A prospective study of paediatric preoperative fasting times at Red Cross War Memorial Children’s Hospital, Cape Town, South Africa

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Background. Fasting for liquids and solids is recommended prior to procedures requiring anaesthesia, to reduce the risk of pulmonary aspiration. Children often experience excessive fasting, which is associated with negative physiological and behavioural consequences, and patient discomfort. The duration of preoperative fasting in children in South Africa (SA) is unknown.

Objectives. To determine compliance with fasting guidelines and fasting times of children prior to elective procedures performed under anaesthesia at a paediatric hospital in Cape Town, SA. The primary focus was fasting for clear liquid. We also intended to identify the most common reasons for prolonged clear liquid fasting.

Methods. Over a 7-week period, we prospectively captured fasting times of consecutive patients undergoing elective surgical, medical and radiological procedures at Red Cross War Memorial Children’s Hospital. Measurement outcomes were defined as the period from the last clear liquid, milk or solid feed to the start of anaesthesia. For analysis of compliance with preoperative fasting guidelines, institutional preoperative fasting target limits were established based on the standard 6-4-2-hour guideline.

Results. The study included 721 elective paediatric cases. The mean (standard deviation (SD)) fasting time for clear liquids (n=585) was 8.0 (4.8) hours, with an adherence rate of 25.5% (95% confidence interval 22 - 29) to the institutional target of 2 - 4 hours. The mean (SD) fasting times for breast milk (n=92), formula milk (n=116) and solid feeds (n=560) were 7.1 (2.8), 8.8 (2.8) and 13.9 (3.6) hours, respectively. The factors associated with clear liquid fasting >4 hours were inadequate fasting instructions, poor adherence to fasting orders, procedural delays and fasting to promote theatre flexibility.

Conclusions. This study demonstrates that children in an SA hospital experience excessive fasting times prior to elective procedures. To reduce fasting durations and improve the quality of perioperative care, quality improvement interventions are required to create an adaptable fasting system that allows individualised fasting. Improving preoperative fasting times in children is the responsibility of all healthcare professionals in the multidisciplinary management team.


The routine application of preoperative fasting, first introduced in 1883, is intended to reduce the morbidity of anaesthesia-related regurgitation and pulmonary aspiration. The current American Society of Anesthesiologists (ASA) preoperative fasting guidelines recommend the following fasting periods: 6 hours for solids and non-human milk, 4 hours for breast milk, and 2 hours for clear liquid. These guidelines apply to patients with oral and nasogastric tube (NGT) intake preparing for elective procedures requiring monitored anaesthetic care. Nasojejunal tube (NJT) feeds should be discontinued 2 hours prior to anaesthesia.

International paediatric literature has highlighted that preoperative fasting durations frequently exceed the minimum durations recommended by these guidelines, with average fasting times for clear liquid of 6.3 - 12.61 hours, breast milk 6.27 - 9.82 hours, formula milk 9.9 hours and solids 10.0 - 14.08 hours. Extended fasting confers no advantages, but rather incites significant physiological, psychological and behavioural perturbations including hypoglycaemia, ketoacidosis, hypotension at induction of anaesthesia, hunger, thirst, sadness, irritability and anxiety. Children aged <36 months are most vulnerable to these complications. In healthy paediatric patients, the risk of aspiration is low and complications from clear liquid aspiration are rare. Reducing preoperative clear liquid fasting times improves patient comfort and quality of care without theatre disruption.

Objectives
Preoperative fasting of children in South Africa (SA) has not been investigated. Clinical observation would suggest that fasting times parallel the international trend. This study aimed to elucidate current fasting practices of children before anaesthesia for elective procedures at Red Cross War Memorial Children’s Hospital (RCWMCH), a tertiary paediatric hospital, as the first step to improving quality of care. The primary objective was to determine the mean duration of preoperative fasting for clear liquid and the percentage of children fasted for 2 - 4 hours, in compliance with institutional fasting standards for clear liquids. The secondary objectives included mean fasting times for breast milk, formula milk and solids, and the percentage compliance with their respective preoperative fasting standards. Reasons for prolonged clear liquid fasting and the incidence of regurgitation and aspiration were reviewed.
Methods
This was a single-centre prospective observational study conducted from 4 October to 23 November 2018 at RCWMCH, Cape Town, SA. The protocol, data collection sheet and consent poster were approved by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (ref. no. 410/2018) and the RCWMCH Research Committee (ref. no. RXH: RCC: 15). The conduct of the study upheld patient privacy and confidentiality.

Participants
Inpatients and outpatients undergoing general anaesthesia or sedation for elective medical, surgical or radiological procedures at RCWMCH were eligible for recruitment. This included patients aged <18 years of all ASA physical status classifications, receiving enteral nutrition via oral, NGT, percutaneous endoscopic gastrostomy (PEG) or NJT. Unscheduled and/or emergency cases and patients receiving total parenteral nutrition (TPN) were excluded. Patients prescribed ‘bowel preparation’ were excluded from all but the clear liquid analysis. Patients whose procedures were postponed or cancelled were excluded owing to time and resource constraints.

Outcome measures
Measurement outcomes were fasting times, defined as the period from the last liquid, milk or solid feed time to the start time of anaesthesia.4-8 For analysis of compliance with preoperative fasting guidelines, institutional preoperative fasting time limits were established using the standard 6-4-2 guideline, plus 2 hours for each category.4-8 This provided a clinically feasible goal without significant negative physiological impact. Acceptable time limits for fasting are defined in Table 1. Non-compliance was defined as fasting times outside these ranges.

Anaesthesiologists recruited consecutive patients and obtained data, including last intake time for clear liquid, milk and/or solids, via in-person interviews with parents immediately preoperatively. A standardised case report form (CRF), based on previous studies,9,10 was used. Parents or guardians provided verbal consent, and opt-out posters were clearly displayed. Translation from English was available and performed as required. The CRF included a checklist of reasons for prolonged clear liquid fasting and an area for additional reasons and/or comments.

Missing data, unrelated to fasting times, were retrospectively acquired from patient records. CRFs with no recorded fasting times were excluded. Patients with NGT and PEG intake were included in the oral intake group, since the same guidelines for preoperative fasting were applied. For children receiving breastmilk and formula milk, the fasting time for the primary feed was assessed. Similarly, the most recent infant formula milk or solid feed was assessed as an additional ‘last feed’ category. Distinguishing between formula milk and solids is of little clinical importance and may be a source of confounding because a mother giving formula milk might omit solid food, giving the impression of prolonged fasting for solids.

<table>
<thead>
<tr>
<th>Table 1. Institutional targets for preoperative fasting durations</th>
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<tbody>
<tr>
<td>Fasting category</td>
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<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Clear liquids</td>
</tr>
<tr>
<td>Breastmilk</td>
</tr>
<tr>
<td>Infant formula milk</td>
</tr>
<tr>
<td>Solids and semi-solids</td>
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<tr>
<td>Nasojejunal tube</td>
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</table>

Sample size
Acceptable compliance with the institutional target fasting time for clear liquids was set at 90%. If the proportion of patients fasted for clear liquid <2 hours and >4 hours was found to be >10%, with a 95% confidence interval (CI) that did not include 10%, RCWMCH would be considered non-compliant for clear liquid fasting. A calculated sample size of 484 cases with enteral clear liquid intake data was required to prove non-compliance, with a two-sided 95% CI of 6%.10 We aimed to recruit an additional 20% (97 cases) to compensate for incomplete data.

Data analysis
All captured data were de-identified, entered into an Excel chart (Microsoft Excel for Mac version 16.40; Microsoft, USA), and analysed using SPSS software, version 25 (IBM, USA). Descriptive analyses were conducted and results appropriately represented as mean and standard deviation (SD) or median and interquartile range (IQR). Frequencies of patients with fasting times consistent with compliance were expressed as percentages derived using the total number of times available for that fasting category. Bivariate analyses were performed using parametric or non-parametric tests as appropriate. Subgroup analysis of clear liquid fasting time was performed according to the age of the children: <12 months, 12 - 36 months, and >36 months. The relationship between age and mean clear liquid fasting time was assessed using the Kruskal-Wallis test. A χ2 test of independence was performed to examine the relationship between age and compliance with clear liquid fasting time. To investigate for confounding variables, an independent-samples t-test was performed, comparing mean clear liquid fasting time in patients with and without preoperative intravenous (IV) maintenance fluids. To identify possible selection bias, characteristics of captured and non-captured cases were assessed as follows: age (two-tailed t-test), sex (Fisher’s exact test), weight (Mann-Whitney U-test), ASA classes (χ2 test) and admission status (Fisher’s exact test).

Results
The study cohort included 721 patients, of whom 585 were eligible for analysis of the primary outcome (Fig. 1). The 100 patients excluded owing to missing data did not differ significantly from recruited patients, with the exception of ASA and inpatient status. The time of last intake of clear liquid was not captured in 136 recruited cases (18.9%), for the following reasons: 2 patients had an ‘unknown time’ of last liquid intake, 23 were exclusively breastfed, 16 were exclusively formula fed, 17 received IV preoperative fluids, and 78 had no oral or IV liquid intake captured preoperatively. This study included 411 ward inpatients, 13 intensive care unit patients and 297 outpatients from a wide range of surgical specialties. Clinical and surgical patient characteristics are summarised in Table 2.

The mean (SD) clear liquid fasting time was 8.0 (4.8) hours (Table 3). The compliance of clear liquid intake with the institutional target was 25.5% (n=149; 95% CI 22 - 29). Clear liquid fasting >4 hours occurred in 73% of cases (n=426; 95% CI 69 - 77), and affected all age groups. Details of clear liquid fasting in age subgroups are shown in Table 4. Age <12 months was significantly associated with improved clear liquid compliance (χ2(2) = 16.06; p<0.01) and reduced mean fasting duration (p<0.001). There was no difference in the mean (SD) clear liquid fasting times of children with and without preoperative IV maintenance fluids (9.0 (5.3) v. 7.9 (4.8) hours, respectively; p=0.20).

The compliance and mean fasting times for breastmilk, formula milk, solids and semi-solids, and NJT feeds, are shown in Table 3.
Twenty-eight captured cases were fasted for a time period shorter than the fasting guidelines. Inadequate fasting times were found in 10 patients (1.7%) receiving clear liquid, 9 (9.8%) breastmilk, 7 (6%) formula milk, and 2 (0.4%) solids. All these fasting times were within 1 hour of the recommended fasting times. No regurgitation events were recorded in this group.

A reason for prolonged clear liquid fasting was identified in 278 of the 426 cases with clear liquid fasting periods >4 hours. The key themes identified were: (i) inadequate preoperative preparation and provision of fasting instructions; (ii) poor adherence to fasting instructions in the immediate preoperative period; (iii) delays at the time of the procedure; and (iv) provisional fasting to permit theatre list flexibility (Table 5). Inadequate preparation for preoperative fasting

**Table 2. Demographic, surgical and anaesthetic information (N=721)**

<table>
<thead>
<tr>
<th>Patient demographic information</th>
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<tbody>
<tr>
<td>Age (months), mean (SD)</td>
<td>53.1 (44.6)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg), median (IQR)</td>
<td>14.4 (10.0 - 20.7)</td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>311 (43.1)</td>
<td></td>
</tr>
<tr>
<td>ASA physical status class, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>369 (51.2)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>224 (31.1)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>121 (16.8)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>7 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

**Surgical information**

- Preoperative location, n (%)  
  - Same-day admission 297 (41.2)  
  - Inpatient 411 (57.0)  
  - Intensive care unit 13 (1.8)  
- Timing of list, n (%)  
  - Morning 334 (46.3)  
  - Afternoon 123 (17.1)  
  - Full day 264 (36.6)  
- Type of procedure or surgery, n (%)  
  - Burns 55 (7.6)  
  - Cardiothoracic 44 (6.1)  
  - Cardiology (cath lab) 26 (3.6)  
  - Dental 24 (3.3)  
  - Ear, nose and throat 118 (16.4)  
  - General 140 (19.4)  
  - Medical specialties* 20 (2.8)  
  - Neurosurgery 25 (3.5)  
  - Ophthalmology 63 (7.7)  
  - Orthopaedic surgery 9 (1.2)  
  - Plastic 75 (10.4)  
  - Radiology 80 (11.1)  
  - Urology 42 (5.8)  
- Anaesthetic information  
  - Anaesthesia technique, n (%)  
    - General anaesthesia 645 (89.2)  
    - Sedation 78 (10.8)  
  - Preoperative IV fluid infusion, n (%)  
    - IV maintenance fluid infusion 67 (9.3)  
    - No IV maintenance fluid infusion 640 (88.8)  
    - Unknown 14 (1.9)  

*Gastroenterology, pulmonology, rheumatology.

**Table 3. Fasting mean times and compliance with preoperative fasting time targets**

<table>
<thead>
<tr>
<th>Fasting category</th>
<th>Fasting time (hours), mean (SD)</th>
<th>Fasting compliance, n (% 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear liquid (N=585)</td>
<td>8.0 (4.8)</td>
<td>149 (25.5; 21.9 - 29.0)</td>
</tr>
<tr>
<td><strong>Secondary outcomes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breastmilk (N=92)</td>
<td>7.1 (2.8)</td>
<td>35 (38.0; 28.1 - 48.0)</td>
</tr>
<tr>
<td>Formula milk (N=116)</td>
<td>8.8 (2.8)</td>
<td>53 (45.7; 36.6 - 54.8)</td>
</tr>
<tr>
<td>Solid feed (N=560)</td>
<td>13.9 (3.6)</td>
<td>49 (8.8; 6.4 - 11.1)</td>
</tr>
<tr>
<td>Last feed (N=639)</td>
<td>12.9 (4.0)</td>
<td>102 (16.0; 13.1 - 18.8)</td>
</tr>
<tr>
<td>Nasojejunal tube (N=5)</td>
<td>6.7 (3.3)</td>
<td>1 (0)</td>
</tr>
</tbody>
</table>

SD = standard deviation; CI = confidence interval.
included no fasting education or instructions being provided to outpatients, fasting instructions not being documented for inpatients, universal fasting times prescribed regardless of the anticipated starting time, and single fasting times ordered for liquids and solids. Parents reported not being informed that clear liquid was allowed on the morning of surgery. In the immediate preoperative period, ward nurses and parents did not follow fasting instructions or ward fasting guidelines in some cases. This may not have been preventable owing to the child being asleep or refusing the clear liquid offered. Further reasons for non-adherence to fasting orders were not investigated. Delays in the starting time of the procedure were reportedly due to problems with surgical consent and other paperwork, the preceding case being of longer duration than anticipated, changes in the order of the list, and emergency cases interrupting the list. Provisional fasting to permit theatre list flexibility was considered responsible when the patient was not offered a drink during the day to promote list adaptability and avoid delays or postponements in the case of list changes.

Regurgitations were recorded in 7 patients receiving general anaesthesia, with no aspirations. All preoperative fasting times for these patients met the minimum times recommended by the ASA.

### Discussion

The principal finding of our study was that compliance with the prespecified clear liquid fasting time target of 2 - 4 hours was 25.5%, which is considerably lower than the acceptable institutional compliance rate of 90%. The mean duration of clear liquid fasting was almost 8 hours. This extended to the majority of children aged <36 months, who are most susceptible to the adverse metabolic effects of prolonged fasting. The subgroup analysis of children aged <12 months suggested better compliance with fasting times, which may indicate awareness that younger children are at increased risk of the adverse effects of prolonged fasting, although the fasting times were still unimpressive, since all age groups showed inadequate compliance and average fasting times >4 hours.

Our study is in keeping with the international published literature, which demonstrates that following a 2-hour clear liquid fasting guideline consistently translates into actual clear liquid fasting times of 6.3 - 10.85 hours, with marginal improvements achieved with quality improvement (QI) interventions.[3-8,11,16]

The four major drivers of prolonged clear liquid fasting identified at RCWMCH are inadequate fasting instructions provided, poor adherence to fasting instructions, delays in procedural starting time, and provisional fasting to promote theatre list flexibility. These barriers to improving clear liquid fasting compliance seem to be largely similar to those experienced in well-resourced settings.[3,15-37] Service delivery issues, including hospital operational factors and resource limitations, and patient-related factors, such as language barriers, cultural beliefs and psychosocial circumstances, may be compounding influences in SA.[29]

This study presents an opportunity to improve the perioperative experience for staff, patients and parents.[39] Reducing the duration of clear liquid fasting confers considerable benefits, mitigating the negative emotional, behavioural, biochemical and haemodynamic effects of fasting, and improving pain scores.[12,15,30-32] To optimise compliance with preoperative fasting guidelines, the areas of failure identified should be systematically addressed using QI methodology with context-appropriate interventions and ongoing monitoring using plan-do-study-act cycles. A successful QI intervention relies on a systems approach, driven by a strong guiding team with commitment from all key stakeholders and role players from ministerial to community level.

Greater adaptability in the fasting system is required to accommodate the changeable nature of theatre lists. Establishing efficient communication and regular updates between theatre staff and ward nurses facilitates appropriate adjustment of fasting times, to reduce liquid fasting times.[32,34] In 2018 the Association of Paediatric Anaesthetists of Great Britain and Ireland and the European Society for Paediatric Anaesthesiology released a joint consensus statement,[31] subsequently endorsed by other international paediatric anaesthesia societies,[39-44] recommending that children receive clear liquid up to 1 hour before elective general anaesthesia. A 1-hour clear liquid fasting protocol enhances theatre flexibility and assists in individualising fasting times,[39,46] thus addressing a major obstacle to improved fasting compliance at RCWMCH. Two paediatric centres[39,44] that adopted this policy demonstrated a 43% and 53% absolute risk reduction in the proportion of patients fasted of clear liquids for >4 hours. Based on the 1-hour fasting recommendation and the two studies with a number needed to treat of two to three, we would expect to improve compliance from 25.5% to between 58.8% and 75.5% (25.5% + (33.3% to 50%)). Liberal liquid fasting policies have not been shown to increase risk of aspiration or theatre interruption, and as such promise a safe and practical strategy to reduce fasting times.[19,23,20,35-37,49-44]

Outdated and widely variable fasting instructions provided by medical staff, and non-adherence to fasting instructions by parents

### Table 4. Clear liquid fasting in age categories

<table>
<thead>
<tr>
<th>Age category (months)</th>
<th>Fasting duration (hours), mean (SD) or median (IQR)</th>
<th>Fasting compliance, n (% 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12 (N=84)</td>
<td>4.3 (3.0 - 6.9)*</td>
<td>36 (42.8; 32.3 - 53.4)</td>
</tr>
<tr>
<td>12 - 36 (N=138)</td>
<td>7.7 (4.6)</td>
<td>34 (24.6; 17.4 - 31.8)</td>
</tr>
<tr>
<td>&gt;36 (N=363)</td>
<td>8.7 (5.0)</td>
<td>79 (21.8; 17.5 - 26.0)</td>
</tr>
<tr>
<td>p&lt;0.001</td>
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</table>

CI = confidence interval, IQR = interquartile range, SD = standard deviation.

*Non-normally distributed.

### Table 5. Reasons for clear liquid fasting >4 hours (N=278)

<table>
<thead>
<tr>
<th>Themes and reasons</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issues with fasting instructions</td>
<td></td>
</tr>
<tr>
<td>Non-individualised fasting instructions</td>
<td>34 (12.2)</td>
</tr>
<tr>
<td>No fasting information or education</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td>Poor adherence to fasting instructions</td>
<td></td>
</tr>
<tr>
<td>Instructions to give clear liquid not followed</td>
<td>77 (27.7)</td>
</tr>
<tr>
<td>Child asleep</td>
<td>29 (10.4)</td>
</tr>
<tr>
<td>Child refused clear liquid</td>
<td>22 (7.9)</td>
</tr>
<tr>
<td>Lack of flexibility in liquid fasting time</td>
<td></td>
</tr>
<tr>
<td>No liquid offered while waiting</td>
<td>83 (29.9)</td>
</tr>
<tr>
<td>Delays</td>
<td>30 (10.8)</td>
</tr>
</tbody>
</table>

"The subgroup analysis of children aged <12 months suggested better compliance with fasting times, which may indicate awareness that younger children are at increased risk of the adverse effects of prolonged fasting, although the fasting times were still unimpressive, since all age groups showed inadequate compliance and average fasting times >4 hours."

"Our study is in keeping with the international published literature, which demonstrates that following a 2-hour clear liquid fasting guideline consistently translates into actual clear liquid fasting times of 6.3 - 10.85 hours, with marginal improvements achieved with quality improvement (QI) interventions."

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"This study presents an opportunity to improve the perioperative experience for staff, patients and parents. Reducing the duration of clear liquid fasting confers considerable benefits, mitigating the negative emotional, behavioural, biochemical and haemodynamic effects of fasting, and improving pain scores. To optimise compliance with preoperative fasting guidelines, the areas of failure identified should be systematically addressed using QI methodology with context-appropriate interventions and ongoing monitoring using plan-do-study-act cycles. A successful QI intervention relies on a systems approach, driven by a strong guiding team with commitment from all key stakeholders and role players from ministerial to community level."

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"Outdated and widely variable fasting instructions provided by medical staff, and non-adherence to fasting instructions by parents..."
and ward nurses, were major contributors to prolonged fasting in this study. Compliance relies greatly on the quality of fasting instructions provided, but is also influenced by personal beliefs, staff availability and parental threat perception, anxiety levels, health literacy, language barriers and recall.[23,25,65-66] Anaesthesiology departments caring for paediatric patients should review their institutional fasting guidelines and develop policies based on the current evidence. Ready available up-to-date preoperative fasting guidelines and educational campaigns for staff can be used to advance knowledge and promote consistent, unified fasting information.[23,67] Verbal preoperative communication with parents, supported by information leaflets, videos or posters in appropriate languages, has been shown to improve parental comprehension and memorisation, reduce anxiety and improve fasting times.[66-68] A phone call or text message reminder the night before surgery has been demonstrated to impact positively on health behaviour and improve adherence.[23,26]

This prospective audit included a large cohort of paediatric in- and outpatients undergoing a broad range of surgical and medical procedures. The results are consistent with the global picture, which may suggest that issues that affect well-resourced environments are relevant in SA. In-person interviews immediately prior to anaesthesia optimised the quality of data collected by avoiding extraction error, allowing clarification of data collection variables, and minimising recall bias. Neither the ward nursing staff nor the parents were aware of the primary objective of the study, to reduce possible bias introduced by the Hawthorne effect.

Study limitations

There are some limitations to this study. Despite a fairly robust sample, 12% of potential cases were not recruited. Analysis revealed an element of selection bias with a greater proportion of sicker sample, 12% of potential cases were not recruited. Analysis revealed an element of selection bias with a greater proportion of sicker patients not recruited for the study, which may be secondary to the urgency of these cases. We do not believe that these missed cases invalidate the findings of this study, as these patients more frequently receive non-standardised fasting protocols or TPN. Secondly, the primary outcome data were missing for 14% (n=97) of the clear fluid eligible patients. However, the study protocol included oversampling to account for incomplete data capture. Finally, while this is a single-centre study, it had a robust sample size to address the study question.

Conclusions

In a prospective observational study of children in an SA hospital, compliance with preoperative fasting guidelines for clear liquid was poor. Non-compliance is driven by inadequate preoperative fasting instructions, poor adherence to fasting instructions, delays in procedural starting times, and fasting to promote theatre list optimisation. Clear fluid fasting durations is a safe way to improve the perioperative quality of care for patients and their parents. Collaborative quality improvement programmes that support and promote the individualisation of clear fluid fasting are likely to have the greatest impact.

Declaration

For the research study was done in partial fulfilment of the requirements for AJK’s MMed (Anaesthesiology) degree at the University of Cape Town.

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Author contributions

The research protocol was developed by AJK and GW. Statistical analysis was performed by MBM. The first draft of the manuscript was written by AJK and revised by KvDS and BMB. All authors participated in critical review of the manuscript.

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