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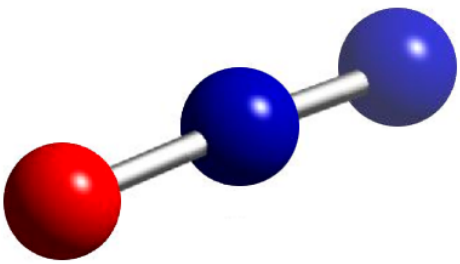
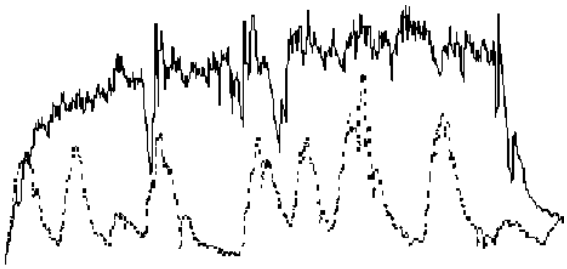
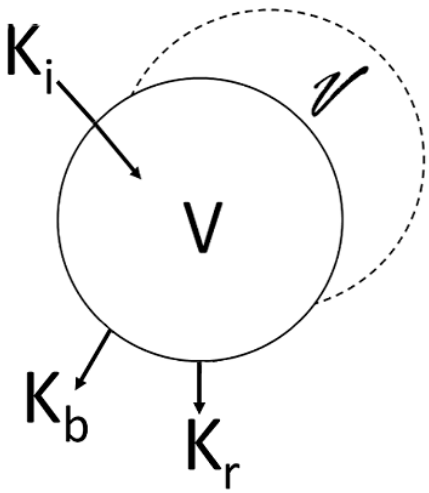
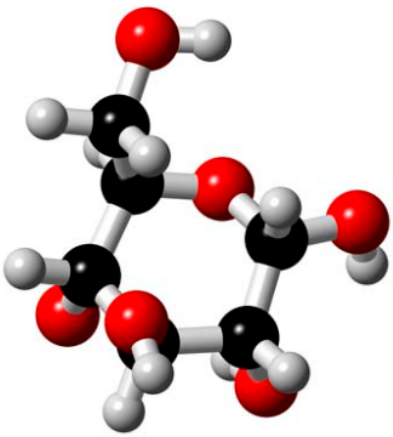
New diagnostic approaches to monitor irrigating fluid absorption









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NEW DIAGNOSTIC APPROACHES TO MONITOR IRRIGATING FLUID ABSORPTION

David Piros

Karolinska Institutet

Stockholm 2019

Cover photo:
Upper left: Nitrous oxide molecule. Upper right: Exhaled breath nitrous oxide concentrations during continuous and intermittent infusions. Lower left: One-compartment turnover model. Lower right: Glucose molecule

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To my wonderful wife and the love of my life, **Anna**

For your constant support and for balancing my fascination in nitrous oxide with a healthy amount of skepticism while giving birth to our two fantastic children, **Hugo and Lisa**

ABSTRACT

New diagnostic approaches to monitor irrigating fluid absorption

Rinsing the endoscopic operating field with an irrigating solution entails the risk of absorption of the fluid. The physiological consequences of such absorption are explored and two new methods for monitoring the amount of absorption are proposed.

Methods: **Paper 1**: 25 anesthetized pigs were randomized to control or continuous infusion of 100 ml/kg over 90 min of either glycine 1.5%, mannitol 3% or mannitol 5%. Several invasive measurements and calculations were performed to describe the pathophysiological processes. **Paper 2**: Exhaled air nitrous oxide (N_2O) concentrations were measured in 12 volunteers receiving intravenous infusions, containing dissolved nitrous oxide and simulating fluid absorption. **Paper 3:** Comparison of N_2O and ethanol for detecting absorption in 86 patients, at 2 centres, undergoing transurethral resection of the prostate (TURP) in spinal anesthesia. **Paper 4:** A 3-part evaluation of glucose as a tracer in fluid absorption detection. Part 1 was a clinical study in 250 patients undergoing TURP with and without a glucose-containing irrigant. Part 2 investigated the glucose kinetics in 10 volunteers receiving 20 ml/kg of acetated Ringer's solution with 1% glucose over 30 min. In part 3, data was used for computer simulations of various absorption patterns.

Results: (Paper 1): Infusions rendered a hypokinetic hypotensive state. Intracellular volume expansion, intracranial pressure elevation and myocardial damage were greater for glycine 1.5%. **(Paper 2):** N_2O is a useful tracer for noninvasive fluid absorption monitoring. It identifies the pattern and the volume of absorption with a 95% predicition interval of ± 200 ml. **(Paper 3):** The N_2O method is feasible in a clinical setting. Agreement with the ethanol method was volume dependent and about twice that of N_2O versus known volume. **(Paper 4):** Sodium and glucose showed a strong inverse linear relation for all patients including diabetics. The glucose levels almost doubled after the experimental infusions, which volume diluted the plasma by 17.7%. Simulations showed that the infused volume correlated with the rise in glucose where an increase by more than 1.4 mmol/L could detect absorption with 95% confidence.

Conclusion: The pathophysiological process and treatment rationale of massive nonelectrolytic irrigating fluid absorption was outlined. The N_2O method allows noninvasive online monitoring of irrigating fluid absorption with better resolution and similar or better prediction of absorbed volume compared to the ethanol method. Glucose can be used as a tracer for retrospective evaluation of irrigating fluid absorption.

LIST OF SCIENTIFIC PAPERS

I. Sandfeldt L, Riddez L, Rajs J, Ewaldsson C-A, Piros D, Hahn RG. High-dose intravenous infusion of irrigating fluids containing glycine and mannitol in the Pig. J Surg Res 2001; 96 (2):114-25.

II. Piros D, Drobin D, Hahn RG. Nitrous oxide for monitoring fluid absorption in volunteers. Br J Anaesth. 2007; 98 (1):53-9.

III. Drobin D, Hjelmqvist H, Piros D, Hahn RG. Monitoring of fluid absorption with nitrous oxide during transurethral resection of the prostate. Acta Anaesthesiol Scand. 2008; 52 (4):509-13.

IV. Piros D, Fagerström T, Collins JW, Hahn RG. Glucose as a marker of fluid absorption in bipolar transurethral surgery. Anesth Analg. 2009; 109 (6):1850-5.

CONTENTS

LIST OF ABBREVIATIONS

AUC	Area under the curve
BW	Body weight
CL	Clearance
CO	Cardiac output
CO_2	Carbon dioxide
ECF	Extracellular fluid
GCP	Good clinical practice
Glc	Glucose
GFR	Glomerular filtration rate
Hct	Hematocrit
Hgb	Blood hemogobin
HR	Heart rate
ICP	Intracranial pressure
ICV	Intracellular (fluid) volume
IV	Intravenous
Kb	Basal diuresis and evaporation
Ki	Infusion rate constant
kPa	Kilo Pascal
Kr	Elimination rate constant
Kt	Distribution rate constant
L	Litres
MCV	Mean corpuscular cell volume
Min	Minutes
Ml	Millilitres = 0,001 liter
Mmol	Millimoles
N_20	Nitrous oxide
NS	Isotonic (Normal) saline
OEL:s	Occupational exposure limits
RBC	Red blood cell count
RS	Lactated or acetated Ringer's solution
SD	Standard deviation
Sec	Seconds
SEM	Standard error of the mean
TBW	Total body water
TCRE	Transcervical endometrial resection
TUR syndrome	Transurethral resection syndrome
TURP	Transurethral resection of the prostate
V	Expanded body fluid volume
V	Not expanded body fluid volume
Vd	The volume of distribution
w/v	Weight per volume

1 THESIS AT A GLANCE

Paper	Aim	Method	Main result
I. High-dose Intravenous Infusion of Irrigating Fluids Containing Glycine and Mannitol in the Pig	To describe the pathophysiological process and compartmental distribution of excessive absorption of two common irrigating solutions	25 anesthetized pigs randomized to control or continuous infusion of 100 ml/kg over 90 min of either glycine 1.5%, mannitol 3 or 5%	Both infusions rendered a hypokinetic hypotensive state. Intracellular volume expansion, elevation of intracranial pressure and myocardial damage were greater for glycine 1,5%.
II. Nitrous oxide for monitoring fluid absorption in volunteers	To investigate if N_2O can be used as a tracer in irrigating solutions and thus allowing online monitoring of fluid absorption	Measurements of N_2O and CO_2 in exhaled air during normal breathing in 12 volunteers receiving iv infusions, containing dissolved N_2O and mimicking fluid absorption	N_2O is a useful tracer for noninvasive monitoring of fluid absorption. It distinguishes different absorption patterns and precisely predicts the total volume absorbed.
III. Monitoring of fluid absorption with nitrous oxide during transurethral resection of the prostate	To compare ethanol and N_2O as tracers for online monitoring of irrigating fluid absorption in a clinical setting	GCP protocolized 2 center study in 86 patients undergoing TURP in spinal anaesthesia	N_2O can be used as a tracer for fluid absorption in a clinical setting. Agreement with the ethanol method was volume dependant and about twice that of N_2O vs known volume.
IV. Glucose as a marker of fluid absorption in bipolar transurethral surgery	To evalute if glucose can be used as a tracer for retrospect analysis of volume of irrigating fluid absorption when sodium dilution is not possible, i.e. during bipolar TURP	Study comprising three parts: 1) A clinical study on 250 patients undergoing TURP with electrolyte free irrigant and randomized to either contain 5% glucose or no glucose. 2) An experimental part on glucose kinetics in 10 volunteers receiving a 30 min iv infusion of 20 ml/kg of R-Ac with 1% glucose. 3) A computer simulation study on various absorption patterns	Sodium and glucose showed a strong inverse linear relation for all patients including diabetics. The glucose levels almost doubled after the experimental infusions and yielded a 17.7% plasma dilution. Simulations showed a volume correlated rise in glucose where an increase by more than 1.4 mmol/L detected absorption with 95% confidence

2 SUMMARY IN SWEDISH (SAMMANFATTNING PÅ SVENSKA)

Vid titthålskirurgi, så kallad endoskopi, används ofta spolvätskor för att vidga och skölja rent operationsfältet. I kontakt med kroppens vävnader finns det då risk att vätskan absorberas d.v.s. tas upp i kroppen. Vid skrapning av prostatan eller livmodern är det vanligt att 9 respektive 15 liter spolvätska används. Om ojordad elektrisk bränning används måste spolvätskan vara saltfri då den annars kan leda ström och explosioner kan uppstå. Nyare operationsmetoder använder jordad bränningsteknik vilket gör att vanligt koksalt då kan användas. Absorption över 3 liter, vilket sker vid upp till 10 av 100 operationer, kan orsaka livshotande tillstånd till följd av akut övervätskning och rubbad saltbalans. Befintliga sätt att mäta graden av absorption är mer eller mindre krångliga och tillförlitliga och används i liten grad. Alkohol kan tillsättas till spolvätskan och koncentrationen i utandningsluften återspeglar då hur stor volym patienten fått i sig. Nackdelar med metoden är att alkohol försvinner långsamt ur kroppen samt att det vid stor absorption kan ge lättare berusning.

Den här avhandlingen består av 4 delarbeten. I arbete 1 studerade vi effekterna av massiv absorption av de saltfria spolvätskorna glycin och mannitol på 25 grisar. Resultaten visade att spolvätskorna ger kraftig urinproduktion med uttorkning och kraftigt försämrad cirkulation som följd. Dessutom sågs glycin ge större cellsvullnad, högre tryck i hjärnan och mer skada på hjärtat. I delarbete 2 testade vi om lustgas kan användas för att diagnostisera spolvätskeabsorption. Vi löste en liten mängd lustgas (slutkoncentration 40 ml/L) i spolvätskan och testade olika tillförseltakter och mätmetoder på 12 försökspersoner. Lustgasmängderna som togs upp i kroppen svarade mot absorptionsmängden och kunde passivt mätas i utandningsluften med hjälp av en syrgasgrimma och en lustgasmätare. Utandningsmönstret visade både den absorberade mängden och om det skett direkt in i blodbanan eller via ansamling i närliggande hålrum. I arbete 3 jämförde vi lustgas och etanolmetoderna på 86 patienter som genomgick skrapning av prostatan. Båda metoderna fungerade bra men stämde inte lika bra överens som då jämförelse gjordes med den kända vätskemängden i arbete 2. Korrelationen varierade med graden av absorption. I arbete 4 utvärderade vi om socker kan tillsättas till spolvätskan för att i efterhand värdera graden av absorption. När saltinnehållande spolvätska används kan inte mätning av salthalten i blodet användas för att värdera om absorption skett. Först studerade vi 250 patienter som lottats till att få sockerhaltig eller sockerfri saltfri spolvätska vid prostataskrapning. Vid absorption av sockerhaltig saltfri spolvätska visade blodprover att blodsockerstegringen svarade mot utspädningen av salt i blodet. Detta stämde även för diabetiker då blodsockernivåerna under operation beror mer på hur tillförda mängder socker omfördelas i kroppen än på insulineffekter. Därefter studerade vi omfördelningen av en 1%-ig sockerlösning i kroppen hos 10 försökspersoner. De erhållna värdena användes i simuleringar av olika vätskemängder och vi såg att ökningen av blodsockret svarade mot absorptionsmängden.

Sammanfattningsvis visar den här avhandlingen mekanismerna bakom skadorna vid saltfri spolvätskeabsorption och visar 2 nya metoder som kan användas för mätning av spolvätskeupptag.

3 GENERAL INTRODUCTION

Endoscopic surgery with the use of irrigating fluids to expand and rinse the operating field has been performed for nearly a century now. Immediate complications include blood loss and the absorption of the irrigating solution. Although several advances have been made as to the understanding of the pathophysiological mechanisms and operating and monitoring methods, there is still an unjustified sense of misunderstanding regarding the risks of absorption. The medical community's perception that adverse reactions are rare and generally avoidable is misleading. Numerous case reports can witness to this. Historically, only electrolyte-free irrigating solutions were used in order to prevent dispersion of the current during electrocautery, but new operating techniques have allowed, since two decades, for the introduction of electrolyte-containing irrigants. These solutions have indeed made absorption less hazardous but complications of fluid overload are still important patient safety issues. Administration of fluids is well known to require conscious strategies and does not allow for unintentional deviation which is the case when the risks of absorption are unaccounted for. This thesis explores the gradual pathophysiologic effects in massive nonelectrolytic irrigating fluid absorption and proposes new diagnostical approaches in monitoring techniques for such events. We investigated the usefulness and feasibility of adding nitrous oxide or glucose as a tracer to the irrigating solutions and thereby allowing sampling of these substances for the detection of absorption.

4 THE USE OF IRRIGATING FLUIDS

4.1 Purpose and Introduction

Irrigating fluids are used in endoscopic surgery to distend the operating area or anatomical space and to rinse the surgical field from blood and debris. Examples of such use is in arthroscopy, liposuction, rinsing of the urinary bladder, lithotripsy, lithotomy, transurethral vaporization of the prostate, transcervical endometrial resection (TCRE) and transurethral resection of the prostate (TURP). Amounts of irrigant being used varies with the type of procedure and the length of the operation. Volumes can be quite large and averages 15 litres (L) during a 45-minute long TURP and 9 L in TCRE [1-4]. Variations in average volumes used may differ significantly between various types of surgery and cohorts, ranging from 2 L to more than tenfold that [1-6]. In a single procedure, the amount can occasionally be around threefold the average. Nearly all of this fluid is directly recovered from the operating field but in the contact with human tissue there is a risk that it is partly absorbed.

Absorption can occur via two routes. Severing of veins, in TURP and TCRE, causes the more commonly seen intravascular absorption which then usually starts halfway into the surgery or later and continues until the end of the operation [7-11]. The pattern of absorption resembles an intermittent intravenous infusion since fluid is driven into the damaged vein every time the irrigating solution pressure exceeds the venous pressure of 1.5 kPa [12]. Absorption via the extravascular route originates from passage through the fallopian tubes or instrumental perforation of the endometrial wall, urinary bladder or prostatic capsule [11, 13-14]. The absorption pattern then looks like a continuous intravenous infusion since the pressure of the irrigant only needs to exceed the intra-abdominal pressure of 0.5 kPa [15].

Main questions to be addressed when choosing an irrigating solution are the surgical needs and the risks of absorption. The latter includes issues regarding the solution specific attributable effects of absorption, how the risks of absorption are assessed and how this is monitored in relation to the timing of what adequate actions should be undertaken.

4.2 Irrigating solutions

Irrigating solutions are used in large quantities. They should allow optimal visualization and not cause additional patient risks. Thus the ideal solution should be sterile, clear, relatively inexpensive, as well as locally and systemically nontoxic. Absorption of water gives hemolysis with subsequent kidney damage due to hemoglobinuria which causes vasoconstriction with hypoxic cellular damage known as lower nephron nephrosis. Isotonic and non-hemolytic irrigating fluids, as proposed by Creevy, Nesbit and Glickman already 70 years ago, are thus preferable if

absorption occurs [16, 17]. Electrically conductive solutions, such as saline, cannot be used if monopolar cautery is needed. This led to the development of the non-conductive and non-hemolytic irrigants glycine, mannitol and sorbitol [17-20]. In the beginning of the 21:st century, the development of bipolar resectoscopes made it possible to irrigate the surgical field with conductive solutions. Isotonic saline (NS) is the most widely used solution for this purpose although Ringer's solution (RS) seems to give similar visibility for the surgeon and less adverse effects if absorption occurs [21-24]. An overview of available irrigating fluids and some of their properties is listed in table 1 below.

Table 1. Specific properties and side effects of available irrigating solutions. All irrigating solutions cause volume effects if absorbed. Glucose 2.5%-4% and urea 1% are not listed in the table since they cause stickiness respectively crystallization on the instruments and are hence not widely used. The half-life of glycine and sorbitol increase with the dose.

	Sterile water	Glycine 1.5%	Mannitol 5%	Sorbitol 3%	Normal saline	Ringer's lactate
Osmolality (mosm/L)	0	220	275	165	308	273
Volume of distribution (L)	42 (TBW)	20	40	23	8.2*	8.1*
Main elimination site	Kidney	Liver	Kidney	Liver	Kidney	Kidney
Metabolites	None	Ammonia	None	fructose, glycogen, CO_2	None	None
Half-life (min)		40	100	30	110*	50*
Additional available irrigating solution preparations	-	1.2%, 2.2%	3%	sorbitol and mannitol mixtures: 2% + 1%, 2.7% + 0.54% (Cytal), 2.75% + 0.54% (Purisole)	-	Ringer's acetate
Specific side effects if absorbed	hemolysis, hyperkalemia, hyponatremia, shock, renal failure, cerebral edema	Hyponatremia, CNS-inhibition, cerebral edema, encefalopathy, myocardial ischemia	Hyponatremia, drives water out of the cells, diuretic effect	Diuretic effect, hyperglycemia, dysnatremia	Hyper-chloremic acidosis, reduces GFR	None

* Pharmacokinetic parameters for isotonic saline and Ringer's lactate are based on volume kinetic data in volunteers [25]. These fluids are not metabolized thus the half-life solely describes distribution and urinary excretion which are influenced by stress, volume status and anesthesia. Abbreviations: Glomerular filtration rate (GFR), total body water (TBW).

4.2.1 General complications of absorption

Using irrigating fluids always entails the risk of absorption. In nearly half of all TURPs performed, an absorbed volume greater than 150 ml can be detected [10]. With increasing volumes, the risk of adverse effects and symptoms progress. Regardless of the solution being used, this affects fluid balance. In addition, homeostasis may be disturbed, especially if a non-electrolyte containing irrigant

is used. Further specific risks varies between different solutions. Based on that massive irrigating fluid absorption was first described during TURP it is referred to in the literature as the TUR-syndrome. Large scale TUR-syndrome, which is most commonly seen during TURP and TCRE, occurs if absorption is in excess of 3 L but this is quite rare [26-28]. An incomplete form, developing in the response to 1-2 L of absorption is more often the case. This is seen in <1 to 10% of all TURPs or equivalent surgeries performed including TCRE [11, 29-34]. The unprecise incidence may be due to that most studies do not include a sufficient amount of patients to give a really good estimate of the frequency of absorption. The risk of absorption is increased in TCRE of fibroids and smokers undergoing TURP [35-37].

Effects on homeostasis include hypocalcemia, dilution coagulopathy, electrolyte and acid-base balance disturbances [36-49]. These changes are directly related to the amount of absorption although they may be further aggravated depending on the type of irrigant used as described in the following sections. All electrolyte-free irrigating solutions cause hyponatremia when absorption occurs. For the intravascular route this is most pronounced at the end of absorption while extravasation causes sodium to flow from the extracellular space to the pool of absorption. This yields a delayed hyponatremia, typically for 2 to 4 hours, and promotes hypovolemia [50-52].

Effects on fluid balance can be viewed both from a local and a systemic perspective. Local symptoms can occur when there is extravasation and this includes pain and obstructed passage in adjacent conduits. Airway obstruction has been reported in shoulder arthroscopy while, in urological or gynecological surgery, local drainage may be attempted [6]. Based on the pathophysiology of extravasation and as shown with increasing amounts of absorption, the risk of hypotension, abdominal pain and bradycardia is increased in case of extravasation [53]. Systemically, fluid absorption causes hypervolemia which obviously entails a risk of fluid overload and pulmonary edema, especially if there is also hyponatremia [54]. However, in many cases this is only transient and followed by a hemodynamically hypokinetic phase with low cardiac output, low blood pressure and hypovolemia [55, 56]. The cause is multifactorial and originates from osmotic diuresis, natriuresis and that with hypo-osmotic irrigants there is also an intracellular uptake of water. The release of endotoxins from the surgical field and changes in homeostasis such as hypocalcemia, hypo-osmolality and mild hypothermia may be of additional importance [56-59].

Hyponatremia and hypo-osmolality can also cause neurological symptoms ranging from mild dizziness to decreased levels of consciousness, epileptic seizures and brain edema [28, 32, 60-61]. Further organ specific effects that may be seen include renal hypoxia and myocardial uptake of water with release of troponin, bradycardia and other ECG changes as signs of strain [26, 47, 50, 62-69]. Myocardial infarction

has been reported in 0.5–3% of TURPs performed [69-71]. Overall mortality from severe TUR-syndrome was estimated to 25% in a French review comprising 24 patients with massive absorption of glycine 1.5% [72].

4.2.2 Non-conducting solutions

Sterile or distilled water was the first irrigating solution used and its use in this context dates back to 1926 when McCarthy introduced the TURP procedure. Since water hemolyse erythrocytes, it provides the best visibility in the surgical field in case of bleeding. If absorption occurs, water is freely distributed in the total body water (TBW) so that hyponatremia may be less pronounced than for other non-electrolytic irrigants. The hemolysis may give hyperkalemia, anemia and cause renal failure since free hemoglobin damages the epithelium of Henle's loop and distal convoluting tubuli [16]. The adverse effects may be fatal and sterile water is now only recommended in diagnostical procedures but, perhaps due to its low cost, it is still used in some TURPs worldwide [73-74].

Glycine is the most inexpensive irrigant after sterile water and has long been dominating the market of electrolyte-free irrigating solutions. It is an endogenous nonessential amino acid with no risk of allergies. Glycine acts as an inhibitory neurotransmitter, especially in the retina and absorption has been shown to cause visual disturbances and transient blindness [75]. Liver metabolization produces glycolic acid, glyoxylic acid, glutamate and ammonia that all have been linked to various neurological symptoms including encephalopathy [76-79]. About 10% of the absorbed volume is eliminated unchanged via the kidneys resulting in osmotic diuresis with loss of sodium [80]. In addition, glycine stimulates secretion of vasopressin and atrial natriuretic peptide which further promotes hyponatremia through water retention respectively natriuresis [81-84]. Compared to absorption of other irrigating solutions, cellular edema becomes more severe with glycine and the risk of myocardial cell damage is increased [85-86]. Glycine has been used in different concentrations from 1.0 to 2.2%. 1.5% is the most commonly used and is slightly hypo-osmotic. The other mixtures offer no advantage and lower concentrations increases the risk of hemolysis while higher can aggravate toxic adverse effects [87-89].

Mannitol is not metabolized in the body but excreted unchanged in the urine. The 5% concentration is the most widely used mannitol solution for irrigation purposes. It is a powerful osmotic diuretic but being nearly iso-osmotic it still does not affect osmolality even though electrolytes are lost. More diluted mannitol solutions are used in the treatment of brain edema and to induce forced diuresis. Compared to glycine, it has no neurological toxic effects, no risk of cerebral edema and causes less myocardial damage although the risk of other circulatory symptoms is equivalent [30].

Sorbitol is metabolized in the liver to fructose and glucose, which in rare cases may result in lactic acidosis respectively hyperglycemia [90]. Just like glycine, about 10% is excreted unchanged in the urine inducing osmotic diuresis [91]. It is available in several mixtures with mannitol and with a total concentration around 3%. Overall symptoms of sorbitol absorption are the same as for glycine [69].

Treatment in case of non-conducting irrigating fluid absorption should focus on the administration of hypertonic saline [92]. If pulmonary edema or prolonged fluid overload is evident, additional treatment with diuretics should be considered but only after stabilization of the hemodynamics [93].

4.2.3 Electrolyte-containing solutions

As stated previously, electrolyte-containing solutions can be used when monopolar cautery is not needed. Isotonic saline (NS) is most commonly used for this purpose but balanced solutions like acetated or lactated Ringer's solution could be a better alternative. There are no studies comparing the absorption effects of balanced crystalloids versus NS when used as an irrigant. However, there are numerous studies comparing the two when used for intravenous infusion therapy and which is the better has been a long ongoing debate.

NS contains 154 mmol sodium and chloride per L yielding a strong iron difference of 0 which in turn promotes hyperchloremic metabolic acidosis. RS, on the other hand, has a sodium content of 130 and chloride 100 mmol/L which gives a more balanced strong iron difference of 30 mmol/L as compared to the normal 38 in plasma. Thus no acidosis is promoted. Of particular interest to the anesthetist are some of the pharmacological effects of acidosis which include attenuated catecholamine response, potassium shift from the cells, increased arrythmogenity of inhalation anesthetics as well as potentiation of opiates and neuromuscular blocking agents. In addition, as renal cells in the macula densa sense an increase in sodium and chloride concentration this triggers the physiological tubuloglomerular feedback mechanism which works to maintain an optimal salt and water balance. The effect is vasoconstriction in the afferent renal arteriole with a reduced glomerular filtration rate (GFR).

NS was found to increase vasopressor requirements, mortality and acute renal failure incidence respectively frequency of major adverse kidney events in three recently published studies comprising goal directed fluid therapy in abdominal surgery and volume substitution in critically ill and non-critically ill adults [22-24]. The average volume administered ranged from 1 to 3.5 L which is in the spectrum of incomplete TUR-syndrome.

4.3 Monitoring of irrigating fluid absorption

The first assessments of fluid absorption were through clinical evaluation of symptoms. Since then this method has evolved with symptoms being more defined and with grading of their severity. Sedation, anesthesia and comorbidities may confound the approach. The clinical symptoms entails chest pain, bradycardia, hypertension, hypotension, poor urine output, blurred vision, nausea, vomiting, uneasiness, confusion, tiredness, decreased levels of consciousness and headache. The duration, severity and number of symptoms correlates with the amount of irrigating solution absorbed. In a study using glycine 1.5% by Olsson et al, the number and severity of symptoms progressed with increasing fluid absorption. The average amount of symptoms was 1.3 for volumes of 0–300 ml, 2.3 for 1–2 L, 3.1 for 2–3 L respectively 5.8 for absorptions exceeding 3 L as measured by the ethanol method [50].

For absorption of non-conductive solutions, blood samples for measurement of sodium dilution or plasma concentration of the irrigating substance at the end of the surgery correlate with the absorbed volume [94-98]. The rate and type of absorption, TBW, intravenous infusions and elapsed time from absorption may influence the detected levels. Analysis of mannitol, sorbitol or glycine is usually not readily available in clinical practice but sodium dilution may be used to validate or refute absorption of electrolyte-free irrigants. When measured at the end of surgery, a depression of sodium by approximately 10-12 mmol/L in males and 20 mmol/L in females corresponds to an absorption of 2 L [95, 99-100].

Direct measurements of fluid absorption includes volumetric, gravimetric and tracer techniques. The advantage is that absorption may be indicated early, before the patient becomes symptomatic and that the further extent of surgery can be modified in order to avoid massive absorption. For volumetric measurements all fluids that enters and exits the patient have to be accounted for. Visual estimates have been found unreliable especially due to spillage on the floor, bag to bag variations in volume and admixing of blood and urine to the retrieved irrigating fluid [101-102]. The method works better for TCRE where there is less blood loss and automated fluid tracking systems are available [103]. Gravimetry uses a bed-scale for repeated weighing of the patient during the operation. The method is rather accurate assuming measurement are performed with an emptied urinary bladder and that blood loss and intravenous infusions are accounted for [104-105].

In tracer techniques a small amount of a traceable substance, i.e. an isotope or ethanol, is added to the irrigating solution. Isotopes reliably detects fluid absorption but radiation hazards makes the method hardly warranted today. The use of ethanol as a tracer was first suggested in 1986 [106]. The technique has since then been used and evaluated in numerous studies and surgeries of the genitourinary tract, especially during TURP and TCRE [11, 107]. Ethanol does not passively

diffuse across the endothelium of these defined anatomical spaces and hence the uptake of ethanol is directly proportional to the absorbed volume. An ethanol concentration of 1% (13.3 ml 95% alcohol per L) in the irrigating solution is used and expired breath concentrations are measured at least every 10 min during surgery. The patient makes an exhaled breath in the alcolmeter during spinal anesthesia and for general anesthesia it is possible to adapt the alcolmeter to the breathing circuit. If the patient exhales too vigorously there may be movement involved that disturbs the surgical procedure. Obtained values are affected by the elimination and distribution kinetics of ethanol. For clinical practice there are available nomograms for quick estimates of the ethanol samples corresponding amount of absorption and degree of hyponatremia [107]. An example of such a nomogram is given in figure 1 below. In scientific studies, an equation is used to give more precise results [108].

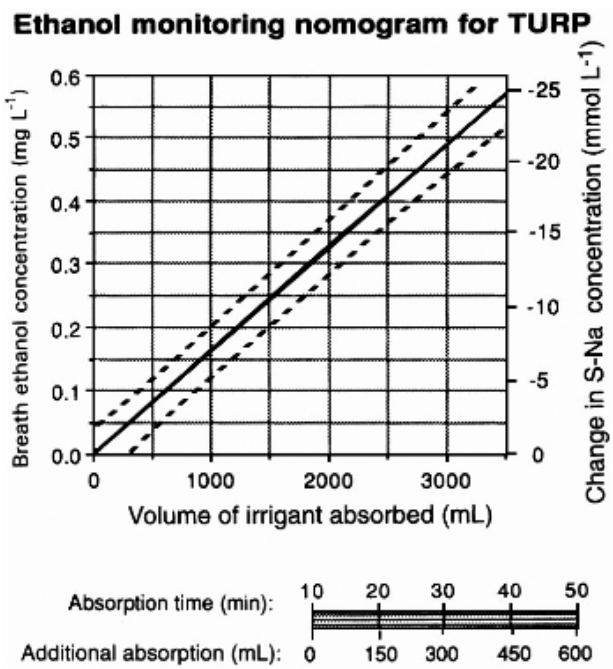
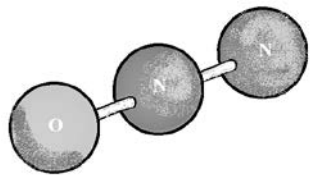


Figure 1. *Nomogram for estimation of the absorbed volume and corresponding change in serum sodium concentration at any given time when an electrolyte-free irrigant containing 1% ethanol is used during TURP. The dotted line indicates the 90% prediction interval. The lower part of the figure indicates a scale for additional corresponding volume based on the distribution and elimination of ethanol over time. I.e. if the measured ethanol concentration is 0.2 the corresponding volume is 1200 ml. If positive tests have been recorded for 30 min an additional volume of 300 ml is added giving a total volume of absorption of 1500 ml [107].*

5 NITROUS OXIDE

5.1 Properties



Nitrous oxide (N_2O) was first discovered in 1793 by the English scientist Joseph Priestley. Initial use was for recreation and public shows. Procedural use, mainly for tooth extraction, commenced in the early 1840s [109]. Main medicinal uses today are for minor pain, sedation, and as complement in anesthesia giving faster and smoother induction, recovery and better hemodynamic stability. N_2O is an inert gas with good analgesic and anxiolytic, but weak anesthetic effects. It is produced through heating of ammonium nitrate to a temperature between 245–270 degrees Celsius. After cooling and purification, N_2O is evaporated and stored compressed in aluminum tanks at a pressure of 50 bar (5000 kPa). The cylinder then contains a mixture of N_2O in its vapor and liquid form and thus the total quantity of gas can only be ascertained by weighing. It is a sweet smelling, colourless, non-irritant, non-flammable gas but it strongly supports combustion, even in the absence of oxygen. Due to this, N_2O should not be used in the presence of sources of ignition, oils, grease, alcohol gels and many plastics [110-111].

The nociceptive mechanisms of action are attributed to spinal inhibition and especially supraspinal activation of opioidergic neurons in the locus ceruleus and periaqueductal grey matter of the brainstem via hypothalamic release of corticotrophin-releasing factor. This activates descending noradrenergic inhibitory pathways [112-116]. In specific this may be related to the presynaptic release of endogenous ligands, such as dynorphine, enkephalin and endorphin that binds to kappa-opioid receptors. Thus, the effect is comparable to that of morphine and may be inhibited by the opioid antagonist naloxone [117-125]. Anxiolytic properties, on the other hand, are mediated through $_Y$-aminobutyric acid $(GABA)_A$ receptors, similar to the mechanism of action for benzodiazepines, and may therefore be inhibited by flumazenil [126-129]. Anesthetic and dissociative effects involve a broader spectra of ligand-gated ion channels, especially the $GABA_A$- and N-methyl-D-aspartate (NMDA)-receptors [130-133]. Thus, N_2O affects several different receptors. These effects are dose dependent.

The potency of an anesthetic gas is related to its partial pressure in the brain. Alveolar concentration is generally accepted as an index of this because it can be actually measured. This potency is described as the Minimal alveolar concentration (MAC), which is defined as the alveolar concentration (in volumes percent at a pressure of 1 atmosphere absolute) of an anesthetic gas at which 50% of patients show no movement upon skin incision. With a MAC of 104%, N_2O is the least

potent of available anesthetic gases. Concentration, described as the fraction of inhaled gases, required for general anesthesia is so high that it does not allow for adequate oxygen delivery. Indeed, early clinical use was disreputed due to the risk of severe hypoxemia. Although N_2O is usually insufficient for providing adequate anesthesia unless combined with another volatile agent it is still a potent analgesic, even in sub anesthetic doses. Several studies have shown clear analgesic effects when N_2O is administered at a concentration of around 15–20%. Psychomotor effects may be seen at even slightly lower doses [134-140].

In this thesis, N_2O was dissolved in fluid and used as a tracer to detect irrigating fluid absorption. The reasons for choosing N_2O were that elimination through urine is negligible, it is not metabolized in the body, concentration in ambient air is negligible, and it has a low blood/gas solubility coefficient of 0,47 at 37 degrees Celsius. The overall physical properties are listed in table 2. Importantly, nitrous oxide can be measured in very low concentrations, down to parts per million (ppm).

Table 2. Physical properties of nitrous oxide [110].

N_2O physical properties			
Molecular weight	44 Da	Ostwald solubility coefficient at 37°C:	
Boiling point	–88°C	Blood/gas	0.47
Saturated vapour pressure	5300 Kpa at 20°C	Fat/blood	2.3
MAC	104%	Oil/gas	1.4
		Oil/H_2O	3.2

The low blood/gas solubility means that cardiac output and minute volume have little effect on the rate of equilibration of inhaled N_2O (figure 2) and that it, like all volatile anesthetics, is quickly eliminated through the lungs. In fact, rise time in alveolar concentration as well as elimination is faster than for any other anesthetic. Although not very soluble, N_2O is still fifteen times more soluble than oxygen and can be dissolved in water and plasma up to 100 respectively 45 volumes percent [111].

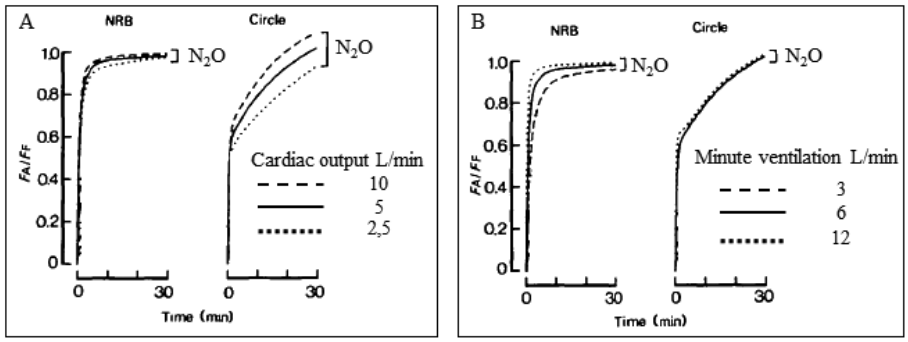


Figure 2. *Influence of cardiac output (A) and minute volume (B) on the rate of equilibration between alveolar/end-tidal (F_A) and inspired (Fi) concentrations of nitrous oxide during non-rebreathing conditions (NRB) and a circle system with a fresh gas flow of 1 L/min. Due to the low solubility of N_2O, changes in alveolar concentration and cardiac output have little effect on the rate of equilibration of inhaled N_2O [Modified from Conway CM. Gaseous homeostasis and the circle system: Factors influencing anaesthetic gas exchange. Br J Anaesth 1986; 58:1167-1180].*

5.2 Side effects and patient risks

The contraindications for using N_2O are few and attributed to the risk of expanding gas filled cavities and affecting vitamin B12. Due to the much higher blood/gas partition coefficient of nitrous oxide as compared to nitrogen (0.015), it passes in to any air filled space faster than nitrogen is eliminated and may give a 3–4 fold increase in volume. This can aggravate numerous conditions i.e. pneumothorax, pneumoperitoneum, trauma with entrapped air in sensitive areas, air embolism, hypoxemia, the use of air filled cuffs, and various types of head surgery [111]. N_2O inactivates cobalamin (vitamin B12) through oxidization of the cobalt I form. It may also generate hydroxyl radicals that irreversibly oxidize cobalamin. This prevents vitamin B12 from, together with 5-methyltetrahydrofolate, acting as a coenzyme for methionine synthase and thus causes a dose dependent inhibition of its enzyme activity. Methionine synthase is essential for generating methyl groups that are necessary for a series of processes such as DNA, RNA, myelin and catecholamine synthesis [141]. Prolonged or frequent use of N_2O may cause agranulocytosis, bone marrow dysplasia, megaloblastic anemia, neuropathies, and degeneration of the spinal cord [142-143]. Nitrous oxide should not be used in patients with abnormalities of the vitamin B12 metabolism, although sub anesthetic or short procedural administrations have not been studied. Teratogenic effects and reproductive toxicity have previously been considered but are now refuted [144-148].

Side effects and adverse events in patients were finally addressed in the ENIGMA-II trial including over 7000 patients randomized to anesthesia with or without N_2O

in major non-cardiac surgery. Apart from a slightly increased N_2O incidence of severe nausea and vomiting, which could be controlled with antiemetic drugs, no statistical differences were seen between any group outcomes [149].

5.3 Occupational and environmental aspects

The risks of occupational exposure to anesthetics has led to the introduction of occupational exposure limits (OELs) which are expressed as a 8-hour time-weighted average. Additionally, some countries have also adopted a short-term exposure limit to set a standard for maximum concentrations not to be exceeded even for short periods. The OEL for nitrous oxide varies, from 25 to 100 ppm, between different countries as it is set by national institutes [150-152]. In comparison, environmental nitrous oxide levels were typically in the range of 1000–2000 ppm before scavenging systems were introduced. Proposed health risks for providers are generally the same as for patients in regard to the effects of inactivation of vitamin B12. Additional concerns have been raised that repeated and prolonged exposure to a mix of volatile anesthetics may cause genotoxicity [153-155]. This effect seems to be reversible and is not apparent when N_2O is used as a sole agent [156]. It has also been proposed that inhibition of methionine synthase may increase the risk of miscarriage and postnatal development impairment. Although several studies have addressed this, no data supports the concern as long as exposure levels are kept within recommendations [157-159]. In summary, there is no evidence of any hematologic, neurologic or reproductive occupational risks as long as OELs are not excessively exceeded to 500 ppm or more [160-163]. Indeed, in 2015, the European Society of Anesthesiology task force on the use of nitrous oxide in clinical anaesthetic practice stated that there is no evidence indicating an increased health risk for patients or providers in a clinically relevant setting [164].

Global environmental N_2O concentrations are steadily rising at a rate of 0.7 to 0.8 parts per billion per year. This is an important issue since N_2O, along with CO_2 and methane, is one of the most influential greenhouse gases that are listed in the different agreements within the United Nations Framework Convention on Climate Change (UNFCC), i.e. the Kyoto protocol, Doha amendment and Paris agreement. The global warming potential of N_2O is attributed to ozone depletion and the obstruction of thermal radiation flux from the earth's surface up through the stratosphere. 1 – 3% of N_2O emissions derive from healthcare [165]. Other main sources are from combustion of fossil fuels and solid waste, fertilizer use, and especially natural microbial actions in soil and water. Although the contribution of N_2O emissions from healthcare are relatively small, the effects on global environment are just as important. Thus, adequate scavenging systems and waste handling systems are necessary together with a conscious and responsible use. The effectiveness of global climate strategies was underlined in a 2018 United Nations report which, for the first time since the 1970s, showed that the ozone layer is now healing [166].

5.4 Measuring nitrous oxide concentrations

Measuring nitrous oxide concentrations is of interest in numerous scientific fields such as in global and local environment issues, occupational exposure and medicine. This has driven the development of several different analytical instruments which all have their pros and cons. Measurements can either be performed by estimating the flux or vertical and horizontal air flow related change over time in a specific area (i.e. Eddy covariance, Eddy accumulation, flux-methods or dispersion techniques) or by collecting samples in a specific liquid or closed chambers. The latter are the methods used in measurements regarding occupational exposure and medicine. The analysis becomes more accurate if the chamber size is increased but at the same time the instrument becomes less portable.

Figure 3. *The NGA 2000 MLT Analyzer (Rosemount Analytical, Hasselroth Germany), used in papers 2 and 3, measures N_2O and CO_2 concentrations via absorption of infrared light.*

The actual analysis of N_2O concentrations is done either through chromatographic, optical or amperometric techniques although additional methods are under development. Gas chromatography, using an electron-capture detector, is the most widely used analytical method. It is accurate and relatively cheap but requires frequent calibration and does not allow for continuous measurements.

For use at room temperature, optical techniques use either infrared light or Quantum-cascade lasers. Absorbance at a specific wave length gives a measurement of the concentration of the gas. For N_2O, this wavelength is around 4 micrometers. Accuracy can be further enhanced by using longer path-lengths, multiple passes or optical lenses and mirrors that increase the points where the beam can be absorbed. Optical techniques have low calibration requirements and allows for quick, continuous measurements. Instruments are, however, more costly than for other analytical methods.

Amperometric sensors measures N_2O concentrations through the current that occurs when N_2O interacts with an electrode. Instruments have a low cost but are sensitive to sensor drift and can only measure dissolved N_2O [167].

6 GLUCOSE HOMEOSTASIS AND KINETICS

In paper IV glucose was investigated as a potential tracer to detect or refute irrigating fluid absorption of electrolyte-containing solutions. Glucose homeostasis is normally regulated through a closed feedback loop including a variety of hormones, especially insulin and glucagon, and a multitude of organ systems such as the liver, pancreas, central nervous system, muscles, and adipose tissue. Increase in blood glucose levels are due to glycogenolysis, gluconeogenesis, or exogenous administration. Glucose concentrations decrease as glucose enters the cells either through facilitated diffusion or active transport involving glucose transporters. If levels are above 12 to 15 mmol/L there is also glucosuria. However, in normal conditions, fastening levels are kept below 7 mmol/L. Iv infusion of a glucose solution usually increases insulin secretion which normalizes blood glucose levels within about 30 min from the end of the infusion [168]. Stress, critical illness and trauma including most types of surgery causes insulin resistance and increased endogenous glucose production which alters these dynamics resulting in a delayed clearance [169]. The plasma volume correlates with the glucose level as its osmotic force influences the distribution of water in the body.

6.1 Pharmacokinetics

Measuring the distribution of a glucose solution in the body can be done by isotope labeling. In addition to the limitations of using radioisotopes there are also the assumptions and issues of the water volume not necessarily distributing in the same way as the labelled molecule and that the techniques are time consuming and cannot describe dynamic processes. An alternative method is that of volume kinetics.

In basic pharmacokinetics, the volume of distribution (Vd) describes the theoretical volume over which a substance is spread. It is thus a description of the relationship between the administered dose and the measured concentration in plasma. Clearance (CL) is the theoretical plasma volume from which the substance is completely removed per unit of time. This reflects both the plasma concentration and the elimination through excretion and metabolization. In volume kinetics, these pharmacokinetic principles are used to describe the turnover of an iv infusion. There are two key differences in this concept; one, the infused volume is an important part of the volume of distribution and two, the dilution effect on endogenous substances is used instead of measuring the drug concentration. Dilution of hemoglobin (Hgb) and sodium correlates to the fractional volume effect in plasma respectively the extracellular space. As the plasma part of blood is 1 minus the hematocrit (Hct), the plasma dilution is derived from the hemodilution by dividing the ratio of starting Hgb/diluted Hgb with 1 – Hct. The plasma dilution variables, glucose concentrations and rate of infusion are entered in a computer program (in this thesis

we used Matlab version 4.2, Math Works, Nastick MA) that performs a random search method for the least-squares fitting procedure to obtain the unknown variables. Compartmental nomenclature is used since volume kinetics is an advanced pharmacokinetic model that describes the expansion of functional rather than physiological fluid spaces and the compliance for volume expansion varies within physiological fluid spaces [170-173]. Depending on the type of fluid, situation and rate of infusion a one, two or even three compartment model may be used [174]. Assuming there is no marked glucosuria, the escape of glucose into the cells can be derived from its Vd and thus the differences in actual blood or plasma glucose concentrations can be converted to the number of glucose molecules in their Vd. Some of the advantages with volume kinetics include that it is minimally invasive, gives a dynamic analysis and that it allows for the prediction of effects of simulated infusions. In paper IV a one compartment turnover model, as described in figure 3 below, was used to describe the kinetics of glucose and the accompanying water volume.

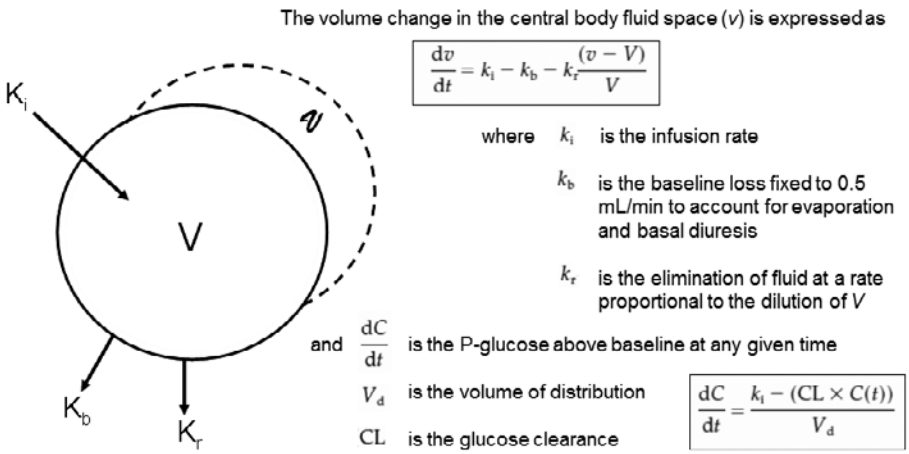


Figure 4. *One compartment turnover model of glucose kinetics and the accompanying water volume.*

7 AIMS

The overall aim of this thesis was to improve patient safety by understanding better the pathophysiology of overload with irrigating fluid and to explore the potential use of two new markers to detect fluid absorption. More specifically this meant to investigate and answer the following questions:

Explore the effects of massive absorption of glycine and mannitol, two commonly used electrolyte-free irrigating solutions. Are there any fluid related differences with regards to the pathophysiology? If so, how are these disturbances gradually developed on an organ specific and whole body level?

Investigate new diagnostic tools for monitoring of irrigating fluid absorption. Can nitrous oxide be added to the solution as a tracer for early detection of absorption? If so, what is the most suitable concentration and sampling device? How well does the nitrous oxide levels correlate to the amount of absorption? Can the method differentiate between the two routes of absorption? Is the technique practically feasible in a clinical context? Are there any adverse effects?

Does the addition of glucose 1% to the irrigating fluid allow for validation or dismissal of suspected absorption when electrolyte changes are absent? If so, how may glucose levels correlate to the total absorbed volume of different patterns?

8 METHODS

Paper 1 was an experimental infusion study in pigs aiming to describe the gradual development of the pathophysiological mechanisms involved in massive irrigating fluid absorption also known as the TUR-syndrome. In papers 2 and 3 we investigated the use of nitrous oxide for instant detection of irrigating fluid absorption. Study 2 was performed in healthy volunteers while the third paper was executed as a good clinical practice (GCP) study in TURP-patients. Here we reported all findings to an external and impartial review board.

One of the reasons why the ethanol method (as described in section 3.3) has not been implemented more widely in clinical practice is that there is no patent for it. Educational costs and technical devices are often sponsored by healthcare companies holding a patent. In hopes of developing a method that would gain as many patients as possible a European patent registration was applied early on. AGA Linde healthcare AB, who manufactured the nitrous oxide used, helped arrange the application and after approval the patent rights were transferred to them. AGA Linde healthcare financially supported papers 2 and 3 by providing some of the equipment for admixing nitrous oxide in the irrigating fluid as well as lending the MLT analyzer, and the accompanying software, used for measuring nitrous oxide concentrations. Results were continuously reported to the company's research and development group although AGA Linde healthcare had no part in the design, execution, interpretation of results or presentation of the studies.

Before study 4 the bipolar TURP technique was introduced. Previously sodium-free irrigating solutions had been used and absorption could then be validated or refuted by measuring the degree of sodium dilution. Since this is not possible when isotonic saline or Ringer's solution is used as the irrigant we wanted to investigate if the addition of glucose 1% could compensate the loss of this diagnostic tool.

8.1 Subjects and irrigating fluid administration

In paper 1, twenty-five pigs of both sexes aged between 8 and 10 weeks and with a mean BW of 22 (19–29) kg were acclimatized in a sty next to the operating room at the animal research facility of Södersjukhuset, Stockholm for at least 3 days before the experiments. Two pigs served as controls, receiving no infusion, and the other 23 were randomized to a 90 min constant rate infusion of 100 mL kg/h of either glycine 1.5% w/v (n = 9), mannitol 5% (n = 8) or mannitol 3% (n = 6). Interspecies scaling, adjusting for differences in metabolic rate and toxicokinetics, suggests that the toxic effect of the infused fluid corresponds to 40 mL/kg/h (≈ 3.2 L/h) in an adult male. The mannitol 3% infusions were administered to compare the effects on intracranial pressure with glycine 1.5% as these solutions have the same osmolality. After anesthetic induction and surgical preparation the

animals were allowed to reach hemodynamic steady state for 30 min before the infusion started. Monitoring and observations continued over a total of 120 min after which the animals were killed with an overdose of thiopental and ethanol.

Paper 2 and the experimental part of study 4 included 12 respectively 10 healthy male volunteers aged 19-52 (median 26) and 20–41 (mean 30) years. Their BWs were 65–88 kg (median 76) respectively 72–111 (mean 85) kg. Volunteers had a light breakfast at home and were not allowed to eat or drink during the experiments, before which they rested supine for 20 min to reach a haemodynamic steady state. Paper 2 experiment were performed at the clinical research and metabolic laboratory facilities of Södersjukhuset, Stockholm while study 4 took place at the Clinical Research Center at Södertälje Hospital in Sweden.

In paper 2 the volunteers underwent four series of experiments aimed at evaluating different aspects of monitoring the administration of iv fluid by means of expired-breath tests using N_2O as a tracer. In unpublished data and a pilot study in pigs it was found that N_2O is not absorbed from the urinary bladder and that infusion of an irrigating solution to which N_2O had been added to a concentration of 10% yielded end expiratory gas concentrations of approximately 300 ppm [175]. Infusion experiments, in volunteers, could thus be used to further investigate the correlation between the amount of irrigating fluid absorbed and exhaled N_2O concentrations. The infusion regimens used were:

1) Seven 5-min continuous rate infusions of Ringer's solution 1 ml/kg for comparing sampling sites. The N_2O was added to Ringer's since hyponatraemia and breath ethanol levels were not assessed as in the other experiments.
2) Continuous infusion of 15 ml/kg of Mannitol 3% and ethanol 1% over 20 min. The total infused volume was 1146 (SD 110) ml.
3) One of three volumes of either 7.5, 12 or 15 ml/kg as an intermittent infusion of mannitol 3% and ethanol 1%. The intermittent infusion paterns are shown in figure 5 below.
4) A intermittent infusion administred in the same regimen as in experiment 3 but with 40% of the volume and 2.5 times higher N_2O concentration.

N_2O was added to a concentration of 40 ml/L in all infusions except in number 4 where the concentration was 100 ml/L. The experiments were performed on separate occasions at least 2 days apart besides in the comparison of sampling sites, which were performed shortly after experiment 3. Randomization for the order of infusions and volumes used in experiments 3 and 4 was made by sealed envelopes. Measurements continued until the N_2O concentration had returned to baseline, which occurred about 10 min after the administration of N_2O had ended, except in experiment 2 where the volunteers were followed for 100 min to allow a follow up of the fluid balance to be made.

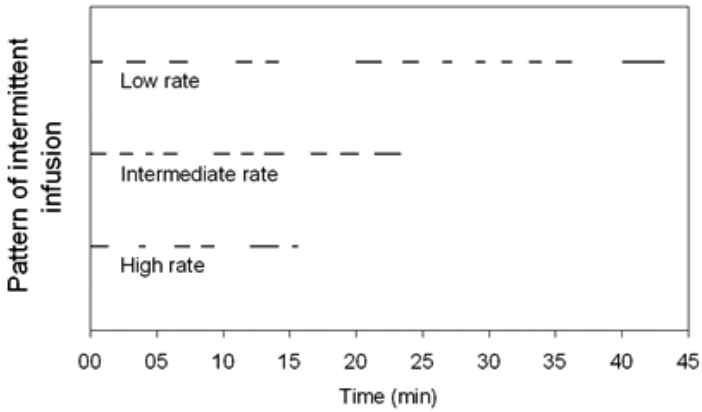


Figure 5. *The intermittent infusion patterns as administred in study 2, experiments 3 and 4.*

The rationale for the infusion regimens used in paper 2 was that the continuous infusions were intended to simulate extravasation. Intraperitoneal infusions of N_2O-containing irrigating fluid in pigs show that a continuous pattern of breath N_2O develops, similar to that when ethanol is used as a tracer. The intermittent infusions mimicked absorption of irrigating fluid by the direct iv route during intermittent filling of the bladder, as such absorption can only occur in bursts whenever the pressure exceeds the prostatic venous pressure of about 1.5 kPa. The three intermittent infusion patterns used were based on actual absorption cases from our clinic in which the pressure in the bladder and fluid uptake had been measured simultaneously. The same data have been used previously for simulation purposes. A maximum amount of 600 ml of N_2O can be dissolved in 1 L of water at room temperature [176]. Mixing is facilitated by letting the gas diffuse through the fluid. A bubble is created in the upper part of the fluid bag, which consists mainly of displaced N_2, with which irrigating fluid is always saturated. The N_2O concentration of only 40 ml/L was chosen because concentrations exceeding 100 ml/L might increase a theoretical risk of explosion during electric cutting. N_2O mixes with gases formed during electric cutting, such as propane, which can gather in air pockets within the area of surgery.

For the experimental part of paper 4, Acetated Ringer's solution, 20 mL/kg, to which 34.5 mL of glucose 300 mg/mL had been added to reach a concentration of 10 mg/mL (1%), was infused at a constant rate over 30 min. Monitoring and observations continued over a total of 160 min. The reason for choosing a glucose concentration of 1% was that 5% glucose, as indicated by the results of the clinical part of the study, could increase plasma glucose to levels far above those that result in glucosuria, and thus seemed unnecessarily high.

Infusion pumps were used in all animal and healthy volunteer experiments for the controlled administration of exact volumes. The mannitol 3% and ethanol 1% solution used has an osmolality of 400 mosm/kg.

Paper 3 and the clinical part of study 4 included 86 respectively 250 males, aged 67 to 85 (median 75) and 48 to 96 (mean 74) years old and scheduled for elective TURP. Their BWs were 73–86 kg (median 80) respectively 55–135 (mean 79) kg. Surgeries were performed at Södersjukhuset and Huddinge sjukhus, Stockholm respectively at Southmead and Torbay Hospitals in the United Kingdom. The size of study 3 was chosen based on an incidence of fluid absorption of 30% and that 10% of the patients absorb > 1 L of fluid [29-30]. Additional comparisons between the ethanol and N_2O method would also be possible during the accumulation of increasing volumes in cases with absorption. Exclusion criteria in the study were renal or liver disease, that general anesthesia (which would not allow for breath sampling during spontaneous breathing) was indicated or mannitol intolerance as the irrigating fluid used was mannitol 3% and ethanol 1% to which N_2O was added to a concentration of 40 ml/L. In paper 4, the patients were randomized to receive either 1.5% glycine or 5% glucose in water as the irrigation fluid. Diabetics were not excluded.

8.2 Techniques and preparations

In paper 1, the anesthesia for all the pigs comprised intramusculary administered premedication with 20–30 mg of diazepam, 400–500 mg of ketamine and 1 mg of atropine. After being weighed to the nearest hectogram on a balance scale, a cannula was inserted into an ear vein for iv induction with 300–600 mg of thiopental, 150–250 mg of ketamine and 5–10 mg of diazepam. Endotracheal intubation was then performed and a Servo Ventilator 900C was used for continuous ventilation with a ventilator rate of 20–24 breaths/min to keep normocapnea. Adequate surgical anesthesia was maintained by 600–900 mg/h of ketamine and intermittent doses of 10 mg of diazepam. The depth of the anesthesia was checked repeatedly by pinching the skin between the hoofs.

The surgical preparation included a neck incision for dissection of the left external jugular vein and placement of three catheters, one with the tip as close as possible to the base of the skull for blood-gas sampling, one directed to the heart for administration of maintenance anesthetics and irrigating fluid and finally a flow-directed thermodilution fiberoptic PA-catheter, size 5.5 F for measurements of mixed venous blood gases, pulmonary arterial pressure, and cardiac output. The right carotid artery was dissected and a perivascular ultrasonic flow probe applied for continuous blood flow rate measurement. Via an incision in the left groin, a catheter was introduced into the femoral artery to monitor the pressure and draw additional blood samples. A 15-cm standard Medtronic PS Medical ventricular

catheter for ICP monitoring was inserted 5 cm via a skin incision and a drilled hole in the parietal bone, about 1 cm lateral to the midline. Its position was verified by X-rays after injecting iohexol contrast through the cannula used for measuring the intracranial pressure. A laparotomy was also performed and a Foley catheter inserted through a cystostomy into the urinary bladder to measure the urinary excretion of fluid and electrolytes. A perivascular ultrasonic flow probe was applied to the abdominal aorta. The abdominal midline incision was then closed. The flow probes were both connected to a Transonic T101 analyzer.

For the infusion experiments in healthy volunteers (papers 2 and 4), a cannula was placed into the antecubital vein for fluid infusion. In paper 2, experiment 2 and paper 4 an additional cannula was placed in the antecubital vein of the opposite arm and used for drawing blood samples.

Adding N_2O to the irrigating fluids, as done in papers 2 and 3, was performed by dissolving N_2O for medical use, under sterile conditions, shortly (at the most 30 min) before each experiment. The N_2O was taken from a gas tube and withdrawn via a 3-way infusion set into a 250 ml graded glass syringe, which was sterilized after each experiment. The valve on the infusion set was then switched, and the withdrawn gas injected manually through a bacterial filter and a sterile needle into the irrigating fluid via the injection port.

The patients in papers 3 and 4 were prepared for undergoing elective TURP according to the hospital routines. In study 3 only spinal anesthesia was used.

8.3 Measurements

For the seventeen pigs receiving mannitol 5% or glycine 1% in paper 1, multiple determinations of blood flow rates, arterial and intracranial pressures, electrolyte status, blood gases, and urinary excretion were performed. For the six pigs receiving an iv infusion of mannitol 3% only the intracranial pressure, electrolyte status, and urinary excretion was studied. The two controls, who did not receive any fluid underwent monitoring of the intracranial pressure and pulse oximetry.

Hemodynamic measurements were effectuated just before and 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 min after the start of the infusion. The measurements included cardiac output, blood pressures in the femoral and pulmonary arteries, blood flow rates in the internal carotid artery and abdominal aorta, and the intracranial pressure. The electrocardiogram was monitored for arrhythmias in all animals, and a printout for detailed analysis was obtained every 10 min in eight of them (three receiving glycine 1.5% and five mannitol 5%). The QT interval was corrected for variations in heart rate (QTc) by dividing the length of the interval between the onset of the QRS complex to the end of the T wave by the square root of the average interval between three consecutive heart beats (RR interval).

Blood was sampled from the femoral artery to determine the B-Hgb concentration by using Multi-Species software on a Technicon H1 on the same occasions as the hemodynamic measurements. Urine samples were also taken and the urine volume was measured before and 10, 20, 30, 40, 50, 60, 70, 80, 100, and 120 min after the start of the infusion. The serum and urine concentrations of sodium and potassium were measured on a Hitachi 917. To detect possible damage to the myocardium, the serum activity of creatine phosphokinase was measured on the Hitachi 917 and the concentration of troponin T in serum by immunoassay on an ES Enzymun system before and 60 min after starting the infusion. Samples for measurements of blood gases were obtained from the femoral artery, right atrium (mixed venous), and from the proximal part of the external jugular vein on the same occasions as the electrolyte concentrations in serum and urine, using an AVL 995-Hb in the operating room.

After the experiments were completed and the animals were killed, the heart was removed from the two controls and 13 of the 17 animals receiving glycine 1.5% or mannitol 5%. After rinsing with saline and fixation in buffered formaldehyde 4%, the hearts were examined, by a treatment blinded investigator, with regards to pathological alterations and weight. Light microscopy was used to investigate the subendocardial, atrial, and ventricular myocardium. Besides routine hematoxylin–eosin staining, Gordon and Sweet's silver impregnation method was used to visualize collagen and reticulum fibers of the myocardial histoskeleton, i.e., the basal membranes of the capillaries and the matrix. Furthermore, an immunohistochemical method for detecting leakage of myoglobin from myocytes was used to demonstrate early stages of myocardial hypoxia. Rabbit anti-human myoglobin, based on a purified immunoglobulin fraction of rabbit antiserum, was tested and found useful for this purpose. Observations were scored as 0 (normal), 1 (mild change), or 2 (severe change). The score was reduced by 50% if the alteration was found only in the subendocardium or deeper in the myocardium.

For the comparison of sampling sites in paper 2, the N_2O concentration was measured, in random order, from a flared nasal cannula, a standard nasal cannula and a Hudson mask during spontaneous ventilation. These experiments were repeated while oxygen therapy was administered through a separate flared nasal cannula at a constant rate of 2 litres of O_2/min. In addition, specific recordings were made every 30 s at the end of forced exhalations. All other N_2O concentration samples in papers 2 and 3 were via a flared nasal cannula.

N_2O and CO_2 concentrations, in papers 2 and 3, were measured every second in the exhaled breath by the N_2O monitor NGA 2000 MLT Analyzer. The monitor consists of a temperature-controlled 30 ml sampling unit with pump valves. Gas concentrations are measured by an absorbance technique using infrared signals which do not interfere with each other. For N_2O, the range was 5–2000 ppm and the pump

was set to a suction rate of 300 ml of air per minute. Data were stored directly on a laptop computer. The end-expiratory ethanol concentration was measured at 0, 5, 10, 20, 30, 40, 50, 60, 80 and 100 min in experiment 2 of study 2 and every 10 min in paper 3. The subject was asked to take a deep breath and perform a single end-expiratory breath alcohol test using a Lion Alcolmeter S-D2.

Blood samples in volunteers in of paper 2, experiment 2 and in paper 4 were drawn from the cannula in the infusion free arm. In study 2 this was done on the same occasions as the ethanol samples and for measurement of the blood Hbg and serum sodium concentrations. Hbg was analyzed on a Technicon H2 and electrolytes on a Hitachi Modular. In the clinical part of paper 4, blood samples were taken just before and 5 min after the end of each operation for serum glucose and sodium concentration measurements, which were performed by the in-hospital routine clinical chemistry laboratory. In the experimental part of the study, blood samples were collected at 5-, 10-, and 20-min intervals between 0 and 60 min, 60 and 100 min, and 100 and 160 min, respectively. Analyses of plasma glucose concentration, Hgb, red blood cell count, mean corpuscular cell volume, and baseline hematocrit were performed by the hospital clinical chemistry laboratory. Additionally, plasma concentrations of sodium and potassium were analyzed at 0, 30, 60, and 160 min. Urine was collected during and immediately after the experiments and analyzed for the content of sodium, potassium, and glucose.

During all human studies, routine monitoring comprised pulse oximetry and non-invasive blood pressure. Patients undergoing surgery were also monitored with electrocardiography.

8.4 Calculations

Oxygen delivery and consumption

Brain and whole body oxygen delivery and consumption were calculated in paper 1 from the measures of cardiac output and the arterial oxygen content (CaO_2) were used to calculate the oxygen delivery (DO_2) and oxygen consumption (VO_2) as follows [177]:

$CaO_2 = 1.34 * B\text{-}Hgb_n * SaO_2 + (0.223 * P_aO_2)$

$DO_2 = \text{cardiac output} * CaO_2/BW$

$VO_2 = \text{cardiac output} * (CaO_2 - CvO_2)/BW$

where the arterial oxygen tension is expressed in kilopascals (kPa) and B-Hgb in g/L of whole blood. To calculate DO_2 and VO_2 for the brain, the input data consisted in the carotic blood flow rate and the blood gas analyses (SaO_2 and P_aO_2) obtained from the samples taken from arterial blood and from the cephalic part of the external jugular vein.

Fluid and glucose distribution

For the distribution of irrigant water, as calculated in paper 1, the baseline blood volume was taken to 7% of the body weight and subsequent changes were estimated from the dilution of the B-Hgb concentration with corrections for the amount of withdrawn blood [178]. Since sodium ions (Na) are distributed throughout the extracellular fluid (ECF) space, the serum sodium concentration during or after infusion of fluid ($S\text{-}Na_t$) at any time t equals the amount of Na in the ECF volume divided by the current ECF volume. Hence,

$$S - Na_t = \frac{(S\text{-}Na_o \times ECF_o - Na_{loss})}{(ECF_o + \text{infused volume} - \text{urine volume} - \Delta ICF)}$$

where $S\text{-}Na_0$ and ECF_0 are the serum sodium concentration and ECF volume at baseline, respectively, Na_{loss} is the natriuresis (in mmol), and ΔICF is the change in the water content of the intracellular fluid compartment. Since ECF_0 corresponds to 20% of the body weight, ΔICF could be calculated by rearrangement of the equation shown above.

$$\Delta ICF = ECF_o + (\text{infused} - \text{urine})\text{volume} - \frac{(S\text{-}Na_o \times ECF_o - Na_{loss})}{S - Na_t}$$

The kinetics of the glucose infusion in paper 4 was studied to characterize how effectively the volunteers handled the glucose molecules and the accompanying water volume. Hence, the pharmacokinetics was analyzed using a one-compartment turnover model in which the plasma concentration of glucose above baseline (C) at any time (*t*) resulting from the infusion rate k_i is calculated by using the following differential equation:

$$\frac{dC}{dt} = \frac{k_i - (CL \times C(t))}{V_d}$$

where V_d is the volume of distribution and CL is the glucose clearance [169].

For the fluid volume, a one-volume kinetic model was used in which fluid infused at a rate k_i expands a central body fluid space *v*, which strives to return to its baseline volume V by two mechanisms: first, elimination of fluid at a rate proportional by a constant kr to the dilution of V, i.e., the rate of elimination at any time was k_r (*v* – V)/V and, second, a baseline loss (k_b) fixed to 0.5 mL/min to account for evaporation and basal diuresis, which occurs at a rate of approximately 700 mL/d in adults [179]. Thus, the parameter k_r is a clearance constant.

The volume change in the central body fluid space (*v*) was then expressed as:

$$\frac{dv}{dt} = k_i - k_b - k_r\frac{(v - V)}{V}$$

The dilution of the plasma was calculated based on the blood chemistry obtained at baseline (time 0) and at any later time (t). The principle is to convert hemodilution into plasma dilution based on the average of the Hgb and RBC dilutions because these are measured by different laboratory methods (photometry and laser beam dispersion, respectively). Hence,

$$\text{Plasma dilution} = 0.5\left(\frac{\text{Hgb}_{\circ}/\text{Hgb}\,(t) - 1}{1 - \text{Hct}_{\circ}} + \frac{\text{RBC}_{\circ}/\text{RBC}(t) - 1}{1 - \text{Hct}_{\circ}}\right)$$

Before use, this expression was corrected for "iatrogenic" dilution resulting from the sampling (7 mL per sample) and also by the ratio $MCV_0/MCV(t)$ to account for changes in erythrocyte cell size [179]. The best estimates and the associated standard deviation for the unknown parameters in these models, being V_d and CL for exogenous glucose and V and k_r for the infused fluid volume, were estimated for each of the 10 experiments individually by applying a nonlinear least squares regression routine based on a Gauss-Newton method to the analytical solutions of equations. 1 and 2 until no parameter changed by more than 0.0001 (0.01%) in each iteration [169, 179-180].

Predictions of the plasma glucose concentration over time for various theoretical infusion regimens were obtained by inserting the best estimates of V_d and CL for the group into the mathematical solution to Equation. 1, using the same computer program (Matlab version 4.2, Math Works, Natick, MA) as the one used for analysis. Simulations included 60-min plots of early (first 30 min), late (last 30 min), and continuous absorption patterns. Volumes of up to 2000 mL were tested in steps of 250 mL for every possible combination of early, late, and continuous absorption. A nomogram was constructed based on the observation that the postsimulation glucose level decreased approximately 30% per 10 min.

Nitrous oxide

In order to get N_2O measures, in part per million (PPM), representative of exhaled air, the output samples in papers 2 and 3 were adjusted for CO_2 levels to account for different breathing patterns and admixture with ambient air. Several ways of minimizing the variability in N_2O were tested and compared based on the data from the continuous infusions in study 2 and we arrived at the following approach. For the end of each 5 min interval of these 12 experiments, the individual area under the concentration curve (AUC) for N_2O was calculated by the linear trapezoid method and based on the samples in which the CO_2 was above the median value. The reason for discarding all values associated with a low CO_2 concentration was to ensure that only the most representative data for exhalation were included. This AUC for N_2O was then divided by the median CO_2 for the remaining sampling points. This algorithm was then applied in all 120 experiments of paper 2, and

its ability to predict the infused fluid volume evaluated by residual plots for each series. To make the low- and high-concentration experiments in paper 2 comparable, fluid volumes used in the latter were too low and therefore multiplied by 2.5 to compensate for the difference in N_2O content between infusions. In paper 3 the individual AUC for the breath N_2O in each patient was calculated in the same fashion but for every second. The data on breath N_2O were then transformed into the volume of irrigant absorbed by applying the following regression equation, which was derived from the infusion experiments in paper 2:

$$Volume_{\mathrm{Abs}}(\mathrm{ml}) = 36 + (6.35 \times AUC_{\mathrm{N_2O}}(\mathrm{p.p.m.})/1000)$$

As N_2O is eliminated quickly from the body, the N_2O integral summarized all absorption events occurring during the operation.

Ethanol

For the ethanol monitoring method, the absorbed volume per 10 min was calculated using the following empirical equation derived during TURP [108]:

$$Volume_{\mathrm{Abs}} = \sum [(2140 + 3430 \times Ethanol) \times \Delta Ethanol] \times (44 + 806 \times Ethanol)$$

where $Volume_{abs}$ is the predicted incremental volume of absorbed fluid (ml) Ethanol is the blood ethanol concentration (mg/ml) as indicated in the breath in the beginning of a 10-min interval, and ΔEthanol is the change in this concentration during the same 10-min interval. The ethanol equation is governed mainly by the distribution function of ethanol in the total body water while elimination of the tracer is quite slow.

8.5 Statistics

Overall, data were normally distributed and expressed as the median or mean with standard deviation (papers 2–4) or standard error of the mean (paper 1).

Differences between sampling sites in paper 2 and between groups as well as changes from baseline in paper 1 were studied by repeated-measures ANOVA. For the changes from baseline this was then followed by Dunnett's test. For paper 1 the repeated-measures ANOVA was specified for the time frame of each analysis, i.e. during infusion, the post infusion period and over the entire experiment.

Linear regression was used for analyzing the correlations between parameters in papers 1, between absorbed volume and N_2O and ethanol concentration in paper 2 and between sodium and glucose changes in the clinical part of paper 4. In paper 2, an algorithm was developed, from the 12 continuous infusion experiments, for the prediction of absorbed volume based on the exhaled air concentrations of N_2O.

The validity of the algorithm was then tested on all the infusion experiments by investigation of the obtained scattering of the residual plots when fitting a linear regression equation to this relationship.

The 95% prediction interval was used for estimation of absorbed volume from breath samples of N_2O and ethanol in papers 2 and 3. The agreement between the two methods in paper 3 was illustrated by Bland-Altman plots.

In paper 2, the coefficient of variation (CV) for breath N_2O was obtained as SD divided by the mean over time. CV was recalculated every 30 s, and the Wilcoxon test was used to compare results between continuous and intermittent infusions.

$P < 0.05$ was considered significant.

9 ETHICAL CONSIDERATIONS

The study protocols for all studies were approved by the regional ethics committees in Stockholm and all volunteers and patients gave their written informed consent. The reference numbers for these approvals are as follows:

1) Paper 1: Stockholm southern animal ethics committee, S66-97
2) Paper 2: Research ethics committee south, Huddinge Hospital, 267/02
3) Paper 3: Research ethics committee south, Huddinge Hospital, 76/03
4) Paper 4: Regional ethics committee, Stockholm, 2007/851-31/4

The known dangers involved in administering high volumes of electrolyte-free irrigating fluids in paper 1 did not allow for this research to be carried out on human subjects. A model with relevant physiology and size as compared to humans was needed in order to be able to study the organ specific and whole body pathophysiology in a meaningful way. The pigs were taken care of by professional animal keepers and were acclimatized in a nearby sty to where the study was conducted. Premedication was given in advance to the experiments and the level of maintenance anesthesia was repeatedly checked. The animals were killed at the end of the investigations and while still under general anesthesia.

The volunteers in papers 2 and 4 had a routine health check-up including laboratory screening of liver function tests, creatinine, sodium, potassium and glucose levels prior to their participation in the respective study. They received two venous cannulas by either a certified nurse or a doctor. This may involve short transient pain but no other adverse reactions occurred. The sampled blood volume was less than 100 ml in all subjects. The infusion regimens with mannitol 3% and ethanol 1% in paper 2 had been used in several previous studies without any adverse effects except maybe slight sensation of being tired which could have been due to that the subjects in those studies were completely fastening. Our subjects were allowed a light breakfast before experiments. The volunteers in paper 2 were asked about the comfort of the various N_2O-sampling methods tested and this played a significant role in the future choice of using a flared nasal cannula.

The patients all followed hospital routines which included the ethanol method in paper 3 and the analysis of pre- and postoperative blood chemistry in study 4. The flared nasal cannula used for N_2O sampling in study 3 was the hospital standard procedure for delivering supplementary oxygen to all patients undergoing spinal anesthesia. In addition, paper 3 was done according to GCP guidelines and an external review board was used for the monitoring of adverse events.

10 RESULTS

The findings with regards to the pathophysiological alterations seen with all irrigating fluids in paper 1 were that blood volume, cardiac output, ICP, mean pulmonary artery pressure, the carotic and aortic blood flow rates increased significantly during the infusions while the heart rate decreased by ≈ 20% ($P < 0.001$) and oxygen delivery to the brain increased transiently ($P < 0.005$). The latter was more consistent in the mannitol 5% group. The parameters showing the most pronounced increase in response to the infusions were the intracranial and pulmonary artery pressures and the blood volume. As the infusions ended, the whole body circulation became hypokinetic. 30 min later, cardiac output and aortic blood flow had dropped to approximately 50% of the baseline, and mean arterial pressure to about 80% ($P < 0.02$) while brain blood flow rates were maintained. There were no changes in pH or base excess in jugular venous blood. Signs of interstitial heart dilatation were found in all groups except the controls although no significant changes in the serum activities of creatine phosphokinase and troponin T were seen. The heart rate corrected QT time increased by 15–20% during both glycine 1.5% and mannitol 5% infusions ($P < 0.03$). The B-Hgb and serum sodium concentrations decreased markedly during both these infusions and the urinary excretion rate and the natriuresis increased progressively ($P < 0.001$). At the end of the experiments, 41, 46% and 30% (mean) of the infused volume of the glycine, mannitol 5% respectively mannitol 3% solutions had been recovered in the urine.

As for the differences between glycine 1.5% and mannitol 5% the peripheral vascular resistance decreased and the blood volume increased most during the mannitol infusions ($P < 0.001$). The pulmonary artery pressures increased most in response to glycine 1.5%. Serum sodium decreased more (from 140 to 98 mmol/L at 80 min) when mannitol was given ($P < 0.002$) as compared to glycine (from 136 to 103 mmol/L at 80 min) but the serum osmolality still only decreased (from 292 to 277.3 mosm/kg) in the glycine group ($P < 0.002$). In spite of increased kaliuresis occurring only in response to glycine ($P < 0.001$), the serum potassium concentration increased from 3.6 to 4.4 mmol/L while it decreased from 3.5 to 3.3 mmol/L during the mannitol infusions. The change in intravascular volume was significantly larger for mannitol during the first 30 min of the infusions ($P < 0.05$). Glycine increased the intracellular fluid volume (ICV) more than mannitol did ($P < 0.002$). After the infusions, the interstitial fluid volume was larger with mannitol ($P < 0.03$). The difference in urinary excretion did not quite reach statistical significance. The whole-body oxygen delivery decreased significantly in the glycine experiments ($P < 0.04$) and the oxygen consumption also tended to decrease ($P = 0.12$) while both these parameters remained unchanged during the mannitol infusions. A slight base deficit developed in arterial and mixed venous blood ($P < 0.001$), in particular when mannitol was infused but pH remained within the normal range. 15 min in to the experiments and onward, ICP was significantly higher in the animals given

glycine and after the infusions, the intracranial pressure remained high only in this group ($P < 0.001$ when compared to baseline). Oxygen consumption in the brain decreased progressively to about 50% of baseline in response to mannitol ($P < 0.001$), while it was more variable and did not change significantly in the glycine group.

Cardiac morphology and conductivity also differed between the irrigating fluids. The highest scores for leakage of myoglobin, focal necrosis, and rupture of the histoskeleton were obtained for glycine 1.5%, whereas mannitol 5% had intermediate and mannitol 3% the lowest scores. There were significant linear correlations between myoglobin leakage, focal necrosis ($P < 0.02$), and rupture of the histoskeleton ($P < 0.006$), whereas interstitial dilatation correlated only with rupture of the histoskeleton ($P < 0.04$). Nearly all observed changes occurred both in the subendocardium and deeper in the myocardium. Mannitol 5% significantly increased the PQ time (+20%, $P < 0.05$ versus glycine) and decreased the QRS amplitude (-20%), while glycine tended to prolong the QRS duration (+25%) and increase the T-wave amplitude (+50%).

As for mannitol 3%, a slightly smaller increase in intracranial pressure than the in glycine pigs was seen (mean 140 versus 170% of baseline, respectively, at 90 min, a nonsignificant difference) but a larger one than in the pigs given mannitol 5% (mean 28%, $P < 0.01$). The changes in heart rate and in the serum sodium (from 138 to 100 mmol/L at 80 min) and potassium concentrations (from 4.0 to 4.1 mmol/L) were similar to those for isotonic mannitol, while the serum osmolality decreased from 292.8 to 278.5 mosm/kg, following the glycine curve. The ICV increase was between 7 and 10% less for mannitol 3% than for the glycine.

Even though correct placement of the intracranial catheter was verified by X-ray examination with contrast medium, the two control pigs showed a slight spontaneous increase in ICP from 7 to 10 mm Hg respectively from 2 to 4 mm Hg over a 110-min observation period.

For the nitrous oxide measurements in paper 2 and 3 no adverse monitoring related events occurred. In the comparison of sampling sites, there were fairly small but still statistically significant differences in the CO_2-adjusted AUC for the N_2O concentration depending on whether sampling was done from a flared nasal cannula (reference), a standard nasal cannula (+14%) or a Hudson mask (+8%). Adding O_2 via a flared nasal catheter marginally changed the AUC obtained via any of these airway devices. In contrast, end-expiratory sampling yielded a 25% lower value ($P < 0.001$). All volunteers agreed that the flared nasal cannula was the most comfortable sampling method. The individual N_2O time–concentration profiles had a different appearance depending on the mode of infusion showing quite variable patterns with bursts in the N_2O concentrations for the three intermittent infusions while in the patterns gradually increased towards a steady state for the continuous

infusions. Mathematically this was also shown as the CV for the N_2O concentration over 3-min intervals was much higher for the intermittent than the continuous infusions (mean 0.70 vs 0.26, $P < 0.001$). The 3-min interval was the shortest that still offered an excellent distinction between the two types of infusions. The CO_2-adjusted AUC for N_2O did not differ significantly when the fluid volume in the high-dose experiment was multiplied by 2.5, i.e. the factor with which the N_2O concentration differed between low- and high-dose experiments. For all infusions, the AUC strongly correlated with the volume of infused N_2O-containing irrigating fluid, regardless of whether the fluid was infused continuously or intermittently with a high or low N_2O concentration. The 95% prediction interval was ± 200 ml, and overall the data showed a normal distribution, when plotting the residuals, around the regression line. Blood Hgb and serum sodium concentrations decreased by 6% during the continuous-infusion experiments (repeated-measures ANOVA, $P < 0.001$), but only Hgb was restored during the follow-up period.

Breath ethanol concentrations obtained during the continuous and intermittent infusions of irrigating fluid in paper 2 slightly overestimated the known infused fluid volume. The 95% prediction interval was approximately 400 ml which is in agreement with previous findings based on a larger number of data points. In the clinical paper 3, thirteen patients (15%) absorbed > 300 ml of fluid as indicated by the control method (ethanol). The median volume was 707 ml (range 367–1422). The time period during which there was absorption during these 13 operations was 40 min (15–90), while the operating time was 65 (50–124 min), i.e. absorption occurred during 58% (22–100) of the period of surgery.

In comparison of the ethanol and the N_2O method in paper 3, the amount of irrigant absorbed was correlated at the end of each 10-min period of surgery during which the ethanol method indicated the presence of absorption (ethanol concentration > 0). The ethanol yielded higher figures for fluid absorption up to about 700–800 ml where after the N_2O method indicated that the absorption was larger. Over the entire range, however, the mean difference between the ethanol and N_2O methods was +45 ml (95% limits of agreement, –479 to +569 ml). When only the last measurements during each operation were compared, the mean difference was – 133 ml (95% limits of agreement, –833 to +587 ml). In 33 patients, the N_2O method indicated small or modest degree fluid absorption, while the ethanol method indicated no absorption. All these cases occurred at centre 2 and is explained by the fact that that ethanol concentrations of 0.04 mg/ml or less were displayed as zero by the alcolmeter used. In the evaluable patients, we found no clear evidence of N_2O being absorbed through the intact bladder.

The most frequent concomitant diseases, in paper 3, were hypertension, 29; angina pectoris, 7; asthma, 3; atrial fibrillation, 2; cerebrovascular accident, 4; and diabetes mellitus, 4. Thirteen patients experienced at least one serious adverse event.

These included haematuria, 5; intraoperative haemorrhage, 1; fever, 1; duodenal ulcer, 1; and syncope, 1.

In the clinical part of paper 4, there was a statistically significant linear relationship showing that each mmol/L decrease in serum sodium concentration increased the serum glucose by 1.5 mmol/L when 5% glucose in water was used as the irrigating solution. For nondiabetic and diabetic patients, respectively, the coefficients of determination were 0.80 ($P < 0.0001$) and 0.92 ($P < 0.0001$). Those patients who received 1.5% glycine but did not absorb the irrigating fluid, as evidenced by a change in serum sodium by no more than 1 mmol/L, had no significant change in serum glucose during monopolar TURP (mean 0.05 mmol/L, SD 0.65). The incidence of TUR-syndrome in 233 of the patients where symptoms could be determined was 2.1%, (95% CI 0.7–5.0) with an average absorbed volume of absorption of 3.6 (2.6–4.1) L as calculated by the degree of sodium dilution. All the background data on these patients have been reported elsewhere [181].

Plasma glucose almost doubled during the experimental infusions and reached a maximum of 8.27 mmol/L (SD 0.95), while the plasma was diluted by 17.7% (3.2%). The excreted urine at the end of the experiments (160 min) had a volume of 1.14 (0.77) L, which represented 66% (37%) of the infused fluid volume. During the infusions, the changes in serum sodium from 140.7 mmol/L (SD 1.5) to 138.8 (2.0) and potassium from 3.87 mmol/L (0.22) to 3.99 (0.22) were not significant. The total urinary excretions were 89.4 mmol/L (SD 35.4) for sodium and 22.8 mmol/L (10.4) for potassium. At the end of experiments, serum sodium was 140.3 mmol/L (SD 2.0) and potassium 4.05 mmol/L (0.21). No glucosuria was found. The hemodynamic variables changed mainly at the beginning of the infusion, averaging +9% for the systolic pressure, +12% for the diastolic pressure, and –7% in heart rate. No adverse reactions were seen during the experimental infusions.

For exogenous glucose, the kinetic analyses of the simulations showed that V_d amounted to 15.52 L (SD 2.15) and CL to 0.56 L/min (0.15). For the infused water volume, the expandable body fluid space was 6.03 L (2.70) and k_r 0.19 L/min (0.12). A nomogram was successfully constructed and showed that, regardless of the simulated pattern of fluid absorption over 1 h, the uptake of 2 L of irrigating fluid containing 1% glucose would have increased plasma glucose by 6.9 (1.7) mmol/L at the end of the surgery. The corresponding increase for 1 L of irrigating fluid was 3.7 (1.6) mmol/L.

11 DISCUSSION

In the clinical study (paper 3), 15% of the patients absorbed > 300 ml of fluid as indicated by the ethanol method. The median volume absorbed was about 700 ml over 40 min of the average operating time of 65 min. In the clinical part of paper 4, 2.1% were diagnosed with the TUR-syndrome. These data are in agreement with other studies in TURP patients [29-30].

All fluids (glycine 1.5%, mannitol 3%, mannitol 5% and RS with 1% glucose) administered in papers 1 and 4 yielded a swift response in hemodynamics with an increase in blood pressure and a decrease in heart rate within 15 min. The hemodynamic variables in the volunteers had returned to baseline at about 30 min after the RS-infusion was discontinued and remained so for the rest of the observation period although the subjects were still slightly overhydrated. In contrast, the flow rates fell promptly when the infusion of the electrolyte-free irrigants was stopped and a low-flow state developed despite the marked overhydration of the animals. The cardiac output and the aortic blood flow rate had fallen to about 50% of baseline 30 min after these infusions, while the carotic blood flow was upheld. This hypokinetic circulation corresponded to intravascular hypovolemia. Low cardiac output, hypotension and hypovolemia has also been seen in clinical studies exploring the TUR-syndrome. [54, 182-183]. The arterial hypotension of sudden onset appearing 30–60 min after surgery is, along with nausea, the most prominent symptom of moderate-sized glycine absorption [53]. The hypovolemia develops in response to water and sodium losses from osmotic diuresis. Mannitol is not reabsorbed in the kidneys and glycine overwhelms [184-185] the reabsorptive mechanisms for amino acids [80]. The decrease in plasma sodium concentrations promotes cellular edema even when the iso-osmotic mannitol 5% is given. Glycine is also taken up by active transport into the cells and it then brings along fluid by virtue of osmosis [186]. Hyperkalemia and kaliuresis was seen in the glycine pigs. This is likely due to the more pronounced intracellular edema which causes the cells to pump out potassium as a means of restoring their volume [187]. The 20 min infusion of 15 ml/kg of mannitol 3% and ethanol 1% rendered a 6% decrease in Hgb and serum sodium levels in the volunteers in paper 2. Hgb but not sodium concentrations returned to baseline during the post-infusion observation period. Hence, sodium losses were apparent here as well.

The fluid shifts can cause cerebral edema due to water intoxication which is thought to be the main reason for nausea, depressed consciousness and late death in the TUR syndrome [28, 100, 188]. We found a more pronounced increase in intracranial pressure for glycine 1.5% which reflects its stronger tendency to cause cellular edema. Mannitol 3%, which had a similar osmolality, produced slightly lower values. Mannitol 5% was associated with the smallest changes in intracranial pressure.

Ringer's solution is also slightly hypo-osmotic which could promote cellular edema but the natriuresis in response to Ringer's solution is smaller than the water excretion [189]. In paper 4, the excreted amount of sodium was 89 mmol, while 205 mmol had been infused (Ringer's solution contains 130 mmol/L). As the serum sodium concentration was virtually the same at end of the study as before it started, simple mass balance calculations [189] indicate that the retained sodium must have bound more water than the retained 400 mL of infused fluid in the extracellular fluid space, and this volume can only have been derived from the intracellular fluid space. Hence, there is no evidence that Ringer's solution with 1% glucose caused intracellular edema but rather that the opposite was the case. Absorption of electrolyte containing irrigating fluids still poses a potential problem with volume overload even though hyponatremia and its consequences with brain edema is prevented. Rapid vascular overload can cause symptoms of swelling, dyspnea and a risk of fatal pulmonary edema [190-192]. Furthermore, absorption of isotonic saline, as compared to RS, is likely to increase mortality and the risk of major adverse kidney events [22-23].

Fluid overload also causes a strain on the heart. In the animal experiments, the most pronounced myocardial damage was seen with glycine 1.5% followed by mannitol 5%. The glycine effects are probably due to cellular edema while the iso-osmolar mannitol 3% may cause less intravascular fluid overload and thus less myocardial strain than mannitol 5%. In addition, the infusions caused prolonged QTc interval which can be explained by the consistent hypocalcemia that was seen [43]. Bradycardia, prolonged PQ interval and a glycine specific increase in QRS duration were also seen. Previous studies have found similar disturbances [12-13].

Two novel approaches to diagnose irrigating fluid absorption were introduced in this thesis. The idea was that measurements of an added tracer amount of either N_2O or glucose to the irrigant would correlate with the volume absorbed. A pre-requisite for these correlations is that the uptake of these tracers only occurs in response and proportion to fluid absorption. Unpublished data in pigs before the start of study 2 and 3 showed that the urinary tract epithelium is impermeable to N_2O. In the clinical investigations of papers 3 and 4 we validated that the measured levels of N_2O respectively glucose only increased in response to absorption. The exhaled air concentrations of N_2O and CO_2 during spontaneous breathing were measured in response to iv infusions to which either 40 ml/L or 100 ml/L of N_2O had been added. The N_2O concentration of 100 ml/L in the infusion did not offer any advantages in respect to accurate predictions of the pattern or volume of infusion. A higher N_2O concentration is also inferior in regards to environmental considerations and especially the theoretical risk of accumulation of explosive gas mixtures during electroresection [195-196]. A 4% solution of N_2O in combination with flammable gases does not produce any such explosive mixtures [175]. Hence, the 4% concentration was used in all other experiments.

An algorithm was developed based on 12 of the 120 infusion experiments, and eventually validated in all of them by demonstrating limited scattering on residual plots. This algorithm adjusted the N_2O data with regard to the depth of breathing and also removed measurements performed during apparent inhalation. Since the AUC for a drug changes in proportion to the dose, we finally used the CO_2-corrected AUC for N_2O concentration as the expression for infused fluid volume. The obtained correlations were slightly influenced by the sampling method and simultaneous oxygen delivery only marginally affected the results. We concluded that further experiments should be performed using a flared nasal cannula, rather than standard nasal cannula or Hudson mask, since it was preferred by the volunteers. End-expiratory sampling yielded lower values than the three modes of continuous measurement. This might be explained by the fact that the measuring chamber for N_2O is larger than that for CO_2. The N_2O chamber then partially equilibrates the concentration over time which yields a falsely low peak at the end of exhalation. The volunteers also had difficulty in providing a forced end expiratory sample every 30 s, which probably made this mode of measuring less accurate.

There was a linear correlation between the infused volume and both the N_2O concentration and ethanol algorithms. In our infusion study, the N_2O method was more precise than the ethanol method with a 95% prediction interval of ± 200 ml respectively ± 400 ml for any new volume estimate. The wider prediction interval of ethanol was based on fewer observations but is in agreement with the 90% prediction interval of ± 300 ml obtained for 160 TURPs [107].

Clinical comparison between N_2O and ethanol in paper 4, showed that the scatter between the ethanol and N_2O methods was about twice as high as between the N_2O algorithm and known amounts of fluid in the volunteer study. The difference in the predictions of fluid absorption was clearly dependent on the absorbed volume. In addition, we found the same agreement between the methods even when the last step of the algorithm, which divides the N2O AUC by the CO_2 median, was omitted. This may be due to that this step primarily compensates for differences between sampling devices and that only the flared nasal cannula was used in the clinical study.

We predicted the fluid absorption by an equation based on ethanol data, obtained every 10 min, in a series of 90 TURP patients in whom the control method was careful measurements of the volumetric fluid balance and serum sodium [108]. The mean deviation between predicted and measured fluid absorption was claimed to be 19%. A dose-dependent bias between the ethanol method and known amounts of infused fluid, which has similarities to the pattern of agreement between N_2O and ethanol in paper 3, has previously been seen in volunteers [197]. Up to 800 ml of fluid, the ethanol method overestimated the absorbed volume by on average 200 ml, while larger volumes were underestimated. This pattern seems to be expected when

the fluid administration is continuous, while fluid absorption during surgery most often occurs in a more haphazard, intermittent manner [10]. Naturally, the bias is larger when N_2O indicated absorption is compared with the ethanol method than with more precisely known amounts of volume. In retrospect, additional tools for estimating fluid absorption might have given a better evaluation of the N_2O method. In paper 2, where the amount of administered fluid was actually known, the N_2O method was clearly the more precise of the two. Still, both methods were quite helpful to the surgical staff by early signaling ongoing fluid absorption. Both offer a possibility to stop further absorption by reducing the fluid pressure and/or the extent of surgery. Treatment can be started early and the optimal level of post-operative care can be chosen [107].

There are also several clinical advantages of the N2O method as compared to the ethanol method. In fluid absorption, ethanol concentrations may increase to levels that causes a slight inebriation and makes car driving illegal. There may also be issues with ethanol use in certain religions and patients with liver impairment. The concentrations of N_2O reached in our studies were in the range of 0–300 ppm and thus within the environmental pollution range. Pharmacological effects of N_2O occurs at a concentration around 15% or higher, which is about 500 times higher than the maximum concentrations measured in our experiments. In addition, N_2O is highly inert with a quick elimination during the first passage over the lungs. This further lowers the exposure to the patient's central nervous system and probably makes any potential accumulation of gas in the intestines unimportant. Exhaled N_2O is also quickly diluted in the ambient air, and any elevated concentration soon becomes impossible to detect. The rapid elimination of N_2O makes distinction between extravasation and intravascular absorption easier than with the ethanol method where elimination is much slower [107]. In the volunteer study in paper 2, the pattern of N_2O concentrations in exhaled air differed considerably between continuous and intermittent infusions. This was also mathematically shown as the coefficient of variation, over each three-minute period, was clearly larger during the intermittent than the continuous infusions. Although not tested in our studies, extravasation results in N_2O, ethanol and serum sodium responses that are only one-third of those found after intravascular absorption [107, 175]. Dangerous symptoms may develop before treatment is started if extravasation is mistaken for intravascular absorption [198]. The end-expiratory breath tests used in spontaneously breathing patients is another downside of the ethanol method since this may cause the patient to move and disturb the surgical procedure. In contrast, N_2O can be monitored continuously without active involvement of the anesthetist, the surgeon or the patient.

The addition of glucose 1% to the irrigating solution has a different approach for its diagnostical purpose. The N_2O and ethanol methods can be used for early detection of absorption and thereby alert the surgical and anesthetic team to initiate

adequate measures. In contrast, addition of glucose as a tracer is used for refuting or validating suspected absorption when indicated by clinical signs or symptoms. For electrolyte-free solutions this can be done by nomograms which correlate the degree of sodium-dilution to the absorbed volume. In the clinical part of paper 4, we found that absorption of an irrigating fluid consisting of isotonic glucose during TURP increases the serum glucose concentration in an inverse linear proportion to the reduction in serum sodium. We conducted experiments in volunteers and kinetic simulations to assess the potential usefulness of glucose as a tracer when electrolyte- containing irrigants are used, i.e. during bipolar TURP, which prevents the detection of sodium dilution. The volume of distribution and clearance of RS with 1% glucose was calculated in 10 healthy volunteers and, based on these data, various absorption patterns were tested in volume steps of 250 ml. The simulations indicated that, regardless of the pattern of absorption during a 1-h operation, absorption of 1 and 2 L would increase the plasma glucose concentration by 3.7 and 6.9 mmol/L, respectively, followed by a decrease of 30% per 10 min. The usefulness of glucose to detect fluid absorption can be judged only after considering the spontaneous variation of plasma glucose during TURP. The clinical trial showed that no consistent change in the glucose level occurs during TURP with no fluid absorption, not even in diabetics. This can be understood from the minor endocrine response associated with this operation [199]. The kinetic method used to analyze the disposal of glucose is independent of the infused amount and infusion rate of glucose [169]. The clearance of glucose, 0.56 L/min, was similar to that found in previous volunteer experiments [169]. The kinetics of the infused fluid volume was analyzed according to a one-volume fluid space model, which is appropriate when elimination of the fluid is prompt [179]. In an individual patient, spontaneous variations may alter glucose levels. Our data indicated that the existence of absorption can be detected with 95% confidence if the plasma glucose concentration increases by more than 1.4 mmol/L even when considering such interindividual variations. As previously described, although absorption of electrolyte-containing solutions does not cause the problems associated with hyponatremia, the effects may still be severe. Absorption may be just as frequent and our study indicates that glucose can be a useful tracer to easily control for the suspicion of such irrigating fluid absorption.

12 CONCLUSIONS

The N_2O technique could serve as a clinical monitoring tool when administration of fluid may occur spontaneously in unknown volumes, such as fluid absorption during endoscopic surgery. Compared to available methods it is likely to offer the following advantages:

1) Immediate detection of amount and route of fluid absorption without active involvement of the patient or operating team.
2) Similar or better prediction of the absorbed volume than the commonly used ethanol method.
3) The linear correlation between accumulated exhaled N_2O concentrations and known amounts of infused fluid does not seem to be volume dependent.
4) The monitoring method does not affect the patient's condition and is not restricted by religious limitations or by concomitant liver disease.

For optimal safety, patient comfort and the best prediction of the absorbed fluid volume, we propose using a N_2O tracer concentration of 40 ml/L and collecting samples via a flared nasal cannula.

The use of an electrolyte-containing solution with 1% glucose for bladder irrigation during bipolar TURP is likely to detect clinically significant fluid absorption (> 1 L) by measuring plasma glucose at least within the first 30 min of the postoperative period. This correlation seems not affected by the infusion pattern.

The fluid distribution, as seen in paper 1, showed that massive infusion of non-electrolyte containing irrigants renders a hypovolemic hypokinetic state. Based on this, we propose the infusion of hypertonic saline to be the first line treatment when such absorption occurs.

Glycine 1.5 was associated with the greatest intracellular swelling, the highest ICP increase and the most damage to the myocardium. Mannitol 3% caused a higher ICP increase than mannitol 5% but less myocardial strain. In regards of adverse reactions, our findings advocate that the irrigating solution glycine 1.5% is abandoned and that irrigation with mannitol 5% is preferable to mannitol 3% when an electrolyte-free solution is indicated.

13 LIMITATIONS

Several limitations of our studies need to be acknowledged. Overall, the experimental studies included few subjects although the study regimens were quite extensive for each subject. In addition, studies in experimental animals and volunteers may not be fully representative of variables seen in a clinical context. This includes such factors as inter species differences, metabolic and endocrine stress response, preoperative status, bleeding, perioperative infusions and drugs, etc.

The use of N_2O as a tracer for detecting fluid absorption precludes the use of N_2O as an adjunct to general anesthesia. Furthermore, one must consider that the regression line describing the correlation between N_2O and the actually infused fluid volume is valid only for the measuring chamber size of 30 ml and a suction rate of 300 ml/min. The slope is likely to be slightly different with other settings. After conclusion of our experiments, a laboratory study revealed that N_2O escapes from plastic fluid bags at a rate of 5% per hour (unpublished data). Administering an exact amount of N_2O to the irrigating solution just prior to use might also be cumbersome. For clinical use, we believe that the N_2O should be added to the fluid immediately before use via a small gas bomb attached to a port on the irrigating system. Additionally, the fluid bags could be made less permeable to N_2O diffusion. This arrangement would also prevent the need for more types of factory-made irrigating fluids. Further studies during actual surgical interventions are also needed to evaluate the correlating amount of absorption in various settings and with a practical low-cost N_2O and CO_2 analyzer.

N_2O may accumulate in gas-filled cavities and diffuse over various barriers in the body which may affect the recordings. The low concentrations seen in our studies and the high first passage elimination over the lungs makes this unlikely to affect the measurements.

The use of glucose as a tracer precludes simultaneous administration of insulin or glucose. This is however rarely indicated in adult endoscopic surgery, even in most diabetics. The metabolic stress response is of particular importance when absorption is traced by glucose concentrations. Fluid absorption or major blood loss shows a metabolic stress response, which is not the case for TURP without these complications [199]. Insulin resistance might also be more common in the elderly. This means that the plasma glucose level would be increased slightly higher during a clinical TURP with fluid absorption than in our volunteer experiments.

In the clinical part of paper 4, no apparent effect of diabetes mellitus was found on the degree of hyperglycemia induced by 5% glucose. This is probably due to the fact that fluid absorption usually occurs during the latter half or at the very

end of the surgery. This allows little time for differences in the half-life of glucose to affect the plasma glucose level. However, the absorbed fluid volume is likely to be progressively more overestimated by our nomogram if sampling of plasma glucose is delayed after the end of TURP. We advise further evaluation of the glucose method in patients with different severity of diabetes before it can be recommended in this patient population. Another issue to consider is a surgical duration longer than 60 min, which is the maximum duration recommended by most urological surgeons. Continuous absorption throughout a TURP procedure is rare. For such events, however, simulations up to 90 min (instead of 60 min) showed that plasma glucose will still increase by > 1.4 mmol/L for practically all patterns of fluid absorption, although the absorbed volume will be less certain. In prolonged surgery, it might be prudent to measure plasma glucose at 60 min if there is any suspicion of fluid absorption.

14 ADDITIONAL AND FUTURE RESEARCH

The studies in this thesis have shown that N_2O and glucose are promising tracers for the early respectively postoperative detection of irrigating fluid absorption. Furthermore, the gradual development of the pathophysiology involved in massive absorption of common electrolyte-free irrigating fluids has been outlined. Still, this thesis only explores a small part of what can be done within this field.

Future research should include the incidence of irrigating fluid absorption and symptoms in large cohorts in order to establish the importance of monitoring such occurrences in modern practice. A clear consensus for the optimal electrolyte-free and electrolyte-containing irrigant is also still lacking and the role of buffered electrolyte-containing irrigants should be further investigated. The validity of the N_2O and glucose methods needs to be validated in various patient groups, kinds of surgery and clinical contexts. Preferably, these results should be compared with as known volumes of absorption as possible. The optimal way of adding and measuring N_2O in irrigating solutions in a clinical context requires further exploration. Automated computerized algorithms would be helpful in clinical decision making.

In addition, N_2O and other inert gases may serve as valuable tools for measuring other unknown local to whole body distributions. This may include the passage through potentially obstructed conduits and monitoring the blood supply in ischemic regions or parts of the body where this is a threat, i.e. skin flaps, peripheral arterial occlusive disease, reimplantation of digits, etc.

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