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# Citrate

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# A CITRATE CONTAINING DIALYSIS FLUID FREE OF ACETATE

The Baxter **SelectBag Citrate** concentrate is a citrate-containing, acetate-free concentrate developed by Baxter for hemodialysis and hemodiafiltration treatments.

Citrate provides energy and buffering capacity to the patient. In addition, citrate is a well-known antioxidant and anticoagulant with potential benefits in dialysis to reduce inflammation, a risk factor for cardiovascular disease.

It is a well-tolerated and biocompatible alternative to regular acetate containing concentrates allowing for individualized treatment.

Patients may benefit from improved hemodynamic stability, improved control of acid-base balance and decreased thrombogenicity.

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## CITRATE VERSUS ACETATE-BASED DIALYSATE IN ON-LINE HAEMODIAFILTRATION. A PROSPECTIVE CROSS-OVER STUDY.

Manuel Molina Nuñez, Rosa de Alarcón, Susana Roca, Gracia Álvarez, María Soledad Ros, Cristina Jimeno, Laura Bucalo, Isabel Villegas, María Ángeles García.

**Background and Aims**: A bicarbonate dialysate acidified with citrate (CD) has been reported to have local anticoagulant effect and improves biocompatibility. This study examines the effect of CD on dialysis efficiency, coagulation, acidbase status, electrolytes, and inflammation in patients in online hemodiafiltration (OL-HDF).

**Methods**: 35 patients in OL-HDF were enrolled in a prospective, cross-over study for a 24-week period and two phases alternating CD and acetate dialysate fluid (AD). Parameters on study were predialysis levels of bicarbonate and ionic calcium, reactive C Protein (CRP), and beta-2 microglobulin ($B_2$MG) and postdialysis levels of activated tromboplastine time, bicarbonate, and ionized calcium.

**Results**: No significant differences in coagulation parameters, pH, and predialysis bicarbonate were found. The postdialysis bicarbonate and postdialysis calcium were lower with CD. Dialysis efficiency was greater with CD. Regarding inflammatory parameters, both CRP and $B_2$MG were lower using CD.

**Conclusion**: The use of CD is safe and effective in OL-HDF, and it improves dialysis efficacy, postdialysis alkalosis, and inflammation.

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## CITRATE DIALYSATE DOES NOT INDUCE OXIDATIVE STRESS OR INFLAMMATION IN VITRO AS COMPARED TO ACETATE DIALYSATE.

Rafael Pérez-García[a], Rafael Ramírez Chamond[b], Patricia de Sequera Ortiz[a], Marta Albalate[a], Marta Puerta Carretero[a], Mayra Ortega[a], M. Caridad Ruiz Caro[a], Roberto Alcazar Arroyo[a].

**Background**: Increased acetataemia during haemodialysis sessions has been associated with a number of abnormalities, including increased oxidative stress, pro-inflammatory cytokines and nitric oxide synthesis. Citric acid may play an alternative role to acetate as a dialysate stabilizer given that the effect of citrate on complement and leukocyte activation is different to that of acetate. The purpose of this study was to compare the inflammatory effect in immunocompetent blood cells of acetate dialysate and citrate dialysate.

**Methods**: The effect of acetate and/or citrate was investigated in the whole blood of uremic patients and in healthy in vitro samples. Four types of dialysate were tested: dialysate 1, acetate-free with 1 mmol/L of citrate; dialysate 2, with 0.8 mmol/L of citrate and 0.3 mmol/L of acetate; dialysate 3, citrate-free with 3 mmol/L of acetate; and dialysate 4, citrate-free with 4 mmol/L of acetate. The cell types used were: human monocyte culture (THP-1); and peripheral blood mononuclear cells (PBMCs) from healthy subjects and uremic patients on haemodialysis. ICAM-1 was determined and levels of reactive oxygen species and total microvesicles were quantified.

**Results**: Unlike the citrate dialysates, the dialysates with acetate (dialysate 3 and dialysate 4) induced increased ICAM-1 expression density in THP-1 cells; an increase in ICAM-1 expression was observed in the immunocompetent cells of healthy subjects with acetate dialysate (dialysate 3 and dialysate 4) but not with citrate dialysate (dialysate 1 and dialysate 2). No significant ICAM-1 differences were found between the different dialysates in the cells of haemodialysed patients. Reactive oxygen species expression and the number of microvesicles increased significantly with acetate dialysate but not with citrate dialysate in the cells of both healthy subjects and haemodialysed patients.

**Conclusion**: At the concentrations in which it is generally used in clinical practice, acetatebased dialysate increases oxidative stress and the total number of microvesicles and may induce another pro-inflammatory stimuli in uremic patients on haemodialysis. Citrate dialysates do not induce this activation, which could make them a suitable alternative in clinical practice.

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## ACUTE EFFECT OF CITRATE BATH ON POSTDIALYSIS ALKALAEMIA.

Patricia De Sequera Ortiz, Marta Albalate Ramón, Rafael Pérez-García, Elena Corchete Prats, Patricia Arribas Cobo, Roberto Alcázar Arroyo, Maira Ortega Díaz, Marta Puerta Carretero.

**Introduction**: The correction of metabolic acidosis caused by renal failure is achieved by adding bicarbonate during dialysis. In order to avoid the precipitation of calcium carbonate and magnesium carbonate that takes place in the dialysis fluid (DF) when adding bicarbonate, it is necessary to add an acid, usually acetate, which is not free of side effects. Thus, citrate appears as an advantageous alternative to acetate, despite the fact that its acute effects are not accurately known.

**Objective**: To assess the acute effect of a dialysis fluid containing citrate instead of acetate on acid-base balance and calcium-phosphorus metabolism parameters.

**Material and methods**: A prospective crossover study was conducted with twenty-four patients (15 male subjects and 9 female subjects). All patients underwent dialysis with AK-200-Ultra-S monitor with SoftPac® dialysis fluid, made with 3 mmol/L of acetate and SelectBag Citrate®, with 1 mmol/L of citrate and free of acetate. The following were measured before and after dialysis: venous blood gas monitoring, calcium (Ca), ionic calcium (Cai), phosphorus (P) and parathyroid hormone (PTH).

**Results**: Differences (p<0.05) were found when using the citrate bath (C) compared to acetate (A) in the postdialysis values of: pH, C: 7.43 (0.04) vs. A: 7.47 (0.05); bicarbonate, C: 24.7 (2.7) vs. A: 27.3 (2.1) mmol/L; base excess (BEecf), C: 0.4 (3.1) vs. A: 3.7 (2.4) mmol/L; corrected calcium (Cac), C: 9.8 (0.8) vs. A: 10.1 (0.7) mg/dL; and Cai, C: 1.16 (0.05) vs. A: 1.27 (0.06) mmol/L. No differences were found in either of the parameters measured before dialysis.

**Conclusion**: Dialysis with citrate provides better control of postdialysis acid-base balance, decreases/avoids postdialysis alkalaemia, and lowers the increase in Cac and Cai. This finding is of special interest in patients with predisposing factors for arrhythmia and patients with respiratory failure, carbon dioxide retention, calcifications and advanced liver disease.

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## A NONINFERIORITY TRIAL COMPARING A HEPARIN-GRAFTED MEMBRANE PLUS CITRATE-CONTAINING DIALYSATE VERSUS REGIONAL CITRATE ANTICOAGULATION: RESULTS OF THE CITED STUDY.

Björn Meijers, Christoph Metalidis, Thomas Vanhove, Ruben Poesen, Dirk Kuypers and Pieter Evenepoel.

**Background**: Anticoagulation is a prerequisite for successful haemodialysis. Heparin and low-molecular weight heparins are routinely used despite increased bleeding risk. Regional citrate anticoagulation (RCA) is efficacious, but is laborious and may induce metabolic disturbances. Heparin-grafted membranes are less efficacious. It is not known whether combining citratecontaining dialysate and a heparin-grafted membrane is a valid anticoagulation strategy.

**Methods**: We performed a randomized crossover noninferiority trial, with a prespecified noninferiority threshold of 10% in maintenance dialysis patients ($n$=25). We compared the combination of citrate-containing dialysate plus a heparin-grafted membrane [CiTrate and EvoDial (CiTED) protocol] with RCA. The primary endpoint was completion of dialysis without significant clotting. Secondary endpoints included time to clotting, achieved $Kt/V_{urea}$, loss of total cell volume, venous air chamber clotting score and systemic-ionized calciumconcentration.

**Results**: In total, 1284 sessions were performed according to study protocol, 636 in the CiTED arm and 648 in the RCA arm. The primary outcome of preterm interruption due to clotting occurred in 36 (5.7%) of sessions in the CiTED arm, and in 40 (6.2%) sessions in the RCA arm, thereby meeting noninferiority criteria (P<0.0001). Most of the clotting events occurred in the fourth hour of dialysis. Repetitive clotting occurred in four patients in the CiTED arm and one patient in the RCA arm. Time to preterm interruption due to clotting and achieved $Kt/V_{urea}$ was not significantly different. Systemic-ionized calcium levels during treatment were significantly lower in the RCA arm and clinically relevant hypocalcaemia was noted only in the RCA arm.

**Conclusion**: The combination of citrate-containing dialysate and a heparin-grafted membrane is a valid alternative to RCA.

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## CITRATE REDUCES CALCIUM PRECIPITATION FORMATION AND PARTICLE INDUCED INFLAMMATION

*Emma Lindeberg, Gunilla Grundström, R&D Gambro Lundia AB, Sweden*

**OBJECTIVE:**
During dialysis calcium precipitates are formed in the fluid path and some of these particles are likely to reach the patient. Circulating particles are known to cause inflammation in diseases such as gout and a major part of atherosclerotic plaques consist of calcium phosphates deposits. Most dialysis patients suffer from both increased inflammation and atherosclerosis and the aim of this study was to investigate whether calcium precipitation may contribute to these conditions. We also wanted to investigate the potential benefits of replacing remaining acetate in dialysis fluids with citrate. Today citrate is mainly used as anticoagulant but if used as an active ingredient in dialysis fluids citrate, being a calcium chelator and antioxidant, has the potential to reduce both particle formation and particle induced inflammation.

**METHODS:**
Calcium precipitates were produced by mixing $NaHCO_3$ (35 mM) and $CaCl_2$ (1-5 mM) in Phosphate Buffered Saline. The inflammatory response to the precipitates was evaluated as well as the cytotoxic effect. To evaluate cell toxicity a murine fibroblast cell line (L929) was exposed to defined amounts of calcium precipitates for 72 h with or without citrate (1 mM) present. Inhibition of Cell Growth (ICG) was calculated as a measure of toxicity. The inflammatory response was investigated by exposing Human Peripheral Blood Mononuclear Cells (PBMCs) from healthy volunteers to defined amounts of particles for 24 h with or without citrate (1 mM) present. Inflammation was measured as increased expression of IL-1ß and IL-6 analyzed by ELISA.

**RESULTS:**
The presence of calcium precipitates significantly induced both cell toxicity and inflammation. Citrate significantly reduced particle formation which did not occur until $CaCl_2$ concentrations above 3mM with citrate compared to 2 mM $CaCl_2$ without. The magnitude of particle induced cell toxicity was reduced by 30 % by citrate and higher levels of calcium was tolerated (50 % ICG at 4,1 mM $CaCl_2$ with citrate vs. 2,4 mM without). Citrate reduced the inflammatory response to calcium precipitates. $CaCl_2$ EC50 values for both IL-1ß (3,4 mM vs. 2,4 mM) and IL-6 (3,3 mM vs. 2,5 mM) were increased with citrate.

**CONCLUSION:**
In this study citrate was shown to significantly reduce particle formation and particle induced cell toxicity and inflammation. The results indicate that citrate in the dialysis fluid may reduce treatment induced inflammation and atherosclerosis.

Poster presented at the 49th ERA-EDTA Congress in Paris, France (2012), Abstract FP031



## LOW CONCENTRATIONS OF CITRATE REDUCE COMPLEMENT AND GRANULOCYTE ACTIVATION IN VITRO IN HUMAN BLOOD.

Shan Huang[1], Kerstin Sandholm[1], Nina Jonsson[1,2], Anders Nilsson[3] Anders Wieslander[3], Gunilla Grundström[3], Viktoria Hancock[3] and Kristina N. Ekdahl[1,2].

**Background**: The use of acetate in haemodialysis fluids may induce negative effects in patients including nausea and increased inflammation. Therefore, haemodialysis fluids where acetate is substituted with citrate have recently been developed. In this study, we investigated the biocompatibility of citrate employing concentrations used in haemodialysis.

**Methods**: The effects of citrate and acetate were investigated in human whole blood in vitro under conditions promoting biomaterial-induced activation. Complement activation was measured as generation of C3a, C5a and the sC5b-9 complex, and granulocyte activation as up-regulation of CD11b expression. For the experimental set-up, a mathematical model was created to calculate the concentrations of acetate and citrate attained during haemodialysis.

**Results**: Citrate reduced granulocyte activation and did not induce higher complement activation compared with acetate at concentrations attained during haemodialysis. Investigating different citrate concentrations clearly showed that citrate is a potent complement inhibitor already at low concentrations, i.e. 0.25 mM, which is comparable with concentrations detected in the blood of patients during dialysis with citrate-containing fluids. Increased citrate concentration up to 6 mM further reduced the activation of C3a, C5a and sC5b-9, as well as the expression of CD11b.

**Conclusion**: Our results suggest that citrate is a promising substitute for acetate for a more biocompatible dialysis, most likely resulting in less adverse effects for the patients.

Clinical Kidney Journal (2014)

## CALCIUM MASS BALANCES IN ON-LINE HEMODIAFILTRATION (HDF) USING CITRATE CONTAINING ACETATE-FREE AND REGULAR DIALYSIS CONCENTRATES.

Denis Steckiph[1], Andrea Bertucci[1], Michele Petrarulo[2], C. Baldini[3], Giovanni Calabrese[4] and Marco Gonella[4].

1 Gambro Hospal s.p.a, Bologna, Italy
2 Renal Stone Laboratory, AO Mauriziano, Turin, Italy
3 Biochemical and Clinic Laboratory, OSPEDALE S. SPIRITO, Casale Monferrato, Italy
4 Nephrology and Dialysis Unit, OSPEDALE S. SPIRITO, Casale Monferrato, Italy

**BACKGROUND:**

**Background.** Citrate-containing acetate-free haemodialysis concentrate may potentially improve removal efficiency and reduce the inflammatory impact of dialysis, but the propensity of citrate to form complexes with calcium (Ca) is likely to affect the Ca transport. We evaluated the mass balance of Ca (CaMB) and Citrate (CitMB) during HDF with a new citrate containing acetate-free dialysis fluid versus a regular dialysis fluid.

**METHODS:**

**Methods:** This randomized cross-over study enrolled 18 stable ESRD pts (71±11 yrs) regularly on 4.5 hours on-line postdilution HDF treatments. Dialysis fluid prepared from SelectBag® Citrate concentrate (1.5 mM Ca, 1 mM citrate, 0 acetate; Cit-) (Gambro) was compared to regular dialysis fluid (1.5 mM Ca, 0 citrate, 3 mM acetate; Ac-). Each patient was treated for one week with Ac-HDF and then switched to Cit-HDF for another week, or viceversa. All patients were treated with 2.1 m2 Polyflux H dialyzers (Gambro). In the mid-week session of each period Ca (total and ionized) and citrate levels were measured in plasma and dialysis fluid at start, at 60 and 120 minutes, after start, and at end of treatment. CaMB and CitMB were calculated from total Ca and citrate levels in dialysis fluid. Citrate in plasma and dialysis fluid was analysed by suppressed-conductivity anion-chromatography. Anion-separator: 0.3x25 cm IonPac Fast Anion IIIA (Dionex Corp., U.S.A.); mobile phase: 20 mmol/L NaOH aqueous solution; flow-rate: 1.0 mL/min.

**RESULTS:**

**Results:** The convective volume, set automatically by TMP biofeedback (UltraControl, Gambro), was 26.3±3.3 in Ac-HDF and. 26.0±3.9 l/session in Cit-HDF (p=0.73). Using 1.5 mM Ca in dialysis fluid, the plasma total Ca level was stable during Cit-HDF treatments (from 2.37±0.14 to 2.42±0.11 mM, p=0.13), while it increased during Ac-HDF treatments (from 2.31±0.12 to 2.63±0.16 mM, p<0.0001). The plasma ionized Ca level decreased during Cit-HDF treatments (from 1.12±0.07 to 1.07±0.03 mM, p<0.001) whereas it increased in Ac-HDF (from 1.13±0.05 to 1.22±0.03 mM, p<0.0001). CaMB was different between the two periods (p<0.0001): removal of 274±260 mg Ca in Cit-HDF versus delivery of 125±174 mg Ca in Ac-HDF. Plasma citrate level increased in Cit-HDF (from 0.12±0.05 to 0.40±0.10 mM, p<0.001), while it was stable during Ac-HDF (from 0.13±0.02 to 0.12±0.05 mM, p=0.24). CitMB indicated that Cit-HDF was associated with a delivery of 5.3±3.8 g citrate, while Ac-HDF a removal of 0.8±0.4 g (p<0.0001).

**CONCLUSION.**

**Conclusion.** With the same Ca concentration as in regular dialysis fluid the use of 1 mM citrate dialysis fluid in on-line postdilution HDF resulted in a different CaMB. Using a 1.5 mM Ca dialysis fluid, the intra-dialysis increase in total and ionized Ca levels seen with Ac-HDF was not seen with Cit-HDF. Our results suggest a need to reevaluate the prescription of Ca in dialysis fluid when shifting to citrate-containing HD concentrates.



## CITRATE DIALYSIS FLUID AND CALCIUM MASS BALANCE

Nilsson A, Sternby J, Grundström G, Alquist M
Gambro Lundia AB

**INTRODUCTION:**

Citrate-containing concentrates have in recent years been introduced for use in hemodialysis. Citrate entering the blood will form complexes with calcium and some of the protein bound calcium will then be released to maintain the equilibrium between protein bound and free ionized calcium. Both free and citrate bound calcium can pass the dialysis membrane which increases the calcium transport from the blood. We developed an algorithm to calculate the mass balance of calcium transfer over the dialysis membrane. The objective was to establish what calcium concentration in citrate containing dialysis fluid gives the same total calcium mass transfer as a citrate-free dialysis fluid. The algorithm takes into account the complex formation between citrate and calcium and also includes other known calcium complexes present in the dialysis fluid, as well as in the blood.

**METHODS:**

The mass transfer area coefficients for ions and complexes were assumed proportional to their diffusive mobility. The electrical potential across the membrane (membrane potential) was considered by requiring electroneutrality. As the transfer of complexes across the membrane affects the concentration gradients for both complexes and individual ions, we included chemical equilibrium equations in the calculations. Each albumin molecule in the blood can bind a large number of (pH dependent) ions like hydrogen, calcium and magnesium with different equilibrium constants. The binding of calcium and magnesium ions to bicarbonate and citrate was also included in the calculations.

The dialyzer was considered being composed of a number of serial subsegments. For each segment the transport of each solute and each complex were calculated separately, taking the membrane potential into account. With the given inlet concentrations for each solute the outlet concentrations for a dialyzer segment were calculated from the transports. The total concentration of each compound was calculated by summing the free concentration and the concentrations of all complexes where they appear. From the total concentration a new distribution between free concentration and complexes was calculated. The recalculated concentrations were used as input to the next subsegments. The following parameters were used: Initial total calcium plasma concentration 2.4 mM (mmol/l), blood flow rate 300 ml/min, dialysis fluid flow rate 500 ml/min, urea KoA 1000 ml/min, calcium concentrations in dialysis fluid without citrate 1.0, 1.25, 1.5 and 1.75 mM, and citrate levels in the dialysis fluid 0.25 – 2 mM.

**RESULTS:**

The need for extra calcium in the dialysis fluid increases almost linearly with the citrate level. Each mM of citrate requires an additional 0.15 mM of calcium to maintain the same calcium mass balance. Other settings of blood and dialysis fluid flow rates, urea KoA, and total calcium concentrations in plasma gave the same result.

**CONCLUSION:**

For each mM of citrate in the dialysis fluid the calcium level should be increased by 0.15 mM to maintain the calcium transport during the treatment.

## ACETATE-FREE, CITRATE-ACIDIFIED BICARBONATE DIALYSIS IMPROVES SERUM CALCIFICATION PROPENSITY—A PRELIMINARY STUDY.

Georg Lorenz, Christopher C. Mayer, Quirin Bachmann, Sarah Stryeck, Matthias C. Braunisch, Bernhard Haller, Javier Carbajo-Lozoya, Alina Schmidt, Simon Witthauer, Jasmin Abuzahu, Stephan Kemmner, Susanne Angermann, Naresh Koneru, Siegfried Wassertheurer, Richard Bieber, Uwe Heemann, Tobias Madl, Andreas Pasch and Christoph Schmaderer.

**Background**: A novel in vitro test ($T_{50}$ test) assesses *ex vivo* serum calcification propensity and predicts mortality in chronic kidney disease and haemodialysis (HD) patients. For the latter, a time-dependent decline of $T_{50}$ was shown to relate to mortality. Here we assessed whether a 3-month switch to acetate-free, citrate-acidified, standard bicarbonate HD (CiaHD) sustainably improves calcification propensity.

**Methods**: $T_{50}$ values were assessed in paired midweek pre-dialysis sera collected before and 3months after CiaHD in 78 prevalent European HD patients. In all, 44 were then switched back to acetate. Partial correlation was used to study associations of changing $T_{50}$ and changing covariates. Linear mixed effect models were built to assess the association of CiaHD and covariates with changing $T_{50}$.

**Results**: A significant intra-individual increase of serum calcification resilience was found after 3months on CiaHD (206 ± 56 to 242 ± 56 min; P<0.001), but not after switching back to acetate (252 ± 63 to 243 ± 64 min; *n*=44; P=0.29). CiaHD, Δ serum phosphate and Δ albumin but not Δ ionized calcium and magnesium were the strongest determinants of changing $T_{50}$. Beneath $T_{50}$, only serum albumin but not phosphate changed significantly during 3months of CiaHD.

**Conclusion**: CiaHD dialysis favourably affected calcification propensity as measured by the $T_{50}$ test.Whether this treatment, beyond established phosphate-directed treatments, has the potential to sustainably tip the balance towards a more anticalcific serum milieu needs to be further investigated.

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## IMPACT OF ACETATE- OR CITRATE ACIDIFIED BICARBONATE DIALYSATE ON EX VIVO AORTA WALL CALCIFICATION.

Ricardo Villa-Bellosta[1], Eduardo Hernández-Martínez[2], Eva Mérida-Herrero[2] & Emilio González-Parra[1].

**Background**: Vascular calcification is highly prevalent in patients with chronic hemodialysis. Increased acetatemia during hemodialysis sessions using acetate-acidified bicarbonate has also been associated with several abnormalities. By contrast, these abnormalities were not induced by citrate-acidified bicarbonate dialysis. Moreover, citrate is biocompatible alternative to acetate in dialysis fluid. However, the effects of citrate on vascular calcification during hemodialysis had not been studied in detail. This study analyzed herein the effects of acetate- or citrate-acidified bicarbonate dialysis on vascular calcification.

**Methods**:
**Hemodialysis conditions and sampling**: Each patient underwent a conventional, purely diffusive 4 hour (mid-week) hemodialysis session without hemodiafiltration, using a high flux helixone dialyzer (Fresenius, CUF, 59 mL/h/mmHg; surface, 1,8 $m^2$) and Dialysis Machine Fresenius 5008 in acetate-acidified bicarbonate dialysis, according to previous studies[3,4,7]. In case of citrate-acidified bicarbonate dialysis, high flux helixone dialyzer (Polyflux 21H, Gambro, CUF 78 ml/min/ mmHg, surface 2.1 $m^2$) and Dialysis Machine Artis Baxter were used. The acetate-acidified bicarbonate dialysate was composed of 3–4 mmol/L acetate, 1.5 mmol/L calcium, 35 mmol/l bicarbonate, 1.5 mmol/L potassium, 0.5 mmol/L magnesium and 140 mmol/L sodium. The citrate-acidified bicarbonate dialysate (SelectCitrate Cx250G, Gambro) contained 1 mmol/L citrate without acetate, 1.65 mmol/L calcium, 35 mmol/l bicarbonate, 2 mmol/L potassium, 0.5 mmol/l magnesium and 140 mmol/L sodium). Blood samples before and after hemodialysis were collected in heparin-containing tubes and centrifuged at 5000 rpm for 5 min at 4 °C, according to previous studies[3,4,7]. Plasma samples were frozen in liquid nitrogen and stored at −80 °C until further use, as previously studies[3,4].
**Aorta isolation and calcification assay**: Rats were euthanized via carbon dioxide inhalation and thoracic aorta tissue was perfused with saline and removed according to previously published protocols[8]. For calcification assays, aortic rings were cultured ex vivo (37 °C. 5% $Co_2$) in Minimum Essential Medium (MEM) Eagle (Gibco, Paisley, United Kingdom) containing 45-calcium as a radiotracer (Perkin Elmer, Boston), 0,1% Fetal Bovine Serum, 1 mmol/L L-glutamine, 100 IU/ mL, penicilin, 100 µg/mL streptomycin and 0.1% fetal bovine serum[7]; and supplemented with indicated concentration of citrate and bicarbonate. After 7 days of incubation aortic rings were dried, weighed and radioactivity was measured via liquid scintillation counting (Perkin Elmer Tri-Carb 2810TR). The half maximal inhibitor concentration (IC50) was calculated with GraphPad Prism 5 software by nonlinear regression using the classical 1-site competition equation, C = bottom + ([top-bottom]/[1–10 [S-logIC50]]), according previously studies[8]. C refers to the calcification (calcium content in the aortic rings); IC50 refers to the concentration of citrate that is required for 50% inhibition of calcification; Top refers to the calcification in the absence of citrate and Bottom refers to maximum inhibition of the calcification.

**Results**: Incubation for 7 days in MEM containing 1.1 mmol/L calcium and the indicated concentration of citrate (pH 7.4) prevented calcium accumulation in rat aortic rings, with an IC50 of 733.2 µmol/L. Moreover, at a constant citrate concentration (733 µmol/L), calcium accumulation correlated positively and dose-dependently with calcium concentration. Plasma bicarbonate levels and pH were significantly higher after than before hemodialysis in all pairs of samples tested (n = 25; Table 1). Interesting, plasma citrate concentrations were significantly higher after (771.6 ± 184.3 µmol/L) than before (145.1 ± 79.8 µmol/L) hemodialysis in all pairs of samples (Table 1). By contrast, plasma calcium concentrations were slightly low (but not significantly different; p = 0.317) after than before hemodialysis (Table 1). The increment in bicarbonate levels after dialysis session (Δ[bicarbonate] = [bicarbonate]post-hemodialysis – [bicarbonate]pre-hemodialysis) were slightly low using citrate-acidified bicarbonate (Δ[bicarbonate] = 5.23 ± 2.86) than acetate-acidified bicarbonate dialysis (Δ[bicarbonate] = 5.62 ± 3.08), but not statistically different (p = 0.484). However, the pH variation during dialysis session (ΔpH = pHpost-hemodialysis – pHpre-hemodialysis) were significantly low (p < 0.001) using citrate-acidified bicarbonate (ΔpH = 0.098 ± 0.043) than acetate-acidified bicarbonate dialysis (ΔpH = 0.171 ± 0.078; Fig. 4). Moreover, the changes in plasmatic ionized calcium levels during dialysis session (Δ[Ca2+] = [$Ca_2$+]post-hemodialysis – [Ca2+]pre-hemodialysis) were significantly low (p < 0.001)using citrate-acidified bicarbonate (Δ[Ca2+] = –0.019 ± 0.089) than acetate-acidified bicarbonate dialysis (Δ[Ca2+] = 0.115 ± 0.118; Fig. 4). Finally, incubation of rat aortic rings in MEM under post-dialysis conditions of citrate-acidified bicarbonate dialysis (27 mmol/L bicarbonate and 772 µmol/L citrate) reduced calcium accumulation when compared with pre-hemodialysis conditions (22 mmol/L bicarbonate and 145 µmol/L citrate; Fig. 5).

**Conclusions**: To our knowledge, our study is the first to show that citrate protects against calcium accumulation in rat aortic walls ex vivo. Therefore, citrate-acidified bicarbonate dialysis may be an alternative approach to reduce calcification in hemodialysis patients without additional cost.

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## REPLACEMENT OF ACETATE WITH CITRATE IN DIALYSIS FLUID: A RANDOMIZED CLINICAL TRIAL OF SHORT TERM SAFETY AND FLUID BIOCOMPATIBILITY.

Gunilla Grundström, Anders Christensson, Maria Alquist, Lars-Göran Nilsson and Mårten Segelmark.

**Background**: The majority of bicarbonate based dialysis fluids are acidified with acetate. Citrate, a well known anticoagulant and antioxidant, has been suggested as a biocompatible alternative. The objective of this study was to evaluate short term safety and biocompatibility of a citrate containing acetate-free dialysis fluid.

**Methods**: Twenty four (24) patients on maintenance dialysis three times per week, 13 on on-line hemodiafiltration (HDF) and 11 on hemodialysis (HD), were randomly assigned to start with either citrate dialysis fluid (1 mM citrate, 1.5 mM calcium) or control fluid (3 mM acetate, 1.5 mM calcium) in an open-labeled cross-over trial (6 + 6 weeks with 8 treatments wash-out in between). Twenty (20) patients, 11 on HDF and 9 on HD were included in the analyses. Main objective was short term safety assessed by acid–base status, plasma ionized calcium and parathyroid hormone (PTH). In addition, biocompatibility was assessed by markers of inflammation (pentraxin 3 (PTX-3), CRP, IL-6, TNF-α and IL-1ß) and thrombogenicity (activated partial thromboplastin time (APTT) and visual clotting scores).

**Results**: No differences dependent on randomization order or treatment mode (HD vs. HDF) were detected. Citrate in the dialysis fluid reduced the intra-dialytic shift in pH (+0.04 week 6 vs. +0.06 week 0, p = 0.046) and base excess (+3.9 mM week 6 vs. +5.6 mM week 0, p = 0.006) over the study period. Using the same calcium concentration (1.5 mM), citrate dialysis fluid resulted in lower post-dialysis plasma ionized calcium level (1.10 mM vs. 1.27 mM for control, p < 0.0001) and higher post-dialysis PTH level (28.8 pM vs. 14.7 pM for control, p < 0.0001) while pre-dialysis levels were unaffected. Citrate reduced intra-dialytic induction of PTX-3 (+1.1 ng/ml vs. +1.4 ng/ml for control, p = 0.04) but had no effect on other markers of inflammation or oxidative stress. Citrate reduced visual clotting in the arterial air chamber during HDF (1.0 vs. 1.8 for control, p = 0.03) and caused an intra-dialytic increase in APTT (+6.8 s, p = 0.003) without affecting post-dialysis values compared to control.

**Conclusions**: During this small short term study citrate dialysis fluid was apparently safe to use in HD and on-line HDF treatments. Indications of reduced treatment-induced inflammation and thrombogenicity suggest citrate as a biocompatible alternative to acetate in dialysis fluid. However, the results need to be confirmed in long term studies.

Trial registration: ISRCTN: ISRCTN28536511

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## CITRATE ANION IMPROVES CHRONIC DIALYSIS EFFICACY, REDUCES SYSTEMIC INFLAMMATION AND PREVENTS CHEMERIN-MEDIATED MICROVASCULAR INJURY.

Sergio Dellepiane[1], Davide Medica[1], Cesare Guarena[1], Tiziana Musso[2], Alessandro Domenico Quercia[3], Gianluca Leonardi[1], Marita Marengo[4], Massimiliano Migliori[5], Vincenzo Panichi[5], Luigi Biancone[1], Francesco Pizzarelli[6], Giovanni Camussi[1] & Vincenzo Cantaluppi[3].

**Background**: Systemic inflammation and uremic toxins (UT) determine the increased cardiovascular mortality observed in chronic hemodialysis (HD) patients. Among UT, the adipokine Chemerin induces vascular dysfunction by targeting both endothelial and vascular smooth muscular cells (EC and VSMC). As Citrate anion modulates oxidative metabolism, systemic inflammation and vascular function, we evaluated whether citrate-buffered dialysis improves HD efficiency, inflammatory parameters and chemerinmediated microvascular injury.

**Methods**: We enrolled 45 pts subjected to HD. Inclusion criteria were: age >18 years, absence of neoplastic or inflammatory diseases, creatinine clearance <5ml/min, treatment with bicarbonate HD or online hemodiafiltration (HDF) (3 times/week, at least 6 months). Pts were treated for 3 months (T1) whit standard HDS containing 3mmol/L of acetate (Select Bag, Baxter Gambro Renal, Deerfield, IL), the next 3 months (T2) with acetate-free HDS containing 1mmol/L of citrate (Select Bag Citrate), and last 3 months (T3) newly with the acetate solution. At the end of each period, we collected laboratory, clinical and HD-related data. In particular, we evaluated inflammatory markers including CRP, fibrinogen, homocystein, beta2-microglobulin and plasma levels of ADMA and of the adipokine chemerin. In vitro, we studied the biological effects of pts' plasma drawn at different time points (T1-T2-T3) on cultured human endothelial and smooth muscle cells.

**Results**: Citrate dialysis increased HD efficacy and reduced plasma levels of CRP, fibrinogen, IL6 and chemerin. In vitro, patients' plasma induced EC and VSMC dysfunction. These effects were reduced by citrate-buffered solutions and paralleled by the decrease of chemerin levels. Consistently, chemerin receptor knockdown reduced EC and VSMC dysfunction.

**Conclusions**: In conclusion, the switch from acetate to citrate buffer increased dialysis efficacy and simultaneously decreased chronic inflammation parameters. Our in vitro data demonstrated that citrate-dialysis prevents EC dysfunction and VSMC osteoblastic differentiation, identifying the adipokine chemerin as a possible target to inhibit micro vascular injury.

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# PROSPECTIVE RANDOMIZED MULTICENTER STUDY TO DEMONSTRATE THE BENEFITS OF HAEMODIALYSIS WITHOUT ACETATE (WITH CITRATE): ABC-TREAT STUDY. ACUTE EFFECT OF CITRATE.

Patricia de Sequera, Rafael Pérez García, Manuel Molina Nuñez, Rosa I. Muñoz González, Gracia Álvarez Fernández, Eva Mérida, M. Jesús Camba, Luís A. Blázquez, M. Paz Alcaide, Rocío Echarri, on behalf of the ABC-treat study group.

**Introduction**: Dialysis fluid (DF), an essential element in hemodialysis (HD), is manufactured in situ by mixing three components: treated water, bicarbonate concentrate and acid concentrate. To avoid the precipitation of calcium and magnesium carbonate that is produced in DF by the addition of bicarbonate, it is necessary to add an acid. There are 2 acid concentrates that contain acetate (ADF) or citrate (CDF) as a stabilizer.

**Objective**: To compare the acute effect of HD with CDF vs. ADF on the metabolism of calcium, phosphorus and magnesium, acid base balance, coagulation, inflammation and hemodynamic stability.

**Methods**: Prospective, multicenter, randomized and crossed study, of 32 weeks duration, inpatients in three-week HD, AK-200-Ultra-S or Artis monitor, 16 weeks with ADF SoftPac®, prepared with 3 mmol/L of acetate, and 16 weeks with CDF SelectBag Citrate®, with 1 mmol/Lof citrate. Patients older than 18 years were included in HD for a minimum of 3 months by arteriovenous fistula. Epidemiological, dialysis, pre and postdialysis biochemistry, episodes of arterial hypotension, and coagulation scores were collected monthly during the 8 months of the study. Pre and post-dialysis analysis were extracted: venous blood gas, calcium (Ca), ionic calcium (Cai), phosphorus (P), magnesium (Mg) and parathyroid hormone (PTH) among others.

**Results**: We included 56 patients, 47 (84%) men and 9 (16%) women, mean age: 65.3 (16.4) years, technique HD/HDF: 20 (35.7%)/36 (64.3%). We found differences ($p < 0.05$) when using the DF with citrate (C) versus acetate (A) in the postdialysis values of bicarbonate [C: 26.9 (1.9) vs. A: 28.5 (3) mmol/L], Cai [C: 1.1 (0.05) vs. A: 1.2 (0.08) mmol/L], Mg [C: 1.8 (0.1) vs A: 1, 9 (0.2) mg/dL] and PTH [C: 255 (172) vs. 148 (149) pg/mL]. We did not find any differences in any of the parameters measured before dialysis. Of the 4416 sessions performed, 2208 in each group, 311 sessions (14.1%) with ADF and 238 (10.8%) with CDF ($p < 0.01$), were complicated by arterial hypotension. The decrease in maximum blood volume measured by Hemoscan® biosensor was also lower [–3.4 (7.7) vs –5.1 (8.2)] although without statistical significance. Conclusion: Dialysis with citrate acutely produces less postdialysis alkalemia and significantly modifies Ca, Mg and PTH. CDF has a positive impact on hemodynamic tolerance.

**Conclusions**: In conclusion, the results of the present work show that dialysis with citrate achieves a better control of post-dialysis acid base balance by decreasing/avoiding postdialysis alkalemia. Post-dialysis levels of Cai and magnesium decrease with CDF and the PTH increases. These results together with lower alkalemia support a less calcifying profile of LD with citrate. Compared with the ADF, the CDF offers greater hemodynamic stability producing fewer episodes of hypotension.

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# AUTHOR INDEX

**Australia**
Baxter Healthcare Pty Ltd
1 Baxter Drive,
Old Toongabbie NSW 2146, Australia
Tel: 1800 BAXTER (1800 229 837)

**New Zealand**
Baxter Healthcare Ltd
33 Vestey Drive, Mount Wellington
Auckland 1060, New Zealand
Tel: 0800 Baxter (0800 229 837)

**renalcare.baxter.com**
Baxter Healthcare Corporation
One Baxter Parkway
Deerfield, IL 60015
USA
1-800-422-9837



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