.

The SCUH ICU conducts CRRT using RCA as a standard and preferred method and as a result, the study should proceed smoothly. The ICU has sufficient machines to treat four patients simultaneously and will have at least a two month stock of the haemofiltration solution.

**8. Study rationale**

The primary objective of the study is to document the metabolites of citrate during CRRT with a haemofiltration solution containing 15.0 mmol/L of sodium citrate as the sole extracorporeal circuit anticoagulant.

Current scientific consensus is that exogenously administered citrate is converted directly into bicarbonate in a 3:1, bicarbonate : citrate, stoichiometric ratio (5, 6). A recent study examining the acid-base effects of two different citrate concentration substitution fluids administered in the context of RCA CRRT found that once the plasma bicarbonate concentration returned to normal about 36 hours after the institution of CRRT, the experimental group receiving the 15 mmol/L solution had both a stable and normal bicarbonate concentration and arterial partial pressure of carbon dioxide even though up to 60 litres of citrate containing substitution fluid was intravenously administered each day until the patients regained independent kidney function (7). If it assumed that 75% to 80% of this infused citrate passes through the hemofilter and into the effluent, the patients still receive an average intravenous load of approximately 150 to 200 mmol (~30 to 40 g) of citrate per 24 hour period, equivalent to 450 to 600 mmol (~40 to 50 g) of bicarbonate using the stoichiometric ratio above. By comparison, patients with chronic kidney disease taking up to 2 grams of sodium bicarbonate per day will increase their plasma bicarbonate concentration from approximately 16 mmol/L to greater than 23 mmol/L (8). If the current opinion is correct, an infusion of 200 mmol of citrate per day in patients with acute kidney failure should result in a very large increase in the plasma bicarbonate concentration.

Under normal physiological conditions, the liver accounts for approximately 90% of citrate clearance with the remainder filtered by the kidneys (9). Furthermore, the liver has the capacity to metabolise up to 2500 mmol of citrate per day (~100 mmoL/hr) with an estimated clearance of 5.70 to 10.45 ml/min/kg (10-13). Therefore metabolising an additional 200mmol of citrate per day should present no problem for a healthy liver. This compares favourably with its capacity to metabolise lactate (120 to 240 mmoL/hr) (14, 15).

Also lacking in the literature is an explanation of the mechanism for the conversion of citrate to bicarbonate. Not only which enzymes are utilised but where in the cell this reaction takes place is yet to be determined. The only possible pathway would involve the intermediate production of carbon dioxide. Because cytoplasmic metabolism of citrate does not generate carbon dioxide, the citrate would need to enter the mitochondria to be metabolised via the Krebs cycle to carbon dioxide and water. As there is no mechanism for the transport of citrate directly into the mitochondria, this would seem unlikely.

Cytoplasmic citrate is converted to acetyl CoA and oxaloacetate by ATP citrate lyase with the oxaloacetate being further converted to malate and finally to pyruvate prior to facilitated entry into the mitochondria by a pyruvate transporter where it takes part in the Krebs cycle. It could be postulated that the extra pyruvate thus produced can be added to the usual load of pyruvate produced by the enzyme phosphofructokinase during glycolysis so as to generate the extra carbon dioxide required to increase plasma bicarbonate concentrations. A limitation of this argument is that the production of pyruvate by glycolysis is tightly regulated and excessive cytoplasmic citrate acts to inhibit phosphofructokinase thus at least damping down, or at most inhibiting, the glycolytic production of pyruvate (16). In addition, rising cytoplasmic levels of malonyl-CoA directly inhibit carnitine palmitoyl transferase 1 which is responsible for the transfer of an acyl group from the free fatty acid chain to carnitine to form acyl-carnitine. Acyl-carnitine is transported through the inner mitochondrial membrane by the carnitine carrier protein whereapon it releases the acyl group to combine with CoA within the mitochondrion and form acetyl-CoA. The carnitine is recycled to the cytoplasm by the same carrier protein. Thus malonyl-CoA inhibits the transfer of acyl groups into the mitochondria further damping the activity of the tricarboxylic acid cycle (17).

A more likely scenario is that the acetyl CoA generated by the action of ATP citrate lyase on citrate is used to fuel the production of free fatty acids and sterols. The pyruvate that results from the production of oxaloacetate is cycled back into the mitochondria but results in no net increase in the production of carbon dioxide (Figure 1). It is the primary intention of this research to either confirm or refute this contention.



Figure 1: Proposed metabolic pathway for exogenously administered citrate taken from work with the common fruitfly *Drosophila melanogaster* (18)

The secondary objective of this study is to measure the concentration of citrate in the plasma. The method has been previously validated (ACCidHF Study, ACTRN 12616000515493) and will be used to document the rise in plasma citrate concentration during RCA CRRT.

This study has not been previously performed and is designed as a prospective trial to be conducted at the SCUH ICU by the Principal Investigator as part of his research higher degree.

**9. Methodology**

*a) Research plan*: Acute kidney injury (AKI) affects up to 30% of ICU patients, with 5% of all patients requiring some form of CRRT (19). Of those patients requiring CRRT there is an associated high mortality rate secondary to their multiple organ failure (20).

CRRT involves an extracorporeal blood circuit through a haemofilter. This circuit requires anticoagulation to prevent filter clotting and subsequent treatment limitation. In Australian ICUs, systemic heparin is the commonest form of circuit anticoagulation but it is not without the risks of bleeding, heparin-induced thrombotic thrombocytopaenic syndrome and inadequate filter life (21, 22). For these reasons, regional anticoagulation with a citrate containing renal substitution solution (haemofiltration fluid – HF fluid) has been widely accepted as a safe alternative option in patients requiring CRRT who are at risk of bleeding (23). Currently though, modalities of CRRT and formulations of citrate HF fluid vary with very real risks associated with administration errors (24).

At the SCUH ICU, citrate HF has been used as our first line anticoagulant for patients requiring CRRT for more than nine years. Furthermore, regional citrate anticoagulation is currently recommended as first line CRRT anticoagulation in the latest KDIGO guidelines (25). By virtue of the sole use of one HF fluid (Baxter NamSol 0K 15.0mmol/l HF) for all patients requiring citrate based CRRT, the citrate haemofiltration protocol used at SCUH ICU has the advantages of simplicity and safety. In our experience, there have been no clinically significant adverse effects with the use of citrate HF fluid. We use a weight based prescription for CRRT as a routine (26).

In the last twelve months the SCUH ICU has placed approximately 60 patients on RCA-CRRT with an average length of time on CRRT of approximately 6 days. At 2.5L/hr this translates to an annual use of HF fluid of about 21600 litres per year. Whilst we make every effort to monitor for side effects and toxicity, we do not know what becomes of the citrate that is returned to the plasma compartment during RCA CRRT nor whether the metabolites have the potential to cause long term harm.

Patients who are enrolled in the study will have one blood sample taken each day when the routine bloods are taken between 0500hrs and 0600hrs. It is planned to take one sample up to 24 hours before the proposed start of RCA CRRT, then one sample per day for five consecutive days, or as dictated by the length of treatment, whilst the patient is being treated with RCA CRRT and finally two samples on consecutive days after the treatment ceases. If the patient continues CRRT beyond five days, samples will only be taken for the first five days. Two extra samples will be taken on Day 3 of CRRT (see below). At most, a total of ten samples will be obtained.

A standard panel of biochemistry (sodium, potassium, chloride, magnesium, calcium, phosphate, albumin, bicarbonate, urea, creatinine, glucose, bilirubin and liver enzymes) as well as routine acid/base parameters (pH, PCO2, standard base excess and lactate) using the ICU based ABL800 Flex acid-base analyser. A subsample of blood (~10.0ml) will be used to assay both the concentration of citrate and a panel of hormones and potential metabolites. These will include insulin, glucagon, parathyroid hormone, adrenaline, noradrenaline, triglycerides, cholesterol, low density lipoprotein, pyruvate, acetoacetate and beta hydroxy butyrate. The paired lactate pyruvate results will be used to calculate the lactate-pyruvate ratio as an indicator of the redox state.

A further 10ml of blood will be collected on Day 3 of CRRT. This blood will be collected as 5ml for the purposes of an organic acid screen and 5ml for an analysis of white blood cell metabolites.

These supplementary assays will be performed at RBWH.

CRRT will be performed using a weight based prescription and patients will be monitored for the end points of filter life, acid-base status, solute clearance and adverse events to ensure effective treatment and safety. The standard protocols for citrate based haemofiltration and calcium replacement will be used. Strict guidelines will be in place for treatment adjustments should there be safety concerns arise.

*b) Screening*: Potentially eligible patients will be identified by their treating Intensivist. For all ICU inpatients, potential patients will be identified and the research team informed.

*c) Informed consent*: Those eligible will be offered the opportunity to participate in the research project. If, for reasons of illness acuity, a patient is unable to consent, the person responsible will be approached, or failing that, the Adult Guardian.

The ICU patient population dictates that many patients who are eligible for this study will not be competent to provide informed consent. Developed from the guidelines in Chapter 4.4. of the NHMRC National Statement (27), the process for obtaining consent when a patient or person responsible is not available will be used according to the following hierarchy:

1. Consent: In accordance with the requirements of the Human Research Ethics Committee approval and applicable legislation, the patient may be enrolled in to the research study only after written consent is obtained from either the patient or their legally authorised representative.
2. Should the patient or person responsible wish to withdraw from the study permission will be sought to utilise the samples and data already obtained. Withdrawal from the CiMet study not affect the CRRT or the clinical care offered to the patient.
3. Should a patient pass away during the study the data and samples already collected will be used for the purpose of this study only.

A patient information sheet will be provided so the patient (or representative) may make an informed decision regarding study enrolment. Once agreement to participate occurs, the patient (or representative) will be asked to sign the study consent form. Once the consent form is signed, one copy is kept with the data, one with the medical record and a third copy is given to the patient.

*d) Randomisation*: Randomisation will not be necessary as this is a single arm study.

*e) Blinding*: Blinding is unnecessary as this is a single arm (before/after) study.

*f) Procedures*: All procedures will be performed in accordance with existing standard CRRT protocols with the decision to commence CRRT based on biochemical and physiological parameters and at the discretion of the treating Intensivist. This is current practice.

(i) Vascular access: Using standard ICU protocols, a double lumen dialysis catheter (Vascath™) will be inserted under aseptic conditions and ultrasound control into one of the jugular veins, femoral veins or subclavian veins. Post-insertion radiography will confirm the position of the catheter and the absence of complications prior to use.

(ii) Haemofiltration: All patients in the study will receive NamSol 0K (15.0mmol/L) at a predilution at a rate determined by a weight formula.

The blood pump will be set to a range of 150 to 250ml/min and all alarms will be activated.

Measurements of ionised calcium and serum magnesium concentrations will be performed according to existing standard protocols.

Haemofiltration will be prescribed in accordance with the standard ICU Metavision order form.

(iii) Cessation of CRRT: The decision to cease citrate CRRT will address the following points

1. A set of biochemical and physiological parameters that suggest that CRRT is no longer necessary. These parameters will be interpreted by the treating Intensivist as per standard care.
2. Where an alternative form of CRRT is deemed more suitable. Such as, in the case of:
   1. Evidence of citrate accumulation with the ratio of total to ionised calcium exceeding 2.5:1.
   2. Metabolic alkalosis with pH > 7.50 or SBE > 10.00mmol/l
   3. Inadequate solute clearance ([urea] persistently higher than 20.0mmol/l)
   4. A preference for intermittent haemodialysis where the patient may be leaving the ICU or is mobile during the day
   5. Recurrent filter clotting resulting in inadequate solute clearance.

(iv) Sample collection: As part of routine care, patients enrolled in the CiMet study will have an intra-arterial catheter in place to facilitate the painless sampling of blood. Arterial blood will be collected using a standard technique prior to and at daily intervals after the commencement of haemofiltration. Approximately 10.0ml of extra blood will be collected once per day and all samples will be analysed using standard biochemical means, that is, a Beckman Coulter™ multianalyser for general serum samples and a Radiometer ABL800 Flex™ for acid-base samples. Blood samples for citrate and metabolite assays will be spun and separated and all samples will be chilled to -80degC and transported to RBWH as necessary for batch analysis. Citrate analysis will take place in the SCHI laboratories and will be performed by the Principal Investigator.

Demographic data including age, sex, reason for admission and relevant co-morbidities will be collected by the research team. In addition, dose and timing of medications, type and volume of intravenous fluids and enteral feeds as well as any blood product transfusions will all be recorded. The progress of their renal function will be assessed by regular blood tests in accordance with standard ICU care.

Anonymity and confidentiality will be maintained throughout the study. All participants will be assigned a unique participant number in place of any identifying information. Should any re-identification be required for data quality and assurance purposes, a separate master log will be maintained to match the participant number with the hospital unit record number. This log will be stored securely in a locked cabinet within the locked Research Office.

**10. Data management and statistical analysis**

General demographic data, indices of severity of illness (APACHE II score), biochemical and acid-base data will be collected. After initial verification, all data will be subsequently re-identifiable.

Currently, the SCUH ICU uses RCA CRRT to treat 40 to 50 patients per year with acute kidney failure, therefore a six to nine month data collection time should suffice.

Statistical analysis will be performed using a proprietary statistical package (STATA version 15.x). Data will be organised and trends reported using standard descriptive statistics (mean (SD), median (IRQ), proportions). More detailed inferential analysis will be done using regression techniques that account for the linear and correlated nature of the data. All data will be analysed on an intention to treat basis.

The project will be managed locally by the principal investigator (CA). General data collection will be the responsibility of the ICU Research staff. Biochemical and acid-base data will be collected by the ICU Nursing staff. Consenting and enrolment will be the responsibility of the Consultant Intensivist or the ICU Research Staff or their delegates on that day.

Governance will be overseen by the local SCHHS Research Board.

Data will be stored on a secure local server. It is proposed to store the data for a maximum of five years after which it will be deleted.

All analyses will be performed at the end of the study and patients will be enrolled on an intention to treat basis.

**11. Human research ethics committee approvals**

An application requesting approval to conduct this study will be submitted to both the HREC at the Prince Charles Hospital and the HREC at the University of Queensland. The second submission is necessary as this study forms part of a research higher degree being conducted by the Principal Investigator, Dr. C Anstey. The content and format of the patient information statements and consent forms will also be submitted.

The Principal Investigator will be responsible for the reporting of adverse events in relation to the performance of CVVHDF in accordance with HREC guidelines. Any amendments to the study protocol and material will be notified to the HREC by the Principal Investigator.

All study records and documents will be securely stored for a minimum of 15 years from the end of the study or for the period required by the HREC.

*a) Withdrawal of consent*: At any time during the study the patient may withdraw consent. This is explained in the informed consent form and the patient information sheet and a withdrawal of consent form is available for signing. Withdrawal of consent will have no impact on quality of care and patients who continue to require CRRT will be no longer have blood sent for citrate or metabolite assays.

*b) Adverse event reporting*: Adverse events will be reported to the HREC according to their guidelines.

*c) Protocol amendments*: Significant study protocol changes will have a written amendment request sent to the HREC for written approval. The approval letter will bear the signature of the HREC Chair and will refer to the protocol number, protocol title, amendment number and amendment date. The protocol amendment can only be implemented after HREC approval.

*d) Study termination*: The study may be terminated for any of the following reasons: study completion, failure of sufficient patient enrolment or at the discretion of the overseeing SCHHS Governance Unit.

*e) Notification of study closure*: Within 3 months of either study completion or termination, the Principal Investigator will notify the HREC of that fact.

**12. Data quality assurance**

Data collection quality will be checked and assurance will be monitored by the trial co-ordinator.

*a) Principles*: The quality management principles will involve a patient focus, demonstration of leadership on the part of the investigator(s), education of both patients and their relatives involved in the study and the use of a systematic and factual approach to decision making. Overall conduct of the study will be overseen by the local Research Board with regular reports on conduct and progress from the investigator.

*b) Safety considerations*: As a routine, all patients receiving CRRT are closely monitored with policies aimed at prevention of adverse events in place. If events occur, mechanisms currently exist to minimise the impact on patient safety and to audit, report and investigate and thus to educate staff and prevent recurrence. At any time, the CVVHDF regime may be stopped at the discretion of the treating Intensivist. All morbidity and mortality is investigated by both local and hospital-wide committees.

*c) Follow up*: No extraordinary follow up will be necessary for this study.

*d) Records retention*: As previously stated, the Principle Investigator will retain and preserve one copy of all data generated during the study for a period of 15 years following study closure.

**13. Publication and presentation**

It is proposed to publish the results in the peer-reviewed scientific literature with appropriate acknowledgement of all investigators. Similarly, it is proposed to present the results at appropriate postgraduate/scientific meetings.

Publication or presentation of the results may see further research undertaken in this area.

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