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INTRAVENOUS ACCESS IN DISTRESSED CHILDREN: EFFECTS OF MIDAZOLAM AND NITROUS OXIDE ON SUCCESS RATE, HORMONE AND METABOLIC STRESS RESPONSES

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S.Kierkegaard

ABSTRACT

Background and Aims:
Intravenous (IV) access is associated with high levels of pain and distress for many children. A stressful IV procedure should be avoided, primarily by for the sake of the children, but it is also of importance for the parents and staff. When testing children with suspected endocrine and metabolic disorders there is a substantial risk that a stressful IV access, as well as the drugs used to reduce pain and stress, might affect hormone release and the metabolic response.
The aims of this thesis were to facilitate painful procedures and IV access in children at a paediatric outpatient clinic and to study the feasibility of nitrous oxide (N_2O), midazolam and EMLA alone in children with endocrine disorders and obesity

Material and Methods:
Children with anxiety or previous difficulties connected with establishing IV access were included (n=140).
Fifty children were openly randomized to EMLA alone (n=25) or EMLA+ N_2O (n=25).
Ninety children (60 obese and 30 growth retarded) were randomized double-blinded to; midazolam, 0.3mg/kg, max 15 mg (n=30), 50% N_2O (n=30), and to 10% N_2O (n=30). All three groups also received EMLA. A subgroup of 20 anxious children undergoing repeatedly painful procedures was also included. These children underwent two procedures with EMLA or EMLA+N_2O, the order of priority being randomized.
Measurements: Number of attempts, defined both as the number required to succeed in setting up double IV lines and as a successful IV line procedure with two attempts for two iv lines vs > two attempts, *IV access time*, the time from the start of setting up the IV lines until two IV lines are established. *Recovery time*, the time from establishment of the IV lines until regained alertness. *Total procedure time,* IV access time plus recovery time. *Evaluation of the procedures;* children's, parents' and nurses' satisfaction with the IV line procedure (Liker Scale). *Pain*, evaluated by the child (VAS/NRS). *Sedation levels,* assessed using the Observer's Assessment of Alertness/Sedation Score.
Blood samples for stress hormones, insulin and glucose were obtained 0–1 min, 5–6 min, 14–15 min, and 29–30 min after achieving venous access and, if possible, after 24 hours.
Analyses were compared between treatments and between treatments over time.
Sixty children with unstressed samples and without any drugs served as controls.

Results:
On comparing all study children together with IV access problems, a significant difference in the number of attempts between the treatments groups was seen ($P<0.001$). The lowest number of attempts was obtained with 50% N_2O. The percentage of successfully IV line procedures was 70% using 50% N_2O and significantly lower with other treatments.
The children's evaluations were significantly more positive for 50% N_2O during IV access and painful procedures.
IV access with 50% N_2O was associated with a lower total procedure time ($P<0.001$).
An unfavourably long procedure time was observed in obese children after midazolam.
Significantly lower cortisol levels were detected with midazolam compared to both 50% and 10% N_2O and to unstressed controls. Glucose levels among growth retarded children increased from 0 to 30 min, whereas the opposite was found in obese children regardless of treatment. The growth hormone levels decreased with time in the midazolam group compared to 50% and 10% N_2O, where the effect of time was reversed.

Conclusion:
50% N_2O in combination with EMLA, was in all aspects superior to midazolam for the facilitation of IV access in distressed children. The IV access procedure was more efficient, with a shorter total procedure time and an increased number of successful IV lines.
Midazolam should only be used exceptionally in obese children due to the long recovery time.
Both treatment with N_2O and midazolam influence the results of hormone analyses with reference to both levels and trends in glucose and stress hormones.

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SAMMANFATTNING

Bakgrund och Syfte:
Smärta och rädsla vid etablering av intravenös infart (IV) och provtagning är välkända problem för många barn. En stressig och smärtsam procedur är inte acceptabel för vare sig barn, föräldrar eller personal. Vid utredningar för metabola och endokrina sjukdomar finns det även en risk att både barnets stress och de smärtstillande läkemedel, som används påverkar frisättningen av både hormoner, insulin och glukos. Syftet med denna avhandling var att studera hur man kan förbättra IV, provtagningar och smärtsamma procedurer hos barn som behandlas på en barnläkarmottagning. Effekterna av lustgas inhalation (N_2O), per olral midazolam och bedövningssalva EMLA studerades hos barn med fetma (obesitas) och hos barn med misstänkt endokrin sjukdom.
Material och Metod:
Rädda barn med tidigare problem i samband med IV eller provtagning inkluderades (n=140).
50 barn blev öppet randomiserade till IV provtagning med EMLA (n=25) eller med EMLA + N_2O (n=25).
90 barn (60 obesa och 30 kortvuxna) randomiserades dubbel-blint till; midazolam, 0.3mg/kg, max 15 mg, (n=30), 50% N_2O (n=30) och till 10% N_2O (n=30).
En grupp med 20 rädda barn, som behandlades regelbundet med smärtsamma injektioner inkluderades också. Barnen genomgick 2 behandlingar en med EMLA och en med EMLA+N_2O, ordningsföljden randomiserades.
Parametrar som studerades*: Antal stickförsök som krävdes för två IV;* definierat både som antal stick för 2 IV samt som andel ”lyckad IV procedur”, dvs två försök för två IV gentemot mer än två försök. *IV procedur tid;* tiden som krävdes för att etablera 2 IV. *Återhämtningstid*; tid från slutförd IV procedur till alert patient. *Total procedur tid;* IV tid + återhämtningstid*. Bedömning av IV proceduren* av barn, förälder och sjuksköterska. *Smärtskattning* av barnet. *Mätning* av allmänpåverkan av läkemedlet.
Blodprov för mätningar av stresshormoner, insulin och blodsocker vid 4 tillfällen under 30 minuter efter etablering av IV, 1; 0–1 min, 2; 5–6 min, 3;14–15 min, och 4; 29–30 min samt efter 24 timmar. Analyserna jämfördes mellan de olika behandlingarna, samt mot en ostressad kontrollgrupp, som inte fick läkemedel i samband med provtagning (40 obesa och 20 kortvuxna barn). Dessutom studerades trendutvecklingen över tid.
Resultat:
Det var en signifikant skillnad i antalet stickförsök som krävdes för IV mellan behandlingsgrupperna vid en jämförelse av samtliga barn ($P<0.001$) där N_2O var bättre än de andra behandlingsmetoderna. 70 % av IV procedurerna lyckades vid behandling med 50 % N_2O. Barnens bedömning av IV procedurer var signifikant bättre när 50 % N_2O användes. Den totala procedurtiden var betydligt kortare vid N_2O behandling ($P<0.001$). Speciellt bland de obesa barnen sågs en extrem lång total procedur tid efter behandling med midazolam.
Signifikant lägre cortisolnivåer påvisades vid behandling med midazolam jämfört med både 50 % and 10 % N_2O och ostressade kontrollbarn. Glukosnivåerna steg de första 30 minuterna hos de smala barnen jämfört med de obesa barnen där nivåerna sjönk.
Nivåerna av tillväxthormon minskade över tid bland barn som behandlades med midazolam jämfört med N_2O grupperna där effekten var den motsatta.
Konklusion:
Behandling med 50 % N_2O vid IV procedurer på stressade barn medförde en kortare total procedur tid, färre antal stickförsök för lyckad nålsättning, samt en bättre bedömning av proceduren av barnen, föräldrar och sköterskor jämfört med midazolam. Behandling med midazolam bör bara i undantagsfall ges till obesa barn, på grund av en extremt lång återhämtningsfas. Behandling med både N_2O och midazolam vid IV provtagning, påverkar analyserna av stress hormoner och glukos, vilket är viktigt att ta hänsyn till vid IV provtagning i samband med endokrina och metabola utredningar.

LIST OF PUBLICATIONS

I. **Ekbom K**. Jakobsson J. Marcus C
Nitrous Oxide Is a Safe Way to facilitate Procedures in Pediatric Outpatient Departments.
Arch Dis Child 2005 Oct; 90(10): 1073-6

II. **Ekbom K.** Kalman S. Jakobsson J. Marcus C
Efficient Intravenous Access Without Distress
A Double-Blind randomized Study of Midazolam and Nitrous Oxide in Children and Adolescents.
Archives of Pediatrics and Adolescent Medicine; Acceppted 2011 Feb

III. **Ekbom K.** Kalman S. Jakobsson J. Marcus C
Effects of midazolam and nitrous oxide on endocrine and metabolic measurments in children
Submitted

IV. **Ekbom K.** Lidman N. Anderson R. Marcus C. Jakobsson J
Health aspects among personnel working with nitrous oxide for procedural pain management in children.
Acta Anaesthesiologica Scand 2008 Apr; 52(4):573-4.

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ABBREVIATIONS

ASA	American Society for Anaesthesia classification of health
BMI	Body Mass Index
BP	Blood Pressure
CAH	Congenital Adrenal Hyperplasia
FTT	Finger Tapping Test
GR	Growth Retarded Children
HPA	Hypothalamic- Pituitary- Adrenal
HR	Heart Rate
IV	Intravenous
LS	Likert Scale
NDIR	Non Depressive Infrared Red
NRS	Numeric Rating Scale
N_2O	Nitrous Oxide
OASS	Observer's Assessment of Alertness/Sedation Scale
OBESE	Obese Children
ppm	Parts per million
SAT	Saturation
SDS	Standard Deviation Score
STEL	Short Term Exposure Levels
TWA	Time Weighted Average
VAS	Visual Analogue Scale
17-OHP	17-hydroxyprogesterone

1 INTRODUCTION

Alleviating pain and distress are important ethical issues in medicine and nursing practice [1]. Historically, in the middle of the 1960s surgical procedures on infants were performed without analgesia, on the assumption that infants were insensitive to pain, because the nervous system was underdeveloped [2]. Previous studies have also reported that children were given less analgesics than adults. It is not known whether the pain relief was considered adequate, because children were not asked about pain [3, 4]. Accumulating evidence during the last few decades has confirmed that pain is perceived early in life and that children's memories of painful experiences can shape their future reactions to painful procedures [5, 6]. There is still a risk, that children are undertreated for pain and distress because it is difficult to distinguish what the pain sensation actually is within the complex mixture of sensations of pain, stress, fear and constraint in children [7].There is also a risk that our limited knowledge about the pharmacokinetics and pharmacodynamics of the drugs given results in under treatment of the children [4].
However, there has been fundamental improvement during the last decade. Pain reduction in paediatric surgery and neonatal care usually is well functioning [8]. An area of concern is intravenous (IV) access in distressed children, as repeated IV access has been identified as one of the most stressful events in hospitalized children [9]. Also within this field, there have been improvements in topical anaesthesia and the development of subcutaneous ports, frequently used in children with chronical diseases. A subcutaneous intravenous access port is ideal when the need for IV access is intermittent for a period of at least 3 months [10]. Psychological support has also been shown to be effective in reducing pain and distress [11], and nowadays facilities for such support are found in all children's hospitals in Sweden. Several studies suggest that breathing exercises, child-directed distraction, nurse-led distraction, and combined cognitive-behavioural interventions are effective [12]. At paediatric hospitals, "lekoteks" i.e. rooms with toys used for children who need to process trauma and fear associated with painful procedures, play therapy and hospital clowns are routine features today to help and support distressed children.
The International Association for the Study of Pain (IASP) defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" and states that it is subjective and best assessed by the patient [13]. To handle all these assessments a widespread use of validated scales for self-reporting methods in children have been designed, the most frequently used ones being the Visual Analogue Scale (VAS) [14] and the Numeric Rating Scale (NRS) [15].

1.1 Procedural pain

A definition of pain for clinical practice, was given by Margo McCaffrey in 1968, who defined pain as *"whatever the experiencing person says it is, existing whenever he says it does."* [16].
Procedural pain is frequently the results of stimulation of nociceptors, an activation in the nervous system, which provide information about tissue damage.

As discussed above, procedures are often accompanied by anxiety, which is a complex combination of fear, apprehension and distress, characterized by somatic, emotional, cognitive, and behavioural components in turn associated with a higher level of pain [17-19]. Descriptions of the pain threshold or intensity of a stimulus causing a painful sensation it only differ slightly between individuals, but the tolerance of pain varies widely and is influenced by the duration of stimuli, age and emotional state and previous experiences [20].
Knowledge of and attention to children's anxiety have increased in recent years and meassuremnents of children's procedure-related anxiety and distress have improved with STAIC (Spielberg State-Trait Anxiety Inventory for Children) being the most frequently used measure. The Visual Analogue Anxiety Scale is another useful instrument for assessing intraoperative anxiety in children [21].
Painful and distressed procedures for children easily lead to conflicting situations, which are difficulty to handle by parents and caregivers. There is not only the physical pain itself that may cause the distressed situation, there are also the experiences surrounding the procedures [22]. Therefore during most situations, it is unacceptable to physically force a child to endure different procedures, such as IV access. It may be unavoidable in an acute situation, but not when children with chronic diseases are treated.
Major improvements have taken place at paediatric outpatient clinics. In Sweden, the use of anaesthetic cream has become routine in paediatric practice, and has significantly reduced the problems associated with IV access and painful injections for the majority of children. [9, 23, 24]. However, the alleviation of pain obtained with anaesthetic cream is sometimes insufficient and not effective enough for distressed children. Some children come repeatedly to the outpatient clinic to undergo different procedures such as blood tests, injections, implantations and to have subcutaneous ports taken care of. Consequently, there is a demand for additional pain relieving methods for children in whom technical difficulties and painful intravenous access can be expected, and for children who are treated on a regular basis with painful procedures and, finally for generally anxious children treated at paediatric outpatient clinics.

1.2 Intravenous access problems

Peripheral intravenous (IV) accesses are difficult to handle in children, because of both a lack of child co-operation by the child and the small size of the veins. Pain, anxiety, distress and difficulties related to IV access and tests are therefore recurrent problems, sometimes resulting in trauma for the children, and sometimes delayed and cancelled procedures [25, 26]. Observational studies and self-reported experiences of children undergoing IV access have demonstrated high levels of pain and distress with IV line insertions being the second most common cause of the "worst pain" experienced by hospitalized children [9, 27].

1.2.1 Technical difficulties

There are several technical difficulties in connection with IV access in distressed children, particlarly when a stressful situation results in a peripheral vasoconstriction [28]. In children with chronic diseases, the blood vessels often become sensitive to pain due to repeated needle

penetrations and the scars make the vessels stiff and difficult to penetrate [5, 29]. In obese children, the difficulty is due to the fact that the veins are hidden deep in the subcutaneous adipose tissue, which obscures their visibility and palpability [30].

1.2.2 Risks and consequences associated with disturbed endocrine and metabolic tests

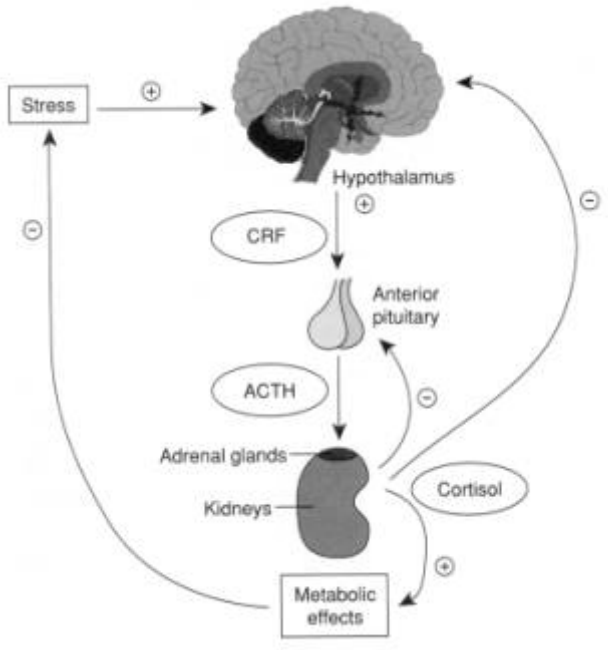
The problems related to IV access and painful procedure might thus lead to a vicious circle, with a stressed child, distressed parents and caregivers and delayed or even cancelled procedures. When started, this circle of reactions is hard to break and requires a lot of patience from all involved [31, 32] and a conflict will easily arise between the needs for speed, efficiency and adequate pain reduction.

1.2.3 Overview of secretion and functions of hormones studied in this thesis

The physiological response to stress involves an interaction of several endocrine systems, primarily the sympathetic system and the hypothalamic- pituitary- adrenal (HPA) axis. Norepinephrine and epinephrine are rapidly released by the sympathetic adrenomedullary system and the HPA axis is activated more slowly [28, 33].
The HPA- axis is a complex set of direct influences and feedback interactions in the hypothalamus, the pituitary gland and the adrenal glands. Corticotropin-releasing hormone (CRH) regulates the secretion of adrenocorticotropic hormone ACTH, thereby stimulating the adrenal cortices to rapidly stimulates the biosynthesis of corticosteroids, such as cortisol, from cholesterol [28]: Figure 1.

Figure 1

Hypothalamic -Pituitary -Adrenal axis.



Cortisol, which increases during stressful situations and stimulates, among other things, gluconeogenesis [33].
A hyperglycaemic response to stress is mediated mainly by catecholamines and cortisol and partly by growth hormone [34]. In addition, insulin, which is the only serum glucose lowering hormone, is suppressed by activation of the sympathetic nervous system, which also may increase the glucolytic response to stress [35, 36].
The catecholamines**,** norepinephrine and epinephrine, are the neuroadrenergic mediators for the induction of the stress response. Norepinephrine is the transmitter substance of the peripheral sympathetic nervous system and is present in the blood, being released from the synapses of the sympathetic system [28]. Epinephrine is secreted from the adrenals. The plasma concentration of norephinephrine is usually much higher than that of epinephrine.
Main indications for analyses of cortisol are a diagnosis of adrenal insufficiency with hypo- or hyper production. Cortisol is also used as an outcome measure to evaluate the effectiveness of pain treatments [37-41].
Normally, cortisol is released by about 15 or more pulsatile bursts in a 24-hour period in children and adults and can be measured in the urine, plasma and saliva [41, 42]. Cortisol levels peak about 30 minutes after awakening for the day, with a 50%–100% increase in levels and reach the lowest point around midnight [33]. In plasma, cortisol is present in free and bound quantities, with at least 90% of it being bound to plasma protein and less than 10% is unbound and biologically active [41].
Insulin is synthesized in the pancreas within the β-cells of the islets of Langerhans. It controls the level of glucose, and for most cells, glucose uptake is insulin-dependent. Exceptions are brain cells and working muscle cells. The ability of the muscle cells and liver and, to a minor extent, fat cells to remove blood glucose is defined as glucose tolerance [43].
Common indications for analyses of insulin and glucose are for the diagnosis of diabetes types 1 and 2. Glucose tolerant tests measure insulin sensitivity and resistance. Insulin sensitivity is defined as the degree to which the body responds to a particular dose of insulin by lowering blood glucose levels. Insulin resistance is defined as a condition when the cells no longer respond well to insulin, resulting in an increased secretion of insulin to reduce blood glucose levels. Insulin resistance is strongly associated with hypertension, elevated triglycerides and low levels of high-density lipoprotein cholesterol, the cardiovascular risk factors of obesity [44].
Growth hormone (GH) stimulates growth, cell reproduction and regeneration. Indications for GH analysis are disorders involving the GH pathway resulting in insulin-like growth factor-I (IGF- I).
Normally, GH is secreted in surges that occur at 3 to 5-hour intervals. The largest and most predictable of these GH peaks occurs about an hour after the onset of sleep [45]. GH secretion is pulsatile throughout the day, with the basal concentration being low. The methods for GH testing therefore include provocative stimulations tests and physiologigal testing with serial sampling. GH is a refractory hormone, i.e., the capacity to secrete a new burst of GH is blunted hours after a series of previous bursts, so there is a risk that a stressfull IV access may affect the results [46].

1.2.4 Situations with a risk for incorrect results of endocrine and metabolic testing in relation to IV access factors

A painful and stressful IV access may affect the release of hormones and the metabolic response.
A new problematic area is childhood obesity. Childhood overweight and obesity are increasing in all western countries with an increased recognition of the metabolic syndrome in children [47]. Type 2 diabetes mellitus is also increasing in younger population, probably due to an increased prevalence of childhood obesity associated with increased insulin resistance [48, 49].
In addition, it is important to identify the obesity-related risk factors early on and therefore there is an increased need for blood sampling and tests to treat and prevent potential complications [50].
For obese children there is a risk that the stressful blood sampling will result in a false high fasting glucose level, which may be misleading. For children in whom hyper-cortisolism is suspected, the stress-induced condition might prompt further investigations, which would otherwise be unnecessary. Stress also increases the release of growth hormone (GH). For children of short stature this might increase the risk of an incorrect diagnosis of growth hormone deficiency since GH secretion is depressed after a previous release of GH [43]. Thus, if GH is released during the IV line insertion the following testing might be inaccurate.
Finally, in children with congenital adrenal hyperplasia (CAH) stress might increase the levels of 17-hydroxyprogesterone (17-OHP), which is the marker of therapeutic efficiency [51].
In addition, drugs used to reduce stress and pain may also affect hormone release. What is known hitherto about drugs used in the present studies is outlined below,
(5.3. Effects of treatments on stress hormones, insulin and glucose levels).

1.3 Treatments used to facilitate intravenous access

1.3.1 Non-pharmacological treatments

Cognitive behavioural therapy is the predominant non-pharmacological strategy used to counteract procedure-related pain in children and adolescents.
Typical components include distraction techniques, breathing exercises and imagery, and others coping skills [52].
Freeze sprays (vapocoolants) evaporate from the skin and are cooling to the point of freezing. The efficacy of vapocoolants is generally considered to be effective and immediate [53], but not always effective for reducing pain associated with IV access [54].

1.3.2 Pharmacological treatments

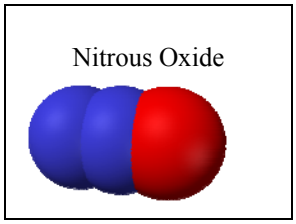
An ideal agent for children should be easy to administer, have a rapid onset and offset, produce no residual symptoms, have minimal side effects, and should be cost-effective.

Pharmacological options for children are available in different forms classified as invasive or non-invasive drugs. The methods of administration may cause different difficulties when using an invasive or a non-invasive drug in the treatment of distressed children.
EMLA, midazolam and nitrous oxide are the intervention treatments used in this thesis (Studies I-III).

Topical anaesthesia
EMLA® cream (Lidokain Prilokain 25mg/g Astra Zeneca, Södertälje, Sweden), the most frequently used anaesthetic cream in Scandinavia, contains two dermal anaesthetics lidocaine and prilocaine. EMLA requires a minimum of 60 minutes to produce a full anlgaesic effect to a deepth of 3 mm and anaesthesia persists for 1 hour [55].
EMLA cream serves as a topical anaesthetic by penetrting the dermal and epidermal layers of the skin into the pain receptors, by blocking them and blocking the ionic fluxes, which are formed and transported through these nerve endings. EMLA may cause local blanching of the skin, followed by redness attributed to vasoconstriction. The side effects associated with EMLA cream and patches are generally mild and transient [56].
Awerness of the risk of an increased methaemoglobin concentration has limited the use of EMLA in newborns, but the risk is minimal if EMLA is used correctly and administrated in adequate doses [57].
Ametop ® is a gel (4% Amethocaine Tetracaine, Smith & Nephew Healthcare Ltd., England) [58]. It takes a minimum of 30 minutes for Ametop to produce an analgesic effect, and anaesthesia persists for 4–6 hours. Studies examining the efficacy of Ametrop have produced mixed results [59].
Ametop was not tested in this thesis.
Rapydan ® patch (Lidocain 70mg Tetracaine 70mg, Eurocept International, Ankeveen, The Netherlands) combines an analgaesic gel with a "Controlled Heat-Assisted Drug Delivery" (CHADD) pod to heat the skin. The Rapydan patch should be applied to the skin for 20 to 30 minutes to provide an analgaesic effect [60]. Rapydan has not been sufficiently tested in children and was not tested in the present thesis.

Injection
Injections of buffered lidocaine, a local anaesthetic consisting of 1 part sodium bicarbonate with 10 parts of 1% lidocaine are used to reduce pain associated with IV access [61]. The pain associated with anaesthetic infiltration is reduced by buffering the pH to 7.4 [62]. This method was not tested in the thesis. It might be considered a non-optimal way to reduce pain associated with injections by using another injection.

Inhalation analgaesics

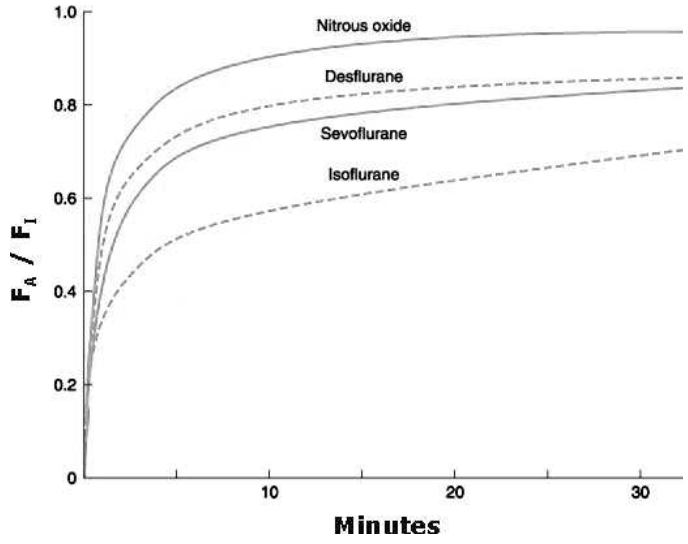


Nitrous oxide (N_2O) is an oxide of nitrogen and a compressed liquefied, colourless non-flammable gas, with a slightly sweet odour and taste. During the last few years N_2O and an oxygen mixture have gained a renewed interest. Administration of N_2O is simple and painless, it has a rapid onset and a short duration of action, and its effects are analgaesic, anxiolytic and sedative [63, 64].

N_2O has the highest relative onset of effect compared with other anaesthetic agents. Depending on the gas tensions throughout the body tissues equilibrate, the inspired gas tension (FI) will equal that in the alveoli (FA): Figure 2.
N_2O is the only inhaled anaesthetic that possesses analgesic properties in a subanaesthetic concentration [65].

Figure 2

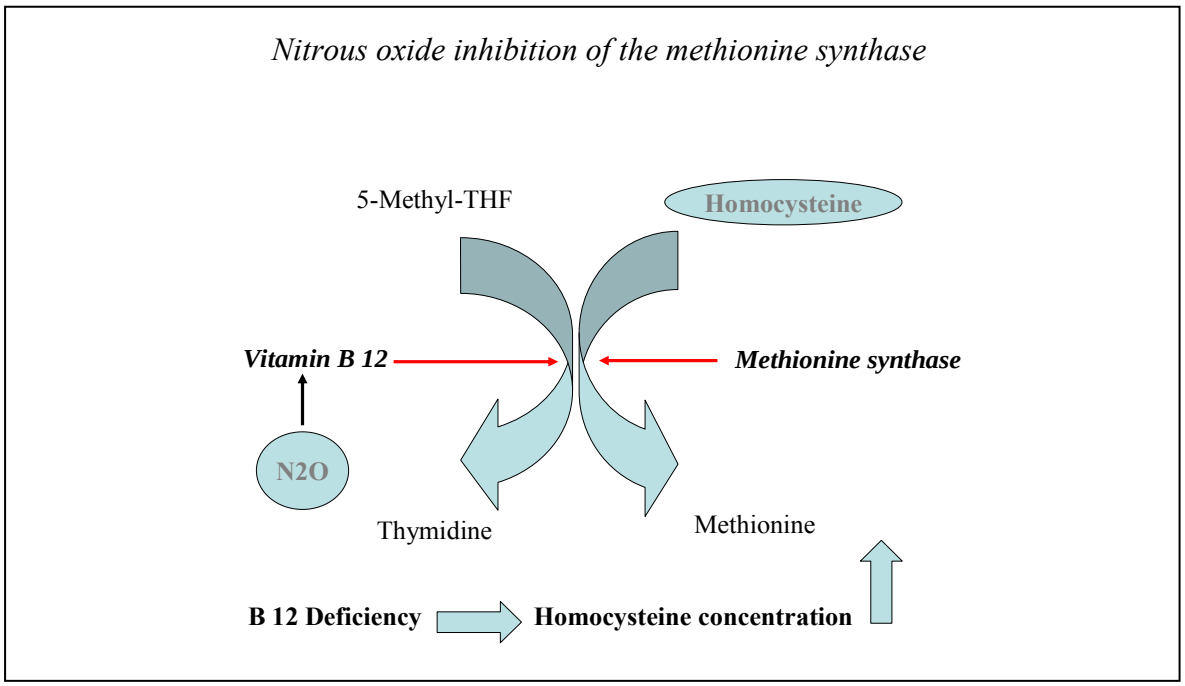
Comparisons of F_A (alveoli gas tension) / F_I (inspired gas tension) between N_2O and other anaesthetic agents [66].



N_2O primary effects are exerted on the central nervous system and the analgesic action is dependent on the inhibition of supraspinal GABA receptors and the activation of spinal GABA receptors [67]. The analgesic effects of N_2O consist of an interaction between the endogenous opioid system and the descending noradrenergic system. It seems that N_2O-induced release of endogenous opioids causes disinhibition of brain stem noradrenergic neurons, which release norepinephrine into the spinal cord and inhibit pain signalling [67]. Exactly how N_2O causes the release of endogenous opioid peptides is not fully known. However, it has been shown to directly modulate a broad range of ligand-gated ion channels, and this probably plays a major role in many of its effects. The euphoric effect of N_2O is induced by dopamine release and the activating of dopaminergic neurons [68].

It is well known that N_2O has a weak emetic effect [64] and that N_2O causes a dose-dependent inhibition of methionine synthase [69]. N_2O irreversibly oxidizes the cobalt atom of vitamin B12 and thereby reduces the activity of B12-dependent enzymes such as methionine and thymidine/ DNA synthesis: Figure 3.

Figure 3



Orally and rectally administered analgesics
Treatments commonly used at paediatric outpatient clinics: Table 1.

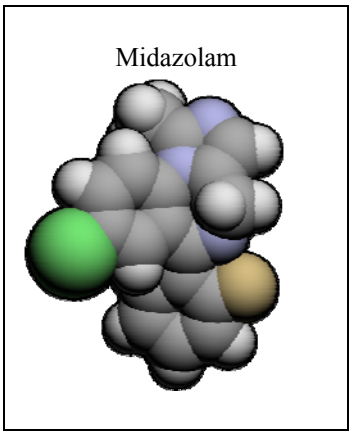
Table 1

Treatment commonly used for sedation, analgesia and at paediatric outpatient clinics.

	Midazolam	**Catapresan**	**Paracetamol**	**NSAID** Non-steroidal Anti-inflammatory drugs
Sedation	x	x		
Analgesia	(x)	x	x	x
Used at paediatric outpatient clinics	x		x	

[70-72].

Midazolam, a benzodiazepine, belongs to the group of central nervous system depressants and has become more popular than other benzodiazepines because it has a shorter half-life and is more potent [71, 73]. Rectal administration has been used primarily, but midazolam is also available as midazolam HCI syrup for oral administration and has been widely used in recent years with demonstrated good clinical efficacy and low toxicity [74]. The main effects of benzodiazepines are sedation, hypnosis, anxiolysis, anterograde and retrogade amnesia, centrally mediated muscle relaxation and anti-convulsant activity [75, 76].



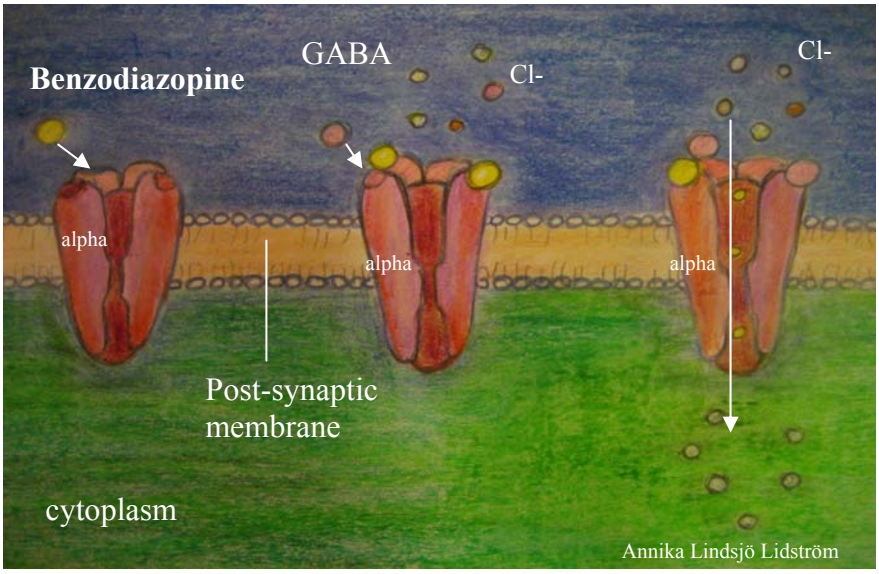
Although midazolam has been proved to be effective, the inter-individual variation of effects and the elimination are high and dose-dependent [30]. The anterograde and retrograde amnesia after midazolam is frequently cited as an advantage of midazoalm [73, 77]. This has been questioned because the amnesic effect mainly affects explicit memory but leaves implicit memory intact [76].

A well-known side effect is post procedure agitation. It occurs in 17% of paediatric patients pre-medicated with midazolam (0.5 mg/kg) and negative behaviour changes have also been reported up to a week after adminstration [78].

The drug exerts its clinical effect by binding to a receptor complex which facilitates the action of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Practically all effects of the benzodiazepines result from their actions on the ionotropic GABA(A) receptors. Benzodiazepines do not activate GABA(A) receptors directly but they require GABA [73, 79] : Figure 4.

Figure 4

GABA A receptors



1.4 Environmental aspects

The possible impact of pharmaceutical substances on the environment is a subject that attracts increasing attention. The bioavailability is rather low for oral midazolam, with a large reported variability [75] and the elimination after oral administration is unclear and little studied [80].
Nitrous oxide (N_2O) has the potential to produce negative health effects if there is chronic workplace exposure to higher concentrations of N_2O [81-83]. Most countries have clear recommendations concerning work place ambient air quality in the form of Time Weight Average(TWA) limits and Short Term Exposure Levels (STEL) [84, 85]. Treatment with N_2O should be administered in rooms with proper ventilation and scavenging equipment in order to comply with national air quality guidelines [83].

There is also an ongoing debate regarding the extent to which inhaled anaesthetics contribute to global climate changes by interfering with the ozone layer and/or acting as green house gases [86]. Most hospitals have, or are planning to have, scavenging systems to reduce the emissions [86, 87]. However, less than 1% of the N_2O in the atmosphere is produced by the use of anaesthetics use, as most of it is produced in agriculture and the burning of fossil fuels [86, 87].

2 AIMS OF THE THESIS

General aims
The overall aims were to study different ways to facilitate painful procedures and IV access in children at a paediatric outpatient clinic and to study the effects of drugs on stress response and hormone release after IV access.

Specific aims were:

- To evaluate wether nitrous oxide, in additional to EMLA, is an effective and feasible pain-relieving treatment (Study I)
- To compare effects of nitrous oxide and midazolam treatment on therapeutic efficiency, success rates and evaluations by children, parents and staff (Study II)
- To study the effects of nitrous oxide and midazolam treatment on endocrine and metabolic measurements in distressed children (Study III)
- To study the effects of nitrous oxide sedation on the working environment in a paediatric outpatient clinic (Study IV)

3 MATERIAL AND METHODS

3.1 Participants and designs

Clinical characteristics of study participants in Studies I-III; Table 2 a and 2 b.
Study designs in Studies I-IV; Table 3.
All children and adolescents were registered at the DEMO (Diabetic, Endocrine, Metabolic, Obesity) clinic at Astrid Lindgrens Children's Hospital, Karolinska University Hospital, Huddinge, Stockholm, Sweden.
The informed written consent of the parents was obtained and the children included gave their verbal consent. The IV procedures were cancelled when the child refused to co-operate.

Table 2 a

Participants in Studies I-III.

	Participants n	Girls n (%)	Boys n (%)	Age. years range	BMI SDS
Study I a					
OBESE	50	23 (46)	27 (54)	13 (6-18)	5.8 (-0.6-10.6)
Study I b					
Injection	20	16 (80)	4 (20)	11 (6-17)	
Study II					
Total	90	39 (43)	51 (57)	12 (5-18)	4.9 (-2.4-8.9)
OBESE	60	27 (45)	33 (55)	14 (8-18)	5.6 (3.4-9.0)
GR	30	12 (40)	18 (60)	8 (5-17)	0.0 (-2.4-3.6)
Study III					
Control	60	24 (40)	36 (60)	11 (4-18)	4.2 (-2.3-9.0)

Children in Study Ia, II and III had two IV lines and children in Study Ib recieved injections.

Table 2 b

Distribution of children with IV difficulties and IV anxiety in Study II.

	Total	**50% N_20**	**10% N_20**	**Midazoalm**
All children	90	30	30	30
Children with IV difficulties	80	28	26	26
No difficulties	10	2	4	4
Children with IV anxiety	82	28	27	27
No anxiety	8	2	3	3

Design

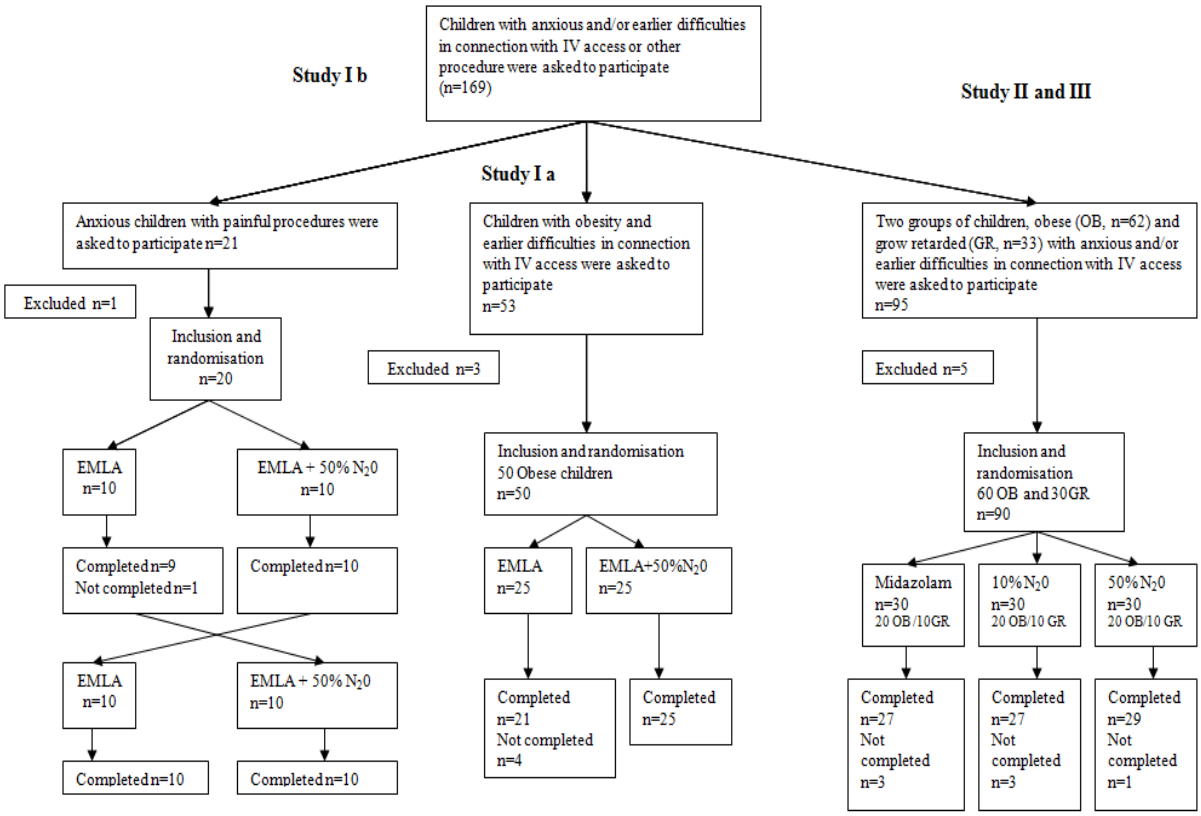
Table 3

Design Studies I – IV.

Study	I a, b	II	III	IV
	Prospective randomized controlled and, in one part, cross-over study	Prospective double-blind randomized controlled study	Prospective double-blind randomized controlled study	Open explorative study

3.2 Flowchart

Figure 5



3.3 Inclusion and exclusion

There were 3 inclusion criteria that patients had to meet to for enrollment in Studies I, II and, consequently in Study III.
(1) IV or injections problems. Patients should have had previous difficulties in connection with establishing IV access, defined as either a previous requirement of several attempts before establishing IV access or as the children rating anticipatory anxiety greater than 4 using a numeric rating scale (0-10) [88] or expressed anxiety about repeated painful injections.
(2) No physiological problems. Patients had to have an American Society for Anaesthesia classification system score of 1 (i.e. a normal healthy child with no physiological disturbances) [13].
(3) Co-operability. Patients had to have the ability to understand and contribute to the different treatments, including using a face mask and the ability to interpret the Numeric Rating Scale (0-10) and the Likert Scale (1-5).
All children were instructed how to handle a face mask and they were asked to use the scales to ensure that they understood the scales before inclusion. Patients were excluded if they (1) did not have previous difficulties in connection with the establishing of IV access and/or had an anticipatory anxiety rating of 4 or less on the Numeric Rating Scale ; (2) had an American Society for Anesthesia classification system status of 2 or greater; and (3) were considered unable to collaborate.

3.4 Intervention and randomization

All studied children received EMLA one hour before IV access and the injection.
EMLA alone is treatment with only EMLA cream or patch.
In Study I a, 50 patients were randomized to EMLA alone (n=25) or nitrous oxide treatment (N_2O) and EMLA.
In Study I b, 20 anxious children undergoing repeateded painful procedures participated. These children underwent two procedures with either/or EMLA/N_2O and EMLA alone, the order of priority being randomized.
For Studies II and III, 90 children were randomly assigned to:
midazolam (0.3 mg/kg, max 15 mg), n = 30, 50% N_2O, n = 30, and to 10% N_2O, n = 30.
20 OBESE and 10 GR (growth-retarded) children were stratified into each treatment group, with body composition providing different IV access problems.
The randomizations were performed using the envelope technique in Studies I–III.
In Study II, a special nurse (N2) performed all the randomization, prepared and administered the midazolam mixture in a separate room with no other staff present.
N2 also set the mixing percentage of nitrous oxide and oxygen according to the randomization and concealed the mixing device. Thereafter, N2 was not further involved in or present during the entire procedure, including the recovery phase.

3.5 Anaesthesia equipment

In Study I, the equipment included an anaesthetic block (Dräger RCD DS3, Lübeck, Germany) with separate rotameters for oxygen/ N_2O /air and a regulator, a fail-safe system which shuts off the N_2O if there is an oxygen pressure decrease. In Study II, oxygen and nitrous oxide were mixed using the Engstrom 2024® device (Engstrom Medical AB, Stockholm, Sweden) connected to 2.5-litre N_2O tubes and oxygen from the wall.
Both systems incorporated a full facemask that covered the nose and mouth and was held in place by the patients, if necessary, assisted by the parents. The gas mixture was delivered by free flow and no on-demand valve was used. It was delivered by a partial rebreathing system, a Bain's circuit. Exhaled N_2O was scavenged by a "double mask" (MEDICVENT® , Umeå, Sweden) system and the scavenging system was attached to the facemask [89].

3.6 Procedure

The children required two peripheral IV lines in preparation for intravenous tests (Studies Ia and II). These children received applications of EMLA at four different locations: on the dorsum of both hands and over the cubital vein on both arms and with subsequent application of adhesive tape during 60 minutes before the procedure (Studies Ia and II). All children in Study II were also given 15 ml of syrup +/- midazolam 40 minutes before the IV line procedure. Midazolam was dosed according to the standard procedure at the clinic i.e. 0.3 mg/kg with a maximum of 15 mg.
In Study I b the children were subjected to two procedures, one with EMLA alone and one with N_2O and EMLA. These children received applications of EMLA at one location 60 minutes before the procedure. The order was randomized. All procedures were performed by the same nurse.
In order to diminish the risk of nausea/vomiting [90], the children were not given any solid food within 4 hours and no liquid within 2 hours preceding the treatment. Altogether study I included 90 procedures.
Oxygen/N_2O was administered 3–5 minutes before the IV line procedure or painful procedure was started [63]. All children breathed into the mask during the time required for the procedures. When the IV access/procedure was finished, the N_2O valve was closed and an additional 3 minutes were allowed for N_2O washout with the child breathing 100% oxygen.
In Study I, a nurse specialized in anaesthesia performed all the N_2O treatments. The concentration was increased in gradual stages to facilitate the co-operation and participation of the child, starting with 2 L N_2O/6 L O_2 (8 L / min fresh gas flow) for 2 minutes, thereafter increasing to 3 L N_2O/5 L O_2 for 2 minutes, and 4 L N_2O/4 L O_2 for 1 minute and then the procedure was performed. Altogether the time required for introduction and emergency administration of N_2O was 8 minutes. The time required to achieve an adequate level of sedation/analgesia was 5 minutes. The anaesthesia nurse established 30 of the IV lines (15 EMLA/15 N_2O) and a general nurse established 20 IV lines (10 EMLA/10 N_2O) using a 22 G catheter.
In Study II, three nurses (N1, N2, N3) were involved with each study patient. N1 and N2 were not exchanged during the study. N1 informed about the study and admitted the patients and

wrote the records. N2 performed the randomization, prepared and administered the midazolam mixture. N3 commenced when the child started to breathe into the mask, setting up two IV lines using a 22 G catheter and evaluated the IV line procedure. The role of N3 involved three different nurses, all with long paediatric experience.
Children who satisfied the inclusion criteria were asked consecutively if they wanted to participate. Nine children did not choose to participate (I a 3: I b 1: II 5). These patients received conventional treatment (EMLA alone). In 3 cases, the procedures were cancelled.

In Study III, blood samples were drawn during 30 minutes at four time points after achieving venous access and, if possible, after 24 hours. The 24-hour sample was regarded as resulting from an unstressed IV access. Analyses were compared between treatments and treatments over time. Stress factors, indicated as children's evaluations of pain and the procedure, were correlated with mean values after IV access. Sixty children aged 4–18 (40 OBESE and 20 GR), who underwent 24- hour blood sampling, served as controls.

3.7 Study IV

The Time Weighed Average (TWA) for an 8-hour working day (n=43) and the Short Term Exposure Levels (STEL) mean concentration for a 15-minute period (n=12), were measured during routine use of nitrous oxide for procedural pain management in children.
Blood samples taken by two nurses, before and after an interval of at least 3 weeks, were analysed for homocysteine, haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration on two occasions.

3.8 Measurements

Timetable showing when the different variables were recorded: Table 5 (page 20).

3.8.1 Attempts

The number of attempts was defined both as the number required to succeed in setting up double IV lines (Studies I and II), and as a successful IV line procedure with 2 attempts for two IV lines vs >2 attempts (Study II).

3.8.2 Procedure time

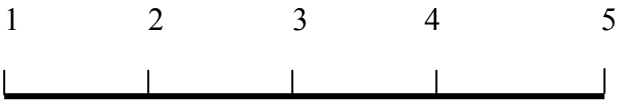
The procedure time was defined as the time required for the IV procedure along with the N_2O procedure (Study I). When comparing midazolam, 50% and 10% N_2O the total procedure time was defined as IV access time plus recovery time (Study II). IV access time was defined as time from the start of setting up the IV lines until two IV lines were established, and recovery time was defined as the time from the establishment of the IV lines until regained alertness. The finger tapping test (FTT) was used as a means to measure alertness and test for

recovery and psychometric function. Recovery was defined as FTT within 10% of the child's baseline value [91]. In the FTT children are instructed to tap a button as fast as they can during a 10-second interval. The baseline number was measured in connection with inclusion in the study. Attempts to measure recovery were made every 15 minutes after establishing IV line access.

3.8.3 Evaluations of the procedure

A Numeric Rating Scale (0–10) was used to measure the children's anticipatory anxiety within the procedure with 0 = no anxiety and 10 = worst possible anxiety.
The Likert scale (1–5), with verbal categorical response options and frequently used for evaluations of different procedures, was used in Studies I and II by the children and parents [92]. The nurses' evaluations of the procedure were assessed by using a 3-point scale (Study I) and by a Likert scale (Study II).

Likert Scale: 1, poor; 2, fair; 3, good; 4, very good; 5.



3-point Scale:
1, procedure without complications; 2, the procedure was performed with difficulties because the child was protesting and found it difficult to remain lying down; 3, the procedure could not be performed.

The children performed the evaluation before the parents and the parents were present when the children made their assessment.

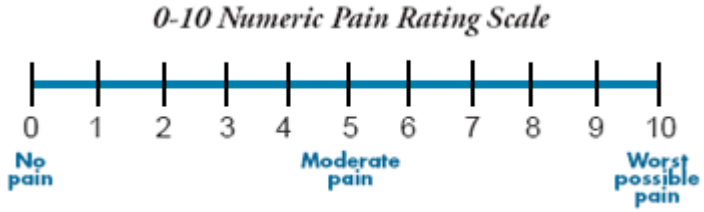
The evaluations were done 5 minutes after performing the procedure or 5 minutes after achieving treatment with N_2O in Study I, and in Study II the evaluations were done after 15 minutes after establishing IV access. The nurses' evaluations were made independently of the children's and parents evaluations and were performed by the nurse who established the IV access. In Study I b the children were followed up at the next visit to the clinic and they were asked which method they would prefer next time.

3.8.4 Pain

The measurement of pain included verbal and numerical self-rating scales [93].
Pain was evaluated by the children using a visual analogue scale (VAS) with a range of 0–10 (Study I) and a numeric rating scale (NRS) of 0–10 (Study II) [15, 94].

Visual analogue scale

Numeric rating scale



3.8.5 Sedation levels

Sedation was estimated in Study II by a research nurse using the Observer's Assessment of Alertness/Sedation Score (OAA/S) of 0–5 [95]: Table 4 .

Table 4

Responsiveness	Score
Responds readily to name spoken in normal tone (Alert)	5
Lethargic response to name spoken in normal tone	4
Responds only after name is called loudly or repeatedly	3
Responds only after mild prodding or shaking	2
Responds only after squeezing the trapezius muscle	1
Does not respond after squeezing the trapezius muscle	0

3.8.6 Body Mass Index and Standard Deviations Scores

WHO (the World Health Organization) defines overweight in adults as a BMI (Body Mass Index) ≥ 25 kg/m^2 and obesity as ≥ 30 kg/m^2. When defining overweight and obesity in children, BMI values are often given as (SDS) [96]. The International Obesity Task Force has published gender and age-specific cut-offs for children and adolescents aged 2–18 years, relating BMI in childhood to BMI in adulthood [97].

3.8.7 Analyses of stress hormones, insulin and glucose

Blood samples were obtained at 4 time points during the first 30 minutes after achieving venous access: 1 (0–1 min), 2 (5–6 min), 3 (14–15 min), and 4(29–30 min) and when possible, after 24 h. This was done to obtain an unstressed blood sample. The stressed sampling in connection with establishing IV access was described by the mean of samples 1–4 (mean sample).

Outcome measurements
Outcome analyses compared treatment effects at different time points and the effects of treatment over time (trends) in all children: n = 83 (seven IV access procedures were not completed). Subgroup analyses compared Obese/GR children, regardless of treatment, between treatment effects at different time points, and treatment trends n=56/27. Mean samples (4 samples after IV access) were compared with 24-hour samples, n = 32. The 24-hour samples were compared with those of a control group of children in whom IV access was not disturbed by pharmacological interventions, IV access difficulties, or anxiety. The control group comprised 60 children (40 Obese and 20 GR) previously tested at our department. The controls were selected consecutively during 1999–2000. All samples used as controls had been collected 10–12 hours after obtaining IV access and between 9 a.m and 10 a.m.
Samples for P-glucose (mmol/L), P-epinephrine and P-norepinephrine (nmol/L) were analysed directly in the Karolinska University Hospital Laboratory. Samples for S-cortisol (nmol/L), P-insulin (pmol/L), and P-growth hormone (microg/L) were centrifuged immediately and stored at -80° C until assayed.

Analysis methods
Glucose in plasma was measured using Modular Analytics P (Roche Diagnostics GmbH, Mannheim, Germany) and epinephrine/norepinephrine in plasma was determined using the HPLC (High Pressure Liquid Chromatography) method (Dionex Corporation. Sunnyvale, CA, USA). The amount of GH in the plasma was determined using Luminex technology in a Milliplex Human Pitutary Immunoassay (Millipore Corporation, 290 Concord Road, Billerica, MA 01821, USA) and of insulin using Milliplex Human Endocrine Immunoassay (Millipore Corporation, 290 Concord Road, Billerica, MA 01821, USA). Cortisol in serum was measured using a commercially available enzyme immunoassay (ELISA kit Labor Diagnostika Nord GmbH & Co, KG. Am Eichenhain 1, 48531 Nordhorn, Germany). Control group samples of S-cortisol were measured in the laboratory of Karolinska University Hospital using AutoDelfia Modular E170 (Roche Diagnostic GmbH, Mannheim, Germany).

S-cortisol analyses were compared between study patients using an enzyme immunoassay (ELISA) kit and a control group using AutoDelfia. We have investigated the source of evidence for method comparisons with study and control samples when both methods are based on antibody techniques.

3.8.8 Safety parameters

Heart rate (HR) and oxygen saturation *(*SAT*)* (Date – Ohmeda TUFF SAT®, New York, NY, USA) were followed throughout the procedure. Hypoxia was defined as a saturation of < 93%. Blood pressure (BP) was followed throughout the procedure (NAIS – Blood Pressure Watch Diagnostic®, Düsseldorf, Germany). Hypotension was defined as an alteration of more than 15% from baseline pressure [98]. Side effects, reported spontaneously or observed, were recorded. Procedure cancellations where counted and recorded.

3.8.9 Environment

The Time Weighted Average (TWA) work place nitrous oxide concentration, during an 8-hour working day was measured using nitrous oxide diffusion samplers supplied by Dräger Safety AG & Co., KGaA, Lubeck, Germany. When exposed to ambient air containing nitrous oxide it is adsorbed by a molecular sieve phase in the sampler. The nitrous oxide is later desorbed and analysed by infrared spectrometry at the sampler supplier's laboratory and a TWA value is calculated.

The sampler was attached to the nurse's working dress collar.

A non-depressive infrared red (NDIR) spectrometer was used to determine the Short Term Exposure Levels (STELs) of nitrous oxide. The instrument type was Rosemount Analytical, NGA 2000, MLT 4, (Emerson Process Management, Health Place, West Sussex, UK). The measuring procedure is sensitive to pressure variations and therefore the analyser is equipped with a pressure sensor. The concentration values are corrected to reflect the barometric pressure. The analyser is connected to a pump for sampling of air via a hose. The equipment also contains a constant flow controller (pressure regulator), a flow meter, and a data logger. During instrument qualification the deviation between the calibration gas value and the measured value was less than 1%, relatively. The sampling hose was attached to the anaesthetic machine at the same level as the face of the attending nurse.

Table 5

Timetable showing when the different variables were recorded.
Time in relation to IV procedure in minutes in Studies I, II and III.

Time, min	-40	0	IV Procedure	+5	+15	+30	
Attempts				I	II		
IV time			I + II				
Finger tapping	II				II	II	->
Recovery time					II	II	->
Total procedure time					II	II	->
Saturation	II	I+II	I +II	I+II	II	II	->
Blood pressure	II	I+II	I+II	I+II	II	II	->
Heart rate	II	I+II	I+II	I+II	II	II	
Sedation	II		II	II	II	II	->
Evaluations				I	II		
Blood samples				III	III	III	->

3.9 Statistical methods

The statistical methods used in this thesis are presented in Table 6.
In Study I, the groups were compared by means of Mann-Whitney test. For comparisons of paired data the Wilcoxon test was used in the second part of the study. All statistical analyses were performed using SPSS for Windows software.
In Study II, the treatments were compared using non parametric statistics comparing independent samples Kruskal-Wallis ANOVA by Rank and Pearson Chi-square. For Post hoc analyses pair wise comparisons were done using the Mann-Whitney U test.
All analyses were performed on the population intended to treat.

Body Mass Index (BMI) was calculated as body weight in kilogram divided by height in meters squared (Kg x m^{-2}) and BMI SDS was calculated according to Rolland-Cachera [99].
In Study III, the parameters were assessed using repeated-measures ANOVA, breakdown one-way ANOVA, t-test for dependent samples and t-test for independent samples. In case of statistical significance ($P < 0.05$), a post hoc analysis was performed using Scheffe´s test. Correlations were assessed using Spearman's rank order correlation.
In Study II and III all statistical analyses were performed using Statistica®, release 8. Statsoft Inc.,Tulsa, OK, USA.

Table 6

Statistical methods used in Studies I–IV.

	Study I	Study II	Study III	Study IV
Descriptive statistics	x	x	x	x
Mann-Whitney U test	x	x		
Wilcoxon test	x			
Kruskal-Wallis ANOVA by rank		x		
Pearson Chi-square	x	x		
General ANOVA			x	
Scheffe's test			x	
T-test for dependent samples		x		
T-test for independent samples		x		
Spearman correlation		x	x	

3.10 Ethic approval

All four studies were approved by the Ethical Committee of the Karolinska University Hospital or by the Regional Ethical Review Board in Stockholm, Dnr 481/01, Dnr 050104 komplettering Dnr 2009/1299-32 and Dnr 2007/944-31/4.

4 RESULTS

The results of Studies Ia and II are presented and summarized in Tables 7 (page 29) and Table 8 (page 31).
The results of Study Ib are presented in Table 9 (page 33).
The results of Study III are presented in Table 10 (page 43).

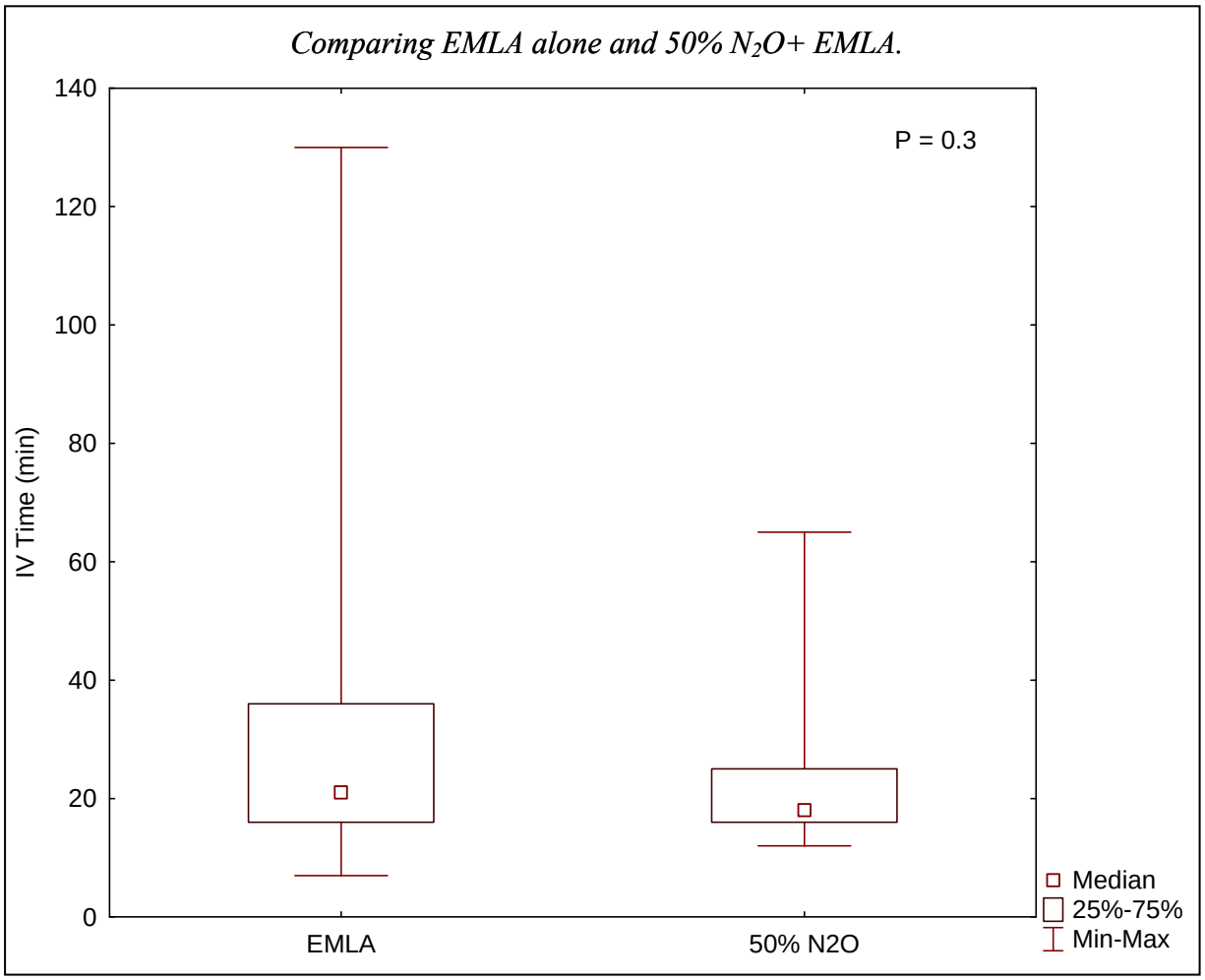
4.1 Number of attempts and success rate for IV access

The number of attempts required to establish double IV lines in children with previous difficulties connected with establishing IV access was significantly lower in the group receiving EMLA + 50% N_2O compared to EMLA alone (P = 0.001) with a higher percentage of successfully IV line procedures (84% and 40% respectively, P=0. 001) [26].
On comparing midazolam, 50% N_2O and 10 % N_2O, in OBESE and GR children, the percentage of successful IV line procedures was significantly higher in those treated with 50% N_2O compared with 10% N_2O and midazolam (67%, 40 %, and 37% respectively, P = 0.04). There was no significant difference in the total number of attempts required for IV access between the treatment arms (P = 0.09). When OBESE and GR groups were analysed separately, a significant difference was seen in the GR group (P = 0.02) with a lower total number of attempts in the 50% N_2O group.

4.2 Procedure time for IV access

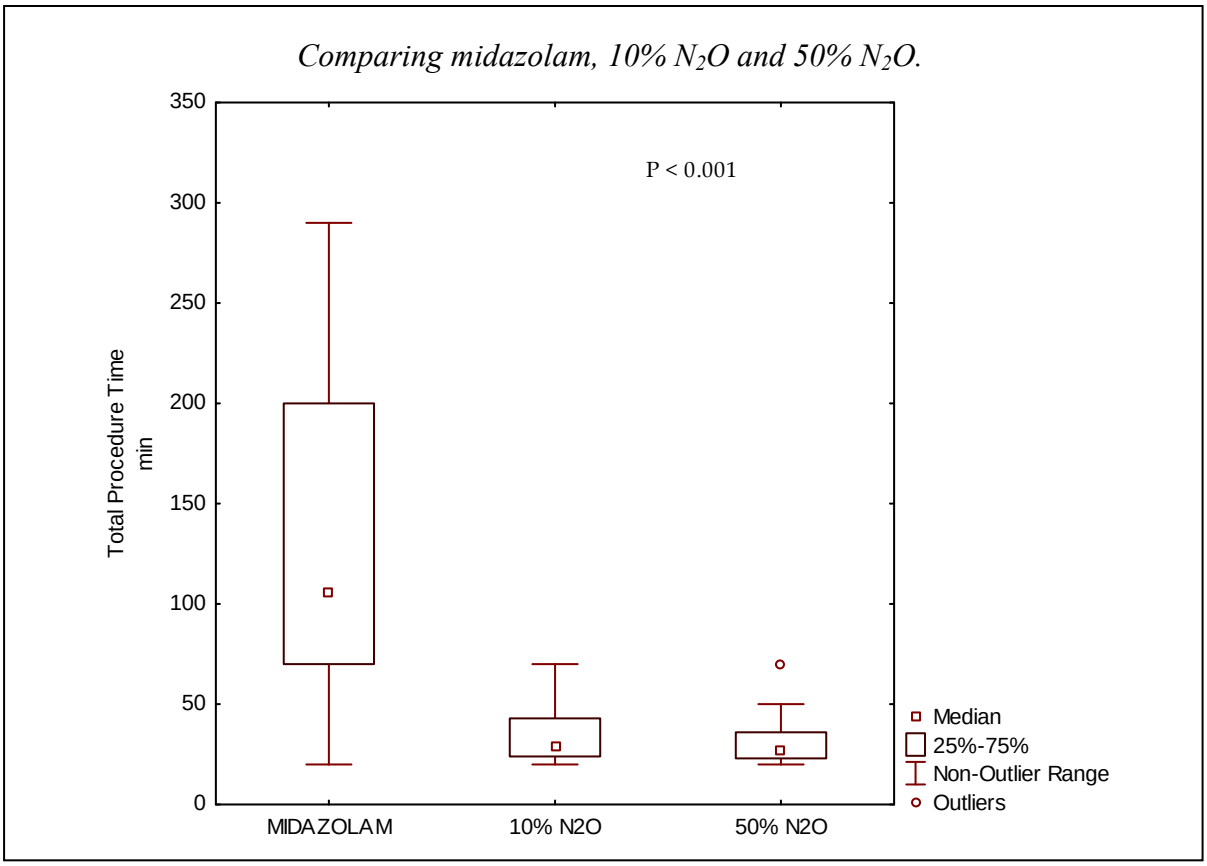
There was no significant difference in the IV time required for achieving IV access on comparing EMLA alone and EMLA+N_2O, including the period of 8 min for induction and completion of N_2O (Study I): Figure 6. If the time for N_2O was excluded the IV time required was significantly lower.

Figure 6
Procedure time for IV access (Study I a)



The total procedure time in Study II, defined as IV time + recovery time, was significantly longer when midazolam was used, compared to 50% N_2O and 10% N_2O ($P < 0.001$): Figure 7.
A subgroup analysis of total procedure time demonstrated a significant difference between OBESE children versus GR children receiving midazolam ($P < 0.05$).

Figure 7
Total procedure time (Study II)



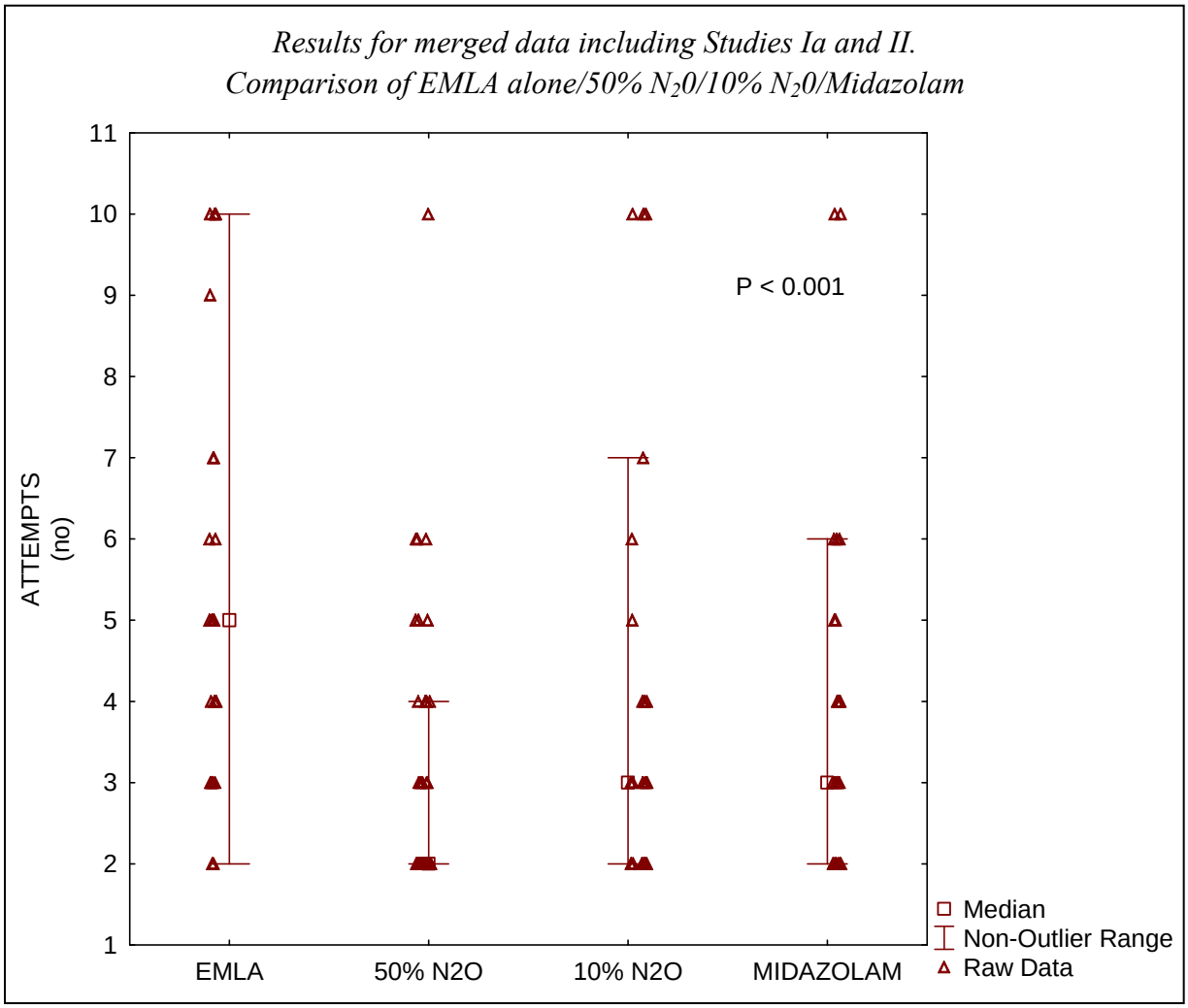
4.3 Evaluations of pain and procedures during IV access and Injections

When EMLA was used alone (Study I) the pain was rated high (VAS ≥ 5), and children and parents considered the IV procedure trying (evaluation ≤ 3). Nine procedures (18%) were accomplished with difficulties. Using N_2O+ EMLA the pain was rated as low (VAS≤ 3) and the children and parents considered the treatment to be tolerable (evaluation ≥4).
In the blinded study (Study II) children's evaluations of the procedure were more positive for 50% N_2O treatment than for both the other treatments (P<0.001). No differences were found in the children's evaluations of midazolam and 10% N_2O. Their evaluations were negatively correlated to the number of attempts (0.6) and pain (0.7). A higher pain score was reported after both midazolam and 10% N_2O (P< 0.05) compared to 50% N_2O. Evaluations were controlled for parental presence. Parents' and nurses' evaluation scores were significantly higher after 50% N_2O treatment than after midazolam and 10% N_2O and no differences were seen in their evaluation of midazolam and 10% N_2O.

4.4 Main results including merged data from Study I and II

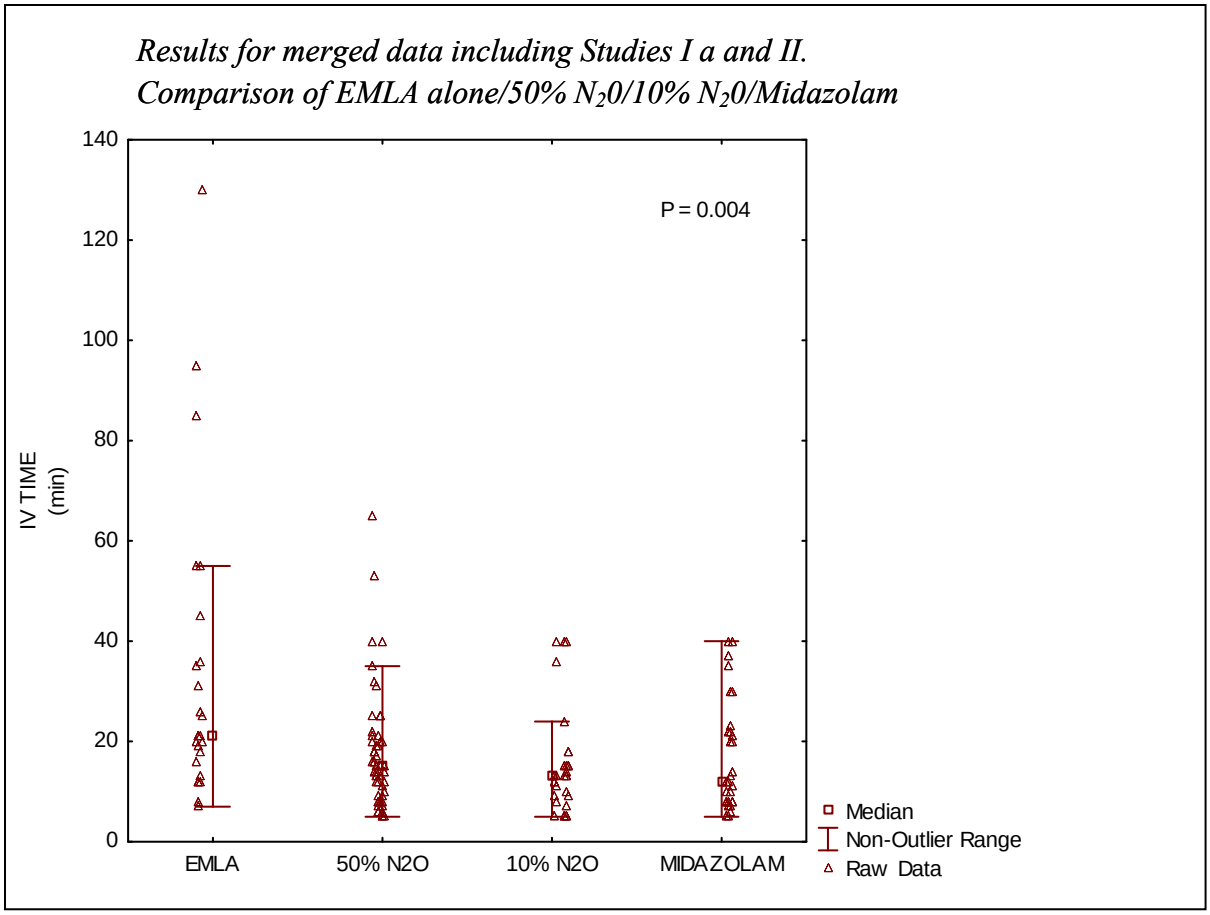
The results are presented in Figure 8-11.
When all children with IV access problems studied in Studies I and II were merged (n = 140, 109 OBESE and 31 GR children) a significant difference in the number of attempts between the treatment groups was found (P < 0.001): Figure 8. Differences were seen between midazolam and N_2O and between EMLA alone and midazoalm, 10%, 50% N_2O. The percentage of successful IV line procedures defined as two successful attempts to establish two IV lines was 70% with 50% N_2O.

Figure 8
Attempts (number)



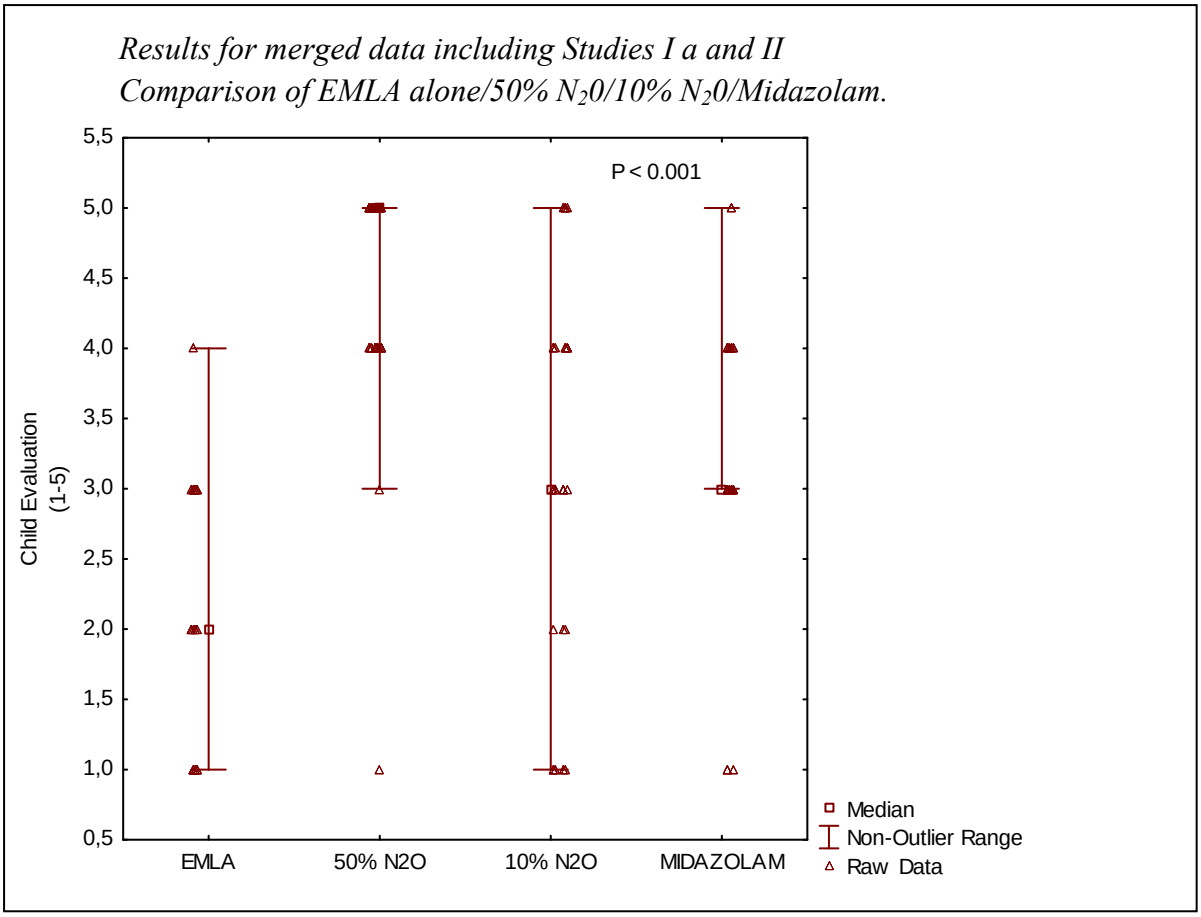
On comparing IV time, defined as time from the start of setting up the IV lines until two IV lines were established, a significantly longer IV time (not including N_2O induction in Study I) was seen using EMLA alone compared to 50% N_2O, 10% N_2O and midazolam (P = 0.004): Figure 9.

Figure 9
IV Time (min)



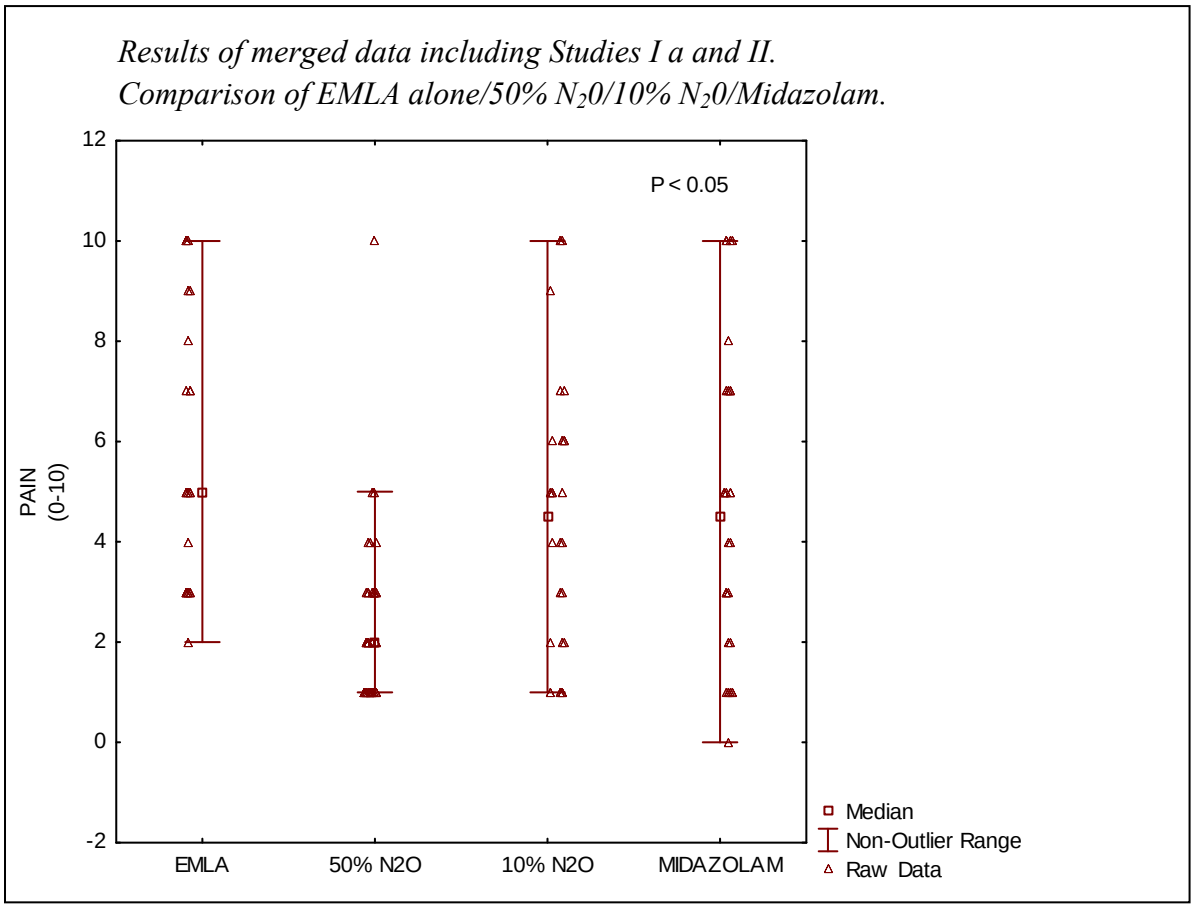
Children's and parents' evaluations of the procedure were more positive for 50% N_2O treatment than for the other treatments ($P < 0.001$):
Figure 10.

Figure 10
Children evaluation of the procedure (1-5)



A higher pain score was reported by the children after both midazolam and 10% N_20 ($P < 0.05$) compared to 50% N_20.

Figure 11
Children's evaluation of Pain (0-10)



Nurses' evaluation scores were significantly higher after 50% N_2O treatment compared to EMLA alone, midazolam and 10% N_2O.

Significant correlations were found between numbers of attempts/pain (0.5), attempts/ children's evaluations (0.6) and parents' evaluations (0.5), pain/children's evaluations (0.7) and BMI SDS/number of attempts (0.2). A significant correlation was also seen between nurse evaluation/attempts in Study I (0.5) and Study II (0.6).
No correlations were found between age and the number of attempts for IV access.

There were no sex differences.

Eleven IV lines procedures (8%) were not completed in the total group of children:
4 in the EMLA alone group, 3 in the midazolam group, 3 in the 10% N_2O group and 1 in the 50% N_2O group. One child did not receive 50% N_2O due to a technical failure revealed after uncoding (Study II). This was due to the fact that there were too many unsuccessful attempts (n=8) or because no attempt at all was allowed by the frightened child (n=3). There were no differences when a specialist nurse or general nurse performed the IV access procedure (Study I) or when three paediatric nurses were involved (Study II).

Table 7

Results for merged data including Studies Ia and II.
Comparison of EMLA alone /50% N_20/10% N_20/midazolam, given to children in the subgroups OBESE and GR children.
Data are presented as the median (range).
Outcome measured as attempts (number), IV procedure time, child/parent evaluations and pain.
Results of the nurses' evaluation and total procedure time are presented separately.

	All n=140	**EMLA alone** n=25	**50% N_20** n=55	**10% N_20** n=30	**Midazoalm** n=30	**P'**
Attempts[1] (no)	3 (2-10)	5 (2-10)	2 (2-10)	3 (2-10)	3 (2-10)	<0.001
IV time	15 (5-130)	21 (7-130)	15 (2-10)	13 (5-40)	12 (5-40)	0.004
Evaluation[2] Children	4 (1-5)	2 (1-4)	5 (1-5)	3 (1-5)	3 (1-5)	<0.001
Evaluation[2] Parents	4 (1-5)	3 (1-4)	5 (1-5)	3(1-5)	3.5 (1-5)	<0.001
Pain[3]	3 (0-10)	5 (2-10)	2 (1-10)	4.5 (1-10)	4.5 (0-10)	<0.001
	Obese n=109	**EMLA** n=24	**50% N_20** n=45	**10% N_20** n=20	**Midazoalm** n=20	**P'**
Attempts[1] (no)	3 (2-10)	4 (2-9)	2 (2-10)	3 (2-10)	3 (2-10)	<0.001
IV Time	16.5 (5-130)	21 (7-130)	17 (5-65)	13 (5-40)	13 (5-40)	0.01
Evaluation[2] Children	4 (1-5)	2 (1-4)	5 (1-5)	4 (1-5)	3 (1-5)	<0.001
Evaluation[2] Parents[4]	4 (1-5)	3 (1-4)	5 (1-5)	3 (1-5)	4 (1-5)	<0.001
Pain[3]	3 (0-10)	5 (2-10)	2 (1-10)	4 (1-10)	4 (0-10)	<0.001
	GR n=30	**EMLA** n=1*	**50% N_20** n=10	**10% N_20** n=10	**Midazoalm** n=10	**P'**
Attempts[1] (no)	2 (2-10)		2 (2-10)	3 (2-10)	3 (2-10)	0.02
IV time	11 (5-40)		9 (5-15)	12 (5-40)	11 (5-40)	0.3
Evaluation[2] Children	4 (1-5)		4 (4-5)	3 (1-4)	3 (1-4)	0.001
Evaluation[2] Parents[5]	4 (1-5)		5 (4-5)	3 (1-5)	3 (1-5)	0.002
Pain[3]	3 (0-10)		2 (1-4)	5 (1-10)	5 (1-10)	0.02

* ITT

Nurses Evaluations	**Treatment**	**Study I** n=50	**P'**	**Study II** n=90	**P'**
		(1-3)		(1-5)	
	All children			4 (1-5)	
	EMLA	2 (1-3)			
	50% N_2O	1 (1)	<0.001	5 (1-3)	
	10% N_2O			3 (1-5)	
	Midazoalm			3 (1-5)	<0.001
	OBESE children				
	EMLA	2 (1-3)			
	50% N_2O	1 (1)	<0.001	5 (1-5)	
	10% N_2O			4 (1-5)	
	Midazolam			3 (1-5)	<0.001
	GR children			4 (1-5)	
	50% N_2O			5 (4-5)	
	10% N_2O			3 (1-4)	
	Midazolam			4 (1-4)	<0.001

Total Procedure Time	**Treatment**	**Study II**	**P'**
	All children	35 (20-290)	
	50% N_2O	27 (20-70)	
	10% N_2O	29 (20-70)	
	Midazoalm	106 (20-290)	<0.001
	OBESE children	40 (20-290)	
	50% N_2O	33 (21-70)	
	10% N_2O	30 (20-70)	
	Midazolam	124 (44-290)	<0.001
	GR children	27 (20-127)	
	50% N_2O	24 (20-30)	
	10% N_2O	28 (20-57)	
	Midazolam	61 (20-127)	<0.001

[1] Attempts at double venous cannulation

[2] Likert Scale. 1-5. 1 = poor and 5 = excellent

[3] Visual analogue scale (Study I). Numeric rating scale (Study II) 0–10. 0 = no pain and 10 = worst possible pain

[4] Parent present during 49 procedures (midazolam, n =16; 50% N_20, n = 15; 10% N_20, n = 18)

[5] Parent present during 29 procedures (midazolam, n =10; 50% N_20, n = 10; 10% N_20, n = 9)

Intention to treat is represented by the worse outcome (attempt, IV access time, pain) when procedures are not completed.

'Kruskal-Wallis ANOVA

Table 8

For Post hoc analyses pair wise comparisons using the Mann-Whitney U test.

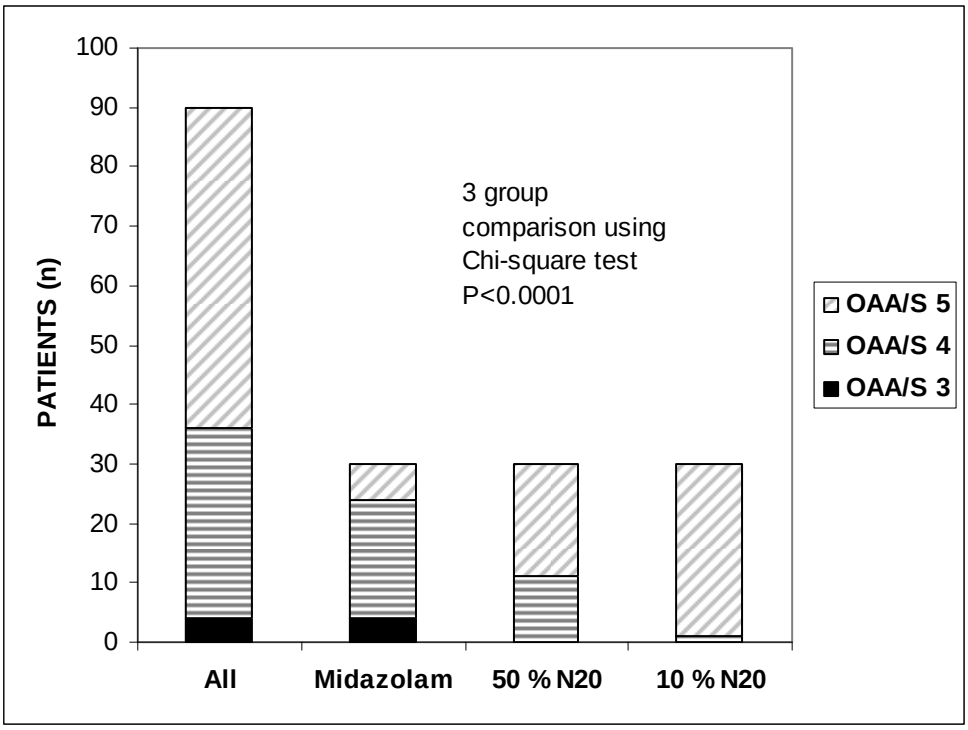
EMLA / 50% N_20	**1**					
EMLA / 10% N_20	**2**					
EMLA / Midazolam	**3**					
50% N_20 / 10% N_20	**4**					
50% N_20 / Midazolam	**5**					
10% N_20 / Midazoalm	**6**					
P < 0.01 = ∗ P < 0.001 = ∗∗						
All Children n=140	**1**	**2**	**3**	**4**	**5**	**6**
Attempts	∗	∗∗	∗	0.2	0.06	0.6
Evaluation Child	∗	∗∗	∗∗	∗	∗∗	0.9
Evaluation Parent	∗	∗∗	∗∗	∗	∗∗	
Pain	∗	0.07	0.09	∗	∗∗	1.0
OBESE Children	**1**	**2**	**3**	**4**	**5**	**6**
Attempts	∗	∗∗	∗	0.2	0.06	0.6
Evaluation Child	∗	∗∗	∗∗	∗	∗∗	0.9
Evaluation Parent	∗	∗∗	∗∗	∗	∗∗	
Pain	∗	0.07	0.09	∗	∗∗	1.0
GR Children	**1**	**2**	**3**	**4**	**5**	**6**
Attempts	∗	∗∗	∗	0.2	0.06	0.6
Evaluation Child	∗	∗∗	∗∗	∗	∗∗	0.9
Evaluation Parent	∗	∗∗	∗∗	∗	∗∗	
Pain	∗	0.07	0.09	∗	∗∗	1.0

4.5 Sedation levels Study II

In study II the sedation levels were measured during and every 15 minutes after the IV procedure using OAA/S (Observer's Assessment of **A**lertness/**S**edation 0–5).
OAA/S 0–2 was not seen in any children and OAA/S 3 was seen in children treated with midazolam, n = 4 (3 OBESE/1 GR).
3 group comparison using Chi-square test, $P < 0.0001$:
Figure 12.

Figure 12
Sedation levels Study II

Comparison of sedation levels using *OAA/S (**O**bserver's Assessment of **A**lertness/**S**edation) between midazolam, 50% N_2O and 10% N_2O 15 min after the IV procedure.*



4.6 Main results for painful injections, Study I b

The results are presented in Table 9.
When studying painful procedures regarding injections and implantations one procedure (5%) could not be performed at all using EMLA alone and on nine occasions (45%) only with difficulties. The pain was estimated to be high according to VAS ≥ 5 and the comments of children and parents indicated that they considered the procedure difficult. The time required for the procedure varied (range 4–95 minutes).

All procedures with N_2O were performed without any problems. The experience of pain was rated lower in all cases. The comments of children and parents indicated that they considered the treatment to be tolerable. The time required for the procedure was significantly lower with N_2O if the time for induction and completion was excluded. Ten minutes after the procedure, all children were able to walk by themselves. Ninety percent of the children who tried both treatments preferred N_2O.

Table 9

Anxious children undergoing painful procedures comparing EMLA alone and 50% N_2O AND EMLA. Effects on procedure time, pain and evaluations scores. Data are presented as the median (range).

	All n=20	EMLA alone n=20	50% N_2O n=20	P [2]
Procedure[1] Time	7 (1-95)	9 (4-95)	5 (1-18)	<0.001
Pain	3 (1-10)	5 (1-10)	1 (1-6)	<0.001
Evaluation Child	3.5 (1-5)	2 (1-3)	5 (4-5)	<0.001
Evaluation Parent	3 (1-5)	3 (1-4)	4 (3-5)	<0.001
Evaluation Nurse (1-3)	1 (1-3)	1.5 (1-3)	1 (1)	<0.005

[1] The time required for the procedure dose not include the time for induction and completion of N_2O (see Material and Methods)

[2] Wilcoxon signed rank test

4.7 Side effects of N_2O and midazoalm

The number of side effects was low. Three complications were documented: tinnitus, which disappeared within 3 minutes after the completion of N_2O, dizziness after midazolam and nausea after 50% N_2O. No cardio respiratory adverse events were observed. No other side effects were reported by the children when they returned for the next treatment.

Altogether, nine children did not choose to participate (Study I, 4 and Study II, 5): in three of these cases, it was not possible to carry out the procedures with conventional treatment using EMLA alone.

4.8 Stress hormones, insulin and glucose levels after IV access

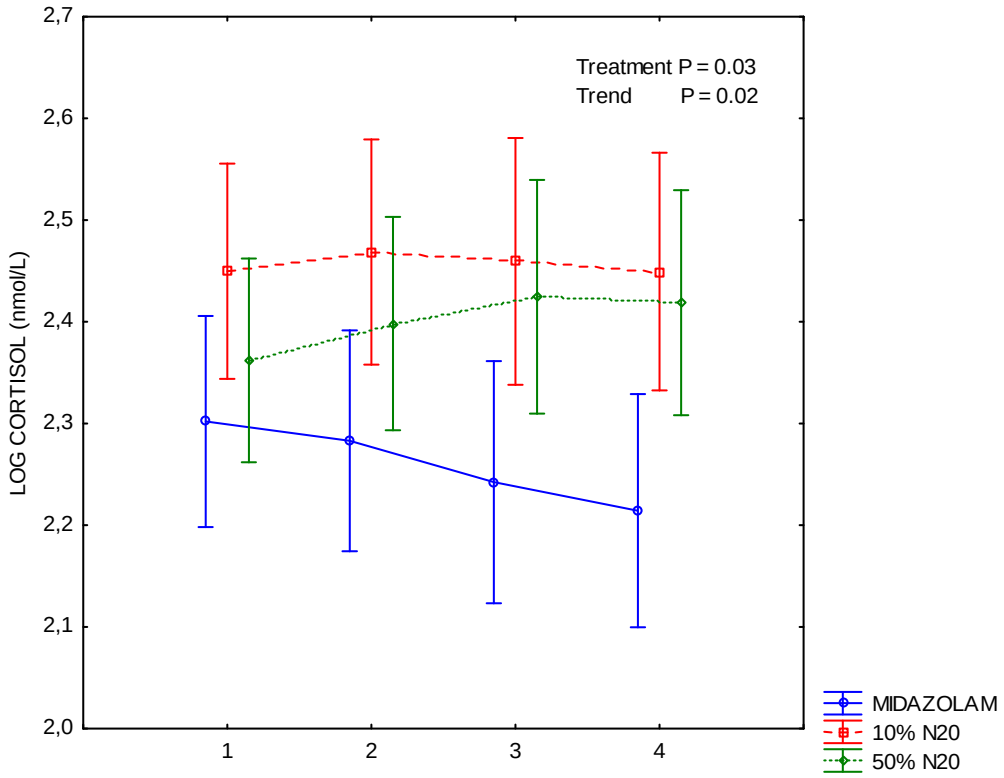
All results are presented and summarized in Table 10 (p 43).

Blood samples were obtained at 4 time points during the first 30 minutes after achieving venous access:
1 (0–1 min),
2 (5–6 min),
3 (14–15 min), and
4 (29–30 min).

Significantly lower levels of *cortisol* were seen when midazolam was used compared to 50% and 10% N_2O and on comparing all children (P = 0.03). Furthermore, there was a significant difference in treatment trends with decreasing cortisol levels in groups given midazolam (P = 0.02), with no difference between GR and OBESE children, regardless of treatment. Post hoc analyses demonstrated similar results between the treatment arms in all subgroups treated with midazolam (P = 0.05): Figure 13.

Figure 13
Cortisol levels

Blood samples were obtained at 4 time points during the first 30 minutes after achieving venous access: 1 (0–1 min), 2 (5–6 min), 3 (14–15 min), and 4 (29–30 min).
Treatment over time in all children comparing midazolam, 10% N_2O and 50% N_2O.

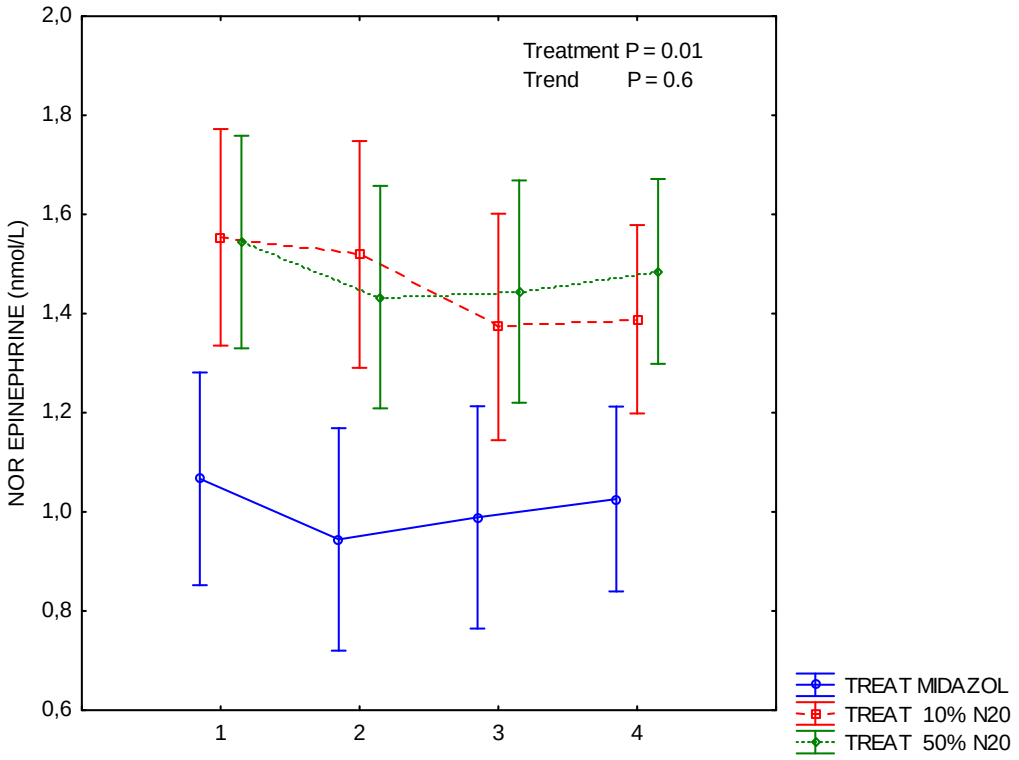


No differences were seen in epinephrine levels.

Significantly lower levels of norepinephrine response were seen in the midazolam group (P = 0.001) compared to the groups receiving N_2O, with no differences in treatment trends.
On comparing subgroups (OBESE/GR) independently of treatment, no differences were seen. Post hoc analyses showed similar results between the treatment arms in all subgroups treated with midazolam (P = 0.003) with no differences in the trends: Figure 14.

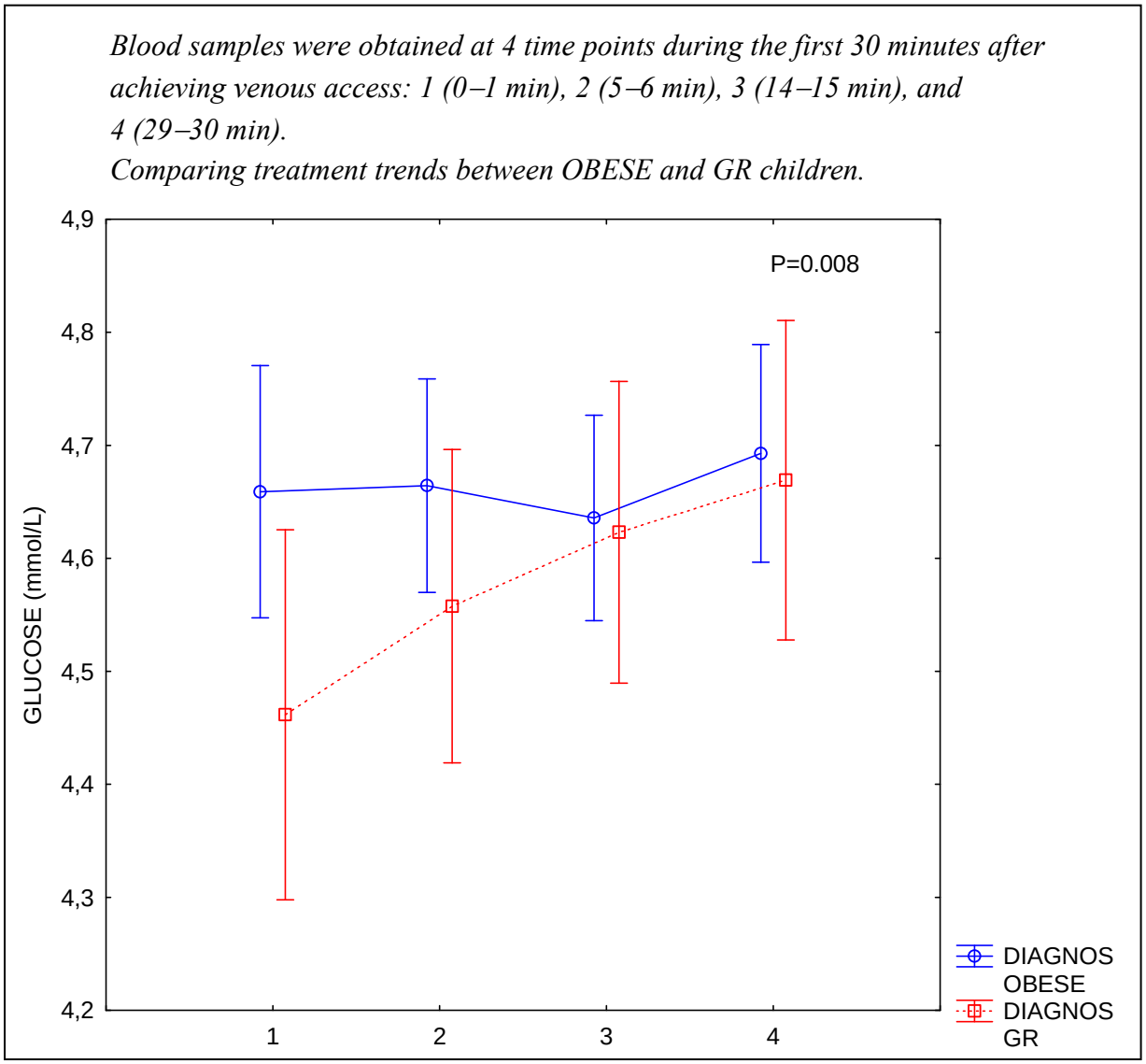
Figure 14
Norepinephrine levels

Blood samples were obtained at 4 time points during the first 30 minutes after achieving venous access: 1 (0–1 min), 2 (5–6 min), 3 (14–15 min), and 4 (29–30 min).
Treatment over time in all children comparing midazolam, 10% N_2O and 50% N_2O.



No differences were found in *glucose* levels between treatments and the treatment trends when all children were studied together. There was a difference between OBESE and GR children (P = 0.008) regardless of treatment, with increasing glucose levels in GR children: Figure 15.

Figure 15
Glucose levels

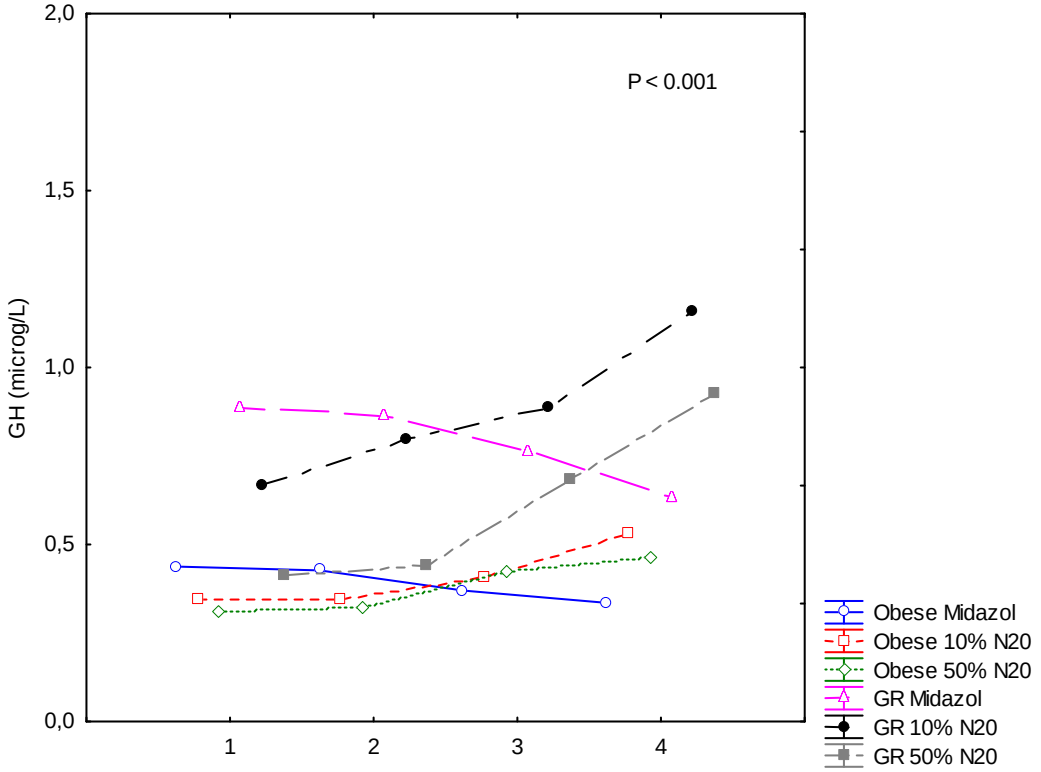


No differences in *insulin* response between treatments and treatment trends over time were seen on comparing all children. When OBESE and GR groups were compared regardless of treatment, significantly lower insulin levels were seen in GR children (P < 0.001).

No differences were seen in *growth hormone* levels between treatments on comparing all children, but the levels decreased over time in the midazolam group (P = 0.001), compared to 50% and 10% N_20 where the effect of time was reversed. When OBESE and GR groups were compared regardless of treatment lower levels of GH were found in OBESE children (P < 0.001). Post hoc analyses showed similar treatment trends in subgroups treated with midazolam: Figure 16.

Figure 16
Growth hormone levels

Blood samples were obtained at 4 time points during the first 30 minutes after achieving venous access: 1 (0–1 min), 2 (5–6 min), 3 (14–15 min), and 4 (29–30 min).
Treatment over time in all children comparing midazolam, 10% N_2O and 50% N_2O with subgroups OBESE and GR children.



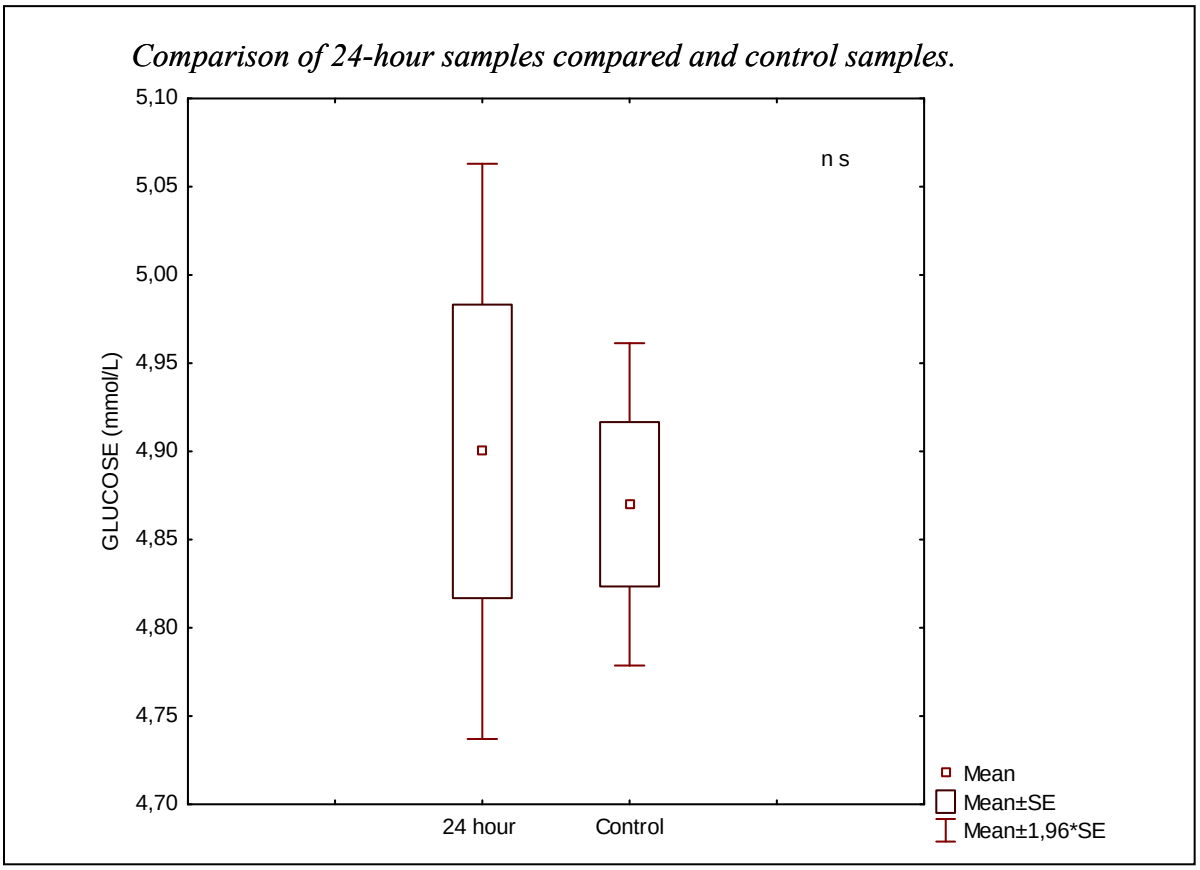
Unstressed blood sampling without any treatment

A control group (40 OBESE and 20 GR), mean age 11.0 (4–18), undergoing a 24-hour endocrine evaluation test) was used to compare P-glucose in samples 24 hours after obtaining IV access.

There were no significant differences between the different samples:

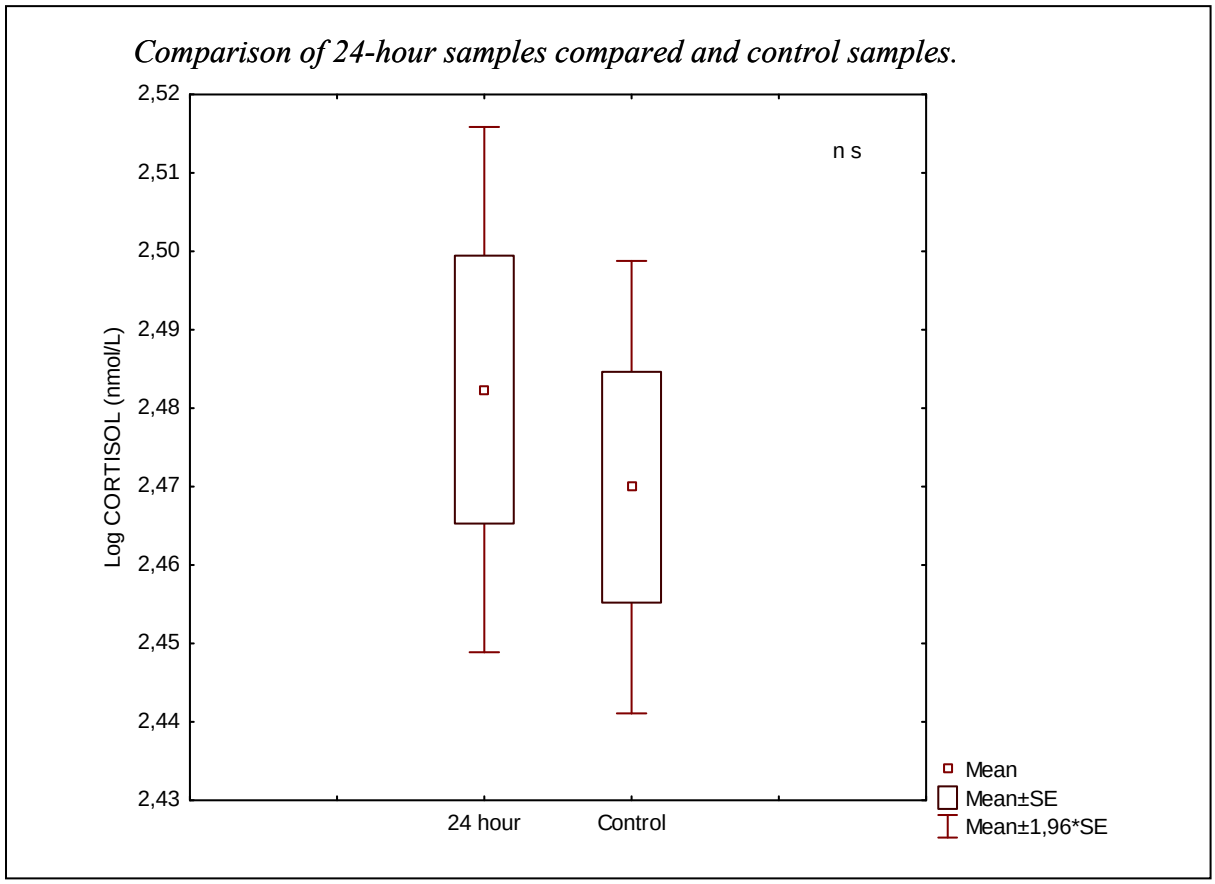
Figure 17.

Figure 17
Glucose



A control group (40 OBESE and 20 GR) , mean age 11.0 (4–18), undergoing a 24-hour endocrine evaluation test) was used to compare S-cortisol in samples 24 hours after obtaining IV access.
There were no significant differences between the different samples:
Figure 18.

Figure 18
Cortisol



The mean of the four samples of cortisol and glucose after obtaining IV access was compared with that of the control group and with an unstressed blood sample obtained after 24 hours. In 32 out of 90 children it was possible to obtain a blood sample 24 hours after establishing IV access (midazolam n=8, 50% N_2O n=14, 10% N_2O n=10).
Significant differences were seen in *cortisol* levels over time (P = 0.02), Figure 19, and in *glucose* levels over time (P = 0.037), Figure 20.
The post hoc analysis showed significantly lower mean levels in children treated with midazolam (cortisol P < 0.001, glucose P = 0.02) compared to N_2O. On comparing 50% and 10% N_2O no differences were seen.

Figure 19
Cortisol comparison

Mean cortisol during 30 minutes after IV access compared with cortisol 24 hours later and cortisol levels obtained in a control group under unstressed conditions.

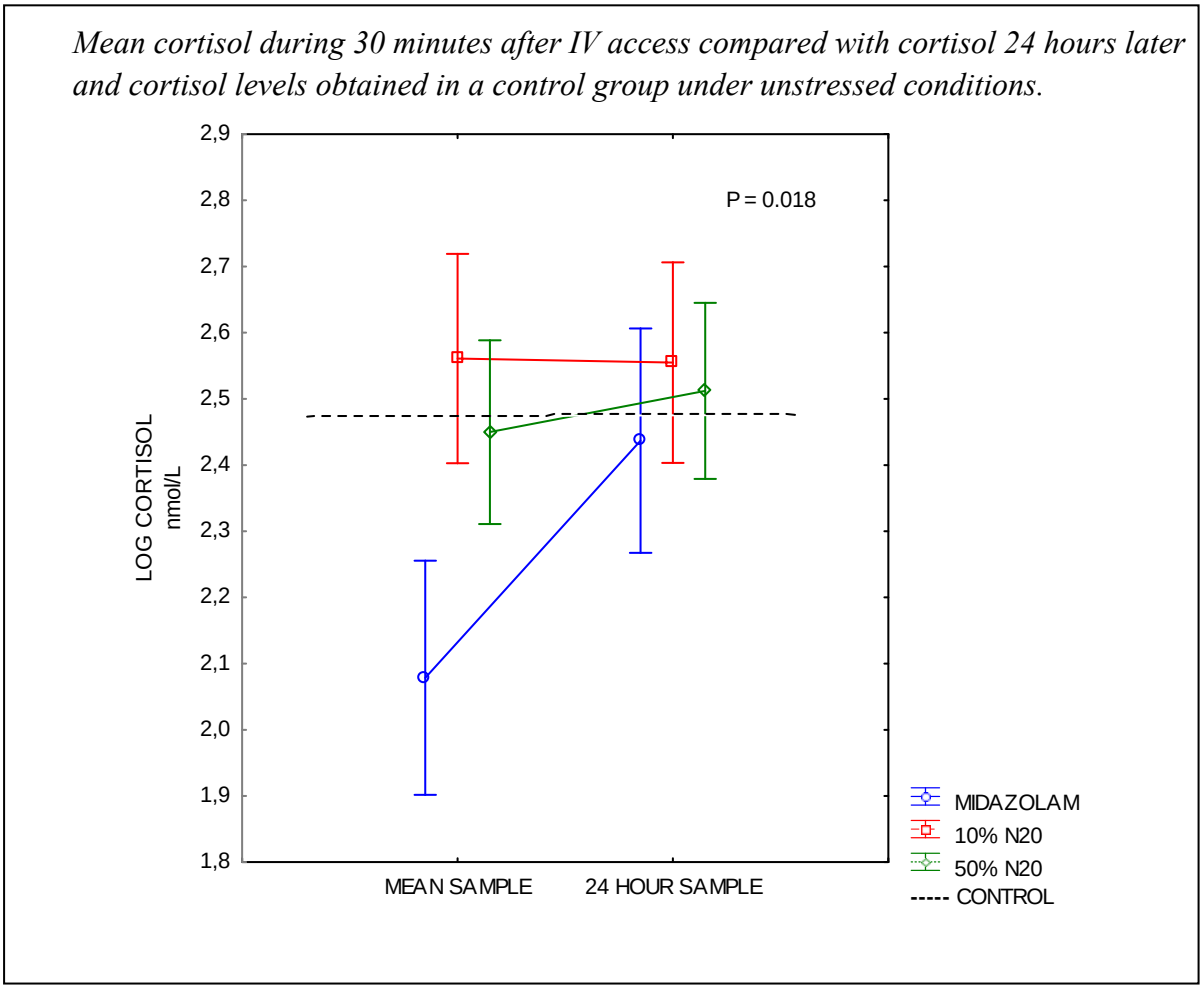
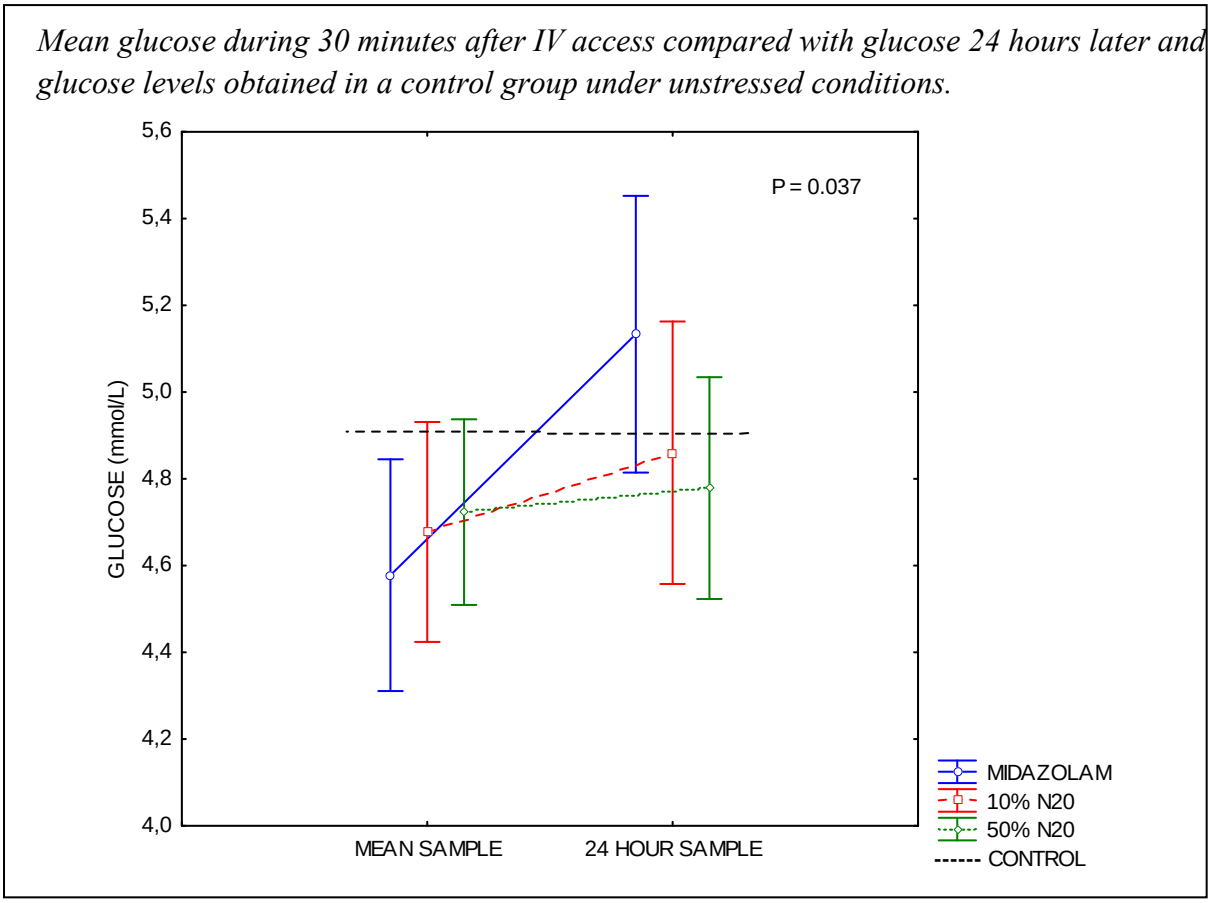


Figure 20
Glucose comparison

Mean glucose during 30 minutes after IV access compared with glucose 24 hours later and glucose levels obtained in a control group under unstressed conditions.



A tendency to higher growth hormone levels after IV access was seen (P=0.07). A post hoc analysis demonstrated significantly higher levels in children treated with midazolam (P=0.02). No differences were seen in *norepinephrine* and *insulin* levels between stressed and unstressed sampling.

Correlations between stress factors and stress response
A significantly lower cortisol response was seen in children treated with midazolam compared to the 10% N_2O group even when the children scored pain and the procedure equally (by the Numeric Rating Scale, 4.5, and by the Likert Scale, 3).
We found a weak correlations between the children's evaluation of pain and mean glucose levels (-0. 3), as well as between the children's evaluation of the procedure and mean glucose levels regardless of treatment. No correlations between cortisol levels and stress effects after obtaining IV access were seen on comparing all children regardless of treatment. Post hoc analyses demonstrated a significant correlation between the children's evaluations of the procedure and mean values of cortisol (-0.53), GH (-0.52) and norepinephrine (-0.5) in children treated with 10% N_2O.

Table 10

Levels of S-cortisol, P-norepinephrine, P-glucose, P-insulin and P-GH given as mean, SD in 90 children, 60 OBESE and 30 grew retarded (GR) children, treated with midazolam, 50% N_2O and 10% N_2O. Blood samples were obtained at 4 time points during the first 30 minutes after IV access; 1, 0-1; 2, 5-6; 3, 14-15; and 4, 29-30 min.

S-cortisol nmol/L	1	2	3	4	**Treat P***	**Trend P****	**Obese/GR Trend P*****
All children n=83	282 (165)	296 (181)	304 (204)	291 (191)	**0.03**	**0.02**	
Midazolam n=27	231 (126)	226 (134)	221 (161)	209 (175)			
50% N_2O n=29	279 (169)	311 (194)	341 (223)	328 (202)			
10% N_2O n=27	337 (182)	353 (190)	351 (202)	335 (175)			
OBESE n=56	272 (160)	288 (174)	292 (208)	279 (195)	0.3	0.05	
Midazolam n=19	241 (133)	237 (141)	224 (168)	207 (169)			
50% N_2O n=19	266 (150)	303 (179)	339 (240)	332 (223)			
10% N_2O n=18	311 (192)	325 (197)	314 (203)	299 (176)			
GR n=27	302 (177)	314 (197)	331 (197)	316 (185)	0.05	0.9	0.4 / 0.5
Midazolam n=9	206 (110)	198 (118)	212 (156)	213 (200)			
50% N_2O n=10	303 (207)	325 (231)	344 (199)	320 (165)			
10% N_2O n=8	397 (152)	417 (168)	432 (186)	415 (154)			
P-norepinephrine nmol/L	1	2	3	4	**Treat P***	**Trend P****	**Obese/GR Trend P*****
All children n=83	1.4 (0.6)	1.3 (0.6)	1.3 (0.6)	1.3 (0.5)	**0.001**	0.6	
Midazolam n=27	1.1 (0.4)	1.0 (0.4)	1.0 (0.4)	1.0 (0.4)			
50% N_2O n=29	1.5 (0.5)	1.4 (0.5)	1.4 (0.4)	1.5 (0.4)			
10% N_2O n=27	1.6 (0.7)	1.5 (0.8)	1.4 (0.9)	1.4 (0.6)			
OBESE n=56	1.4 (0.6)	1.2 (0.5)	1.1 (0.4)	1.2 (0.5)	**0.01**	0.3	
Midazolam n=19	1.1 (0.5)	0.9 (0.4)	0.9 (0.4)	1.0 (0.4)			
50% N_2O n=19	1.5 (0.6)	1.3 (0.5)	1.3 (0.4)	1.4 (0.4)			
10% N_2O n=18	1.6 (0.7)	1.4 (0.5)	1.2 (0.5)	1.3 (0.5)			
GR n=27	1.4 (0.5)	1.5 (0.8)	1.5 (0.8)	1.4 (0.6)	0.06	0.9	0.1 / 0.09
Midazolam n=9	1.1 (0.4)	1.0 (0.5)	1.2 (0.5)	1.1 (0.4)			
50% N_2O n=10	1.6 (0.3)	1.6 (0.4)	1.6 (0.3)	1.6 (0.3)			
10% N_2O n=8	1.5 (0.7)	1.8 (1.2)	1.7 (1.4)	1.6 (0.9)			

Levels of S-cortisol, P-norepinephrine, P-glucose, P-insulin and P-GH given as mean, SD in 90 children, 60 OBESE and 30 grew retarded (GR) children, treated with midazolam, 50% N_2O and 10% N_2O. Blood samples were obtained at 4 time points during the first 30 minutes after IV access; 1, 0-1; 2, 5-6; 3, 14-15; and 4, 29-30 min.

P-glucose mmol/L	1	2	3	4	**Treat P***	**Trend P****	**Obese/GR Trend P*****
All children n=83	4.6 (0.4)	4.6 (0.4)	4.6 (0.4)	4.7 (0.4)	0.1	0.9	
Midazolam n=27	4.5 (0.4)	4.5 (0.4)	4.5 (0.3)	4.6 (0.3)			
50% N_2O n=29	4.7 (0.3)	4.7 (0.3)	4.7 (0.3)	4.8 (0.3)			
10% N_2O n=27	4.6 (0.5)	4.7 (0.4)	4.7 (0.4)	4.7 (0.5)			
OBESE n=56	4.7 (0.4)	4.7 (0.3)	4.6 (0.3)	4.7 (0.3)	0.3	0.3	
Midazolam n=19	4.6 (0.3)	4.6 (0.3)	4.6 (0.3)	4.6 (0.3)			
50% N_2O n=19	4.8 (0.3)	4.8 (0.3)	4.7 (0.3)	4.7 (0.3)			
10% N_2O n=18	4.6 (0.5)	4.6 (0.3)	4.6 (0.4)	4.1 (0.4)			
GR n=27	4.4 (0.5)	4.5 (0.5)	4.6 (0.4)	4.7 (0.4)	0.3	0.6	0.30 / **0.008**
Midazolam n=9	4.2 (0.5)	4.3 (0.4)	4.4 (0.4)	4.5 (0.3)			
50% N_2O n=10	4.5 (0.4)	4.6 (0.4)	4.7 (0.4)	4.8 (0.3)			
10% N_2O n=8	4.5 (0.6)	4.8 (0.4)	4.7 (0.4)	4.8 (0.6)			
P-insulin pmol/L	1	2	3	4	**Treat P***	**Trend P****	**Obese/GR Trend P*****
All children n=83	100 (102)	110 (102)	105 (100)	106 (108)	0.3	0.6	
Midazolam n=27	104 (75)	110 (88)	108 (76)	115 (89)			
50% N_2O n=29	115 (147)	132 (137)	120 (142)	121 (147)			
10% N_2O n=27	81 (55)	87 (56)	85 (52)	81 (61)			
OBESE n=56	127 (112)	140 (108)	134 (107)	137 (118)	0.3	0.7	
Midazolam n=19	132 (68)	141 (83)	134 (69)	150 (82)			
50% N_2O n=19	150 (172)	171 (154)	159 (164)	156 (172)			
10% N_2O n=18	99 (54)	106 (53)	106 (47)	104 (61)			
GR n=27	40 (26)	44 (35)	38 (18)	39 (24)	0.1	0.7	**<0.001** / 0.6
Midazolam n=9	27 (15)	26 (13)	27 (9)	26 (13)			
50% N_2O n=10	49 (27)	58 (42)	46 (16)	54 (29)			

Levels of S-cortisol, P-norepinephrine, P-glucose, P-insulin and P-GH given as mean, SD in 90 children, 60 OBESE and 30 grew retarded (GR) children, treated with midazolam, 50% N_2O and 10% N_2O.
Blood samples were obtained at 4 time points during the first 30 minutes after IV access; 1, 0-1; 2, 5-6; 3, 14-15; and 4, 29-30 min.

10% N_2O n=8	40 (30)	42 (32)	36 (21)	33 (17)			
P-GH mikrog/L	1	2	3	4	**Treat P***	**Trend P****	**Obese/GR Trend P*****
All children n=83	0.5 (1.0)	0.6 (1.7)	0.7 (1.1)	0.9 (0.5)	0.8	**<0.001**	
Midazolam n=27	0.8 (1.2)	1.1 (2.7)	0.6 (1.0)	0.5 (0.9)			
50% N_2O n=29	0.3 (0.8)	0.3 (0.7)	0.6 (1.0)	0.9 (1.2)			
10% N_2O n=27	0.5 (1.0)	0.6 (0.9)	0.8 (1.4)	1.2 (1.7)			
OBESE n=56	0.3 (0.7)	0.3 (0.6)	0.4 (0.7)	0.5 (0.9)	0.9	<**0.001**	
Midazolam n=19	0.4 (0.6)	0.4 (0.5)	0.3 (0.4)	0.2 (0.4)			
50% N_2O n=19	0.4 (1.0)	0.3 (0.8)	0.6 (1.0)	0.6 (1.0)			
10% N_2O n=18	0.2 (0.3)	0.2 (0.3)	0.4 (0.8)	0.7 (1.0)			
GR n=27	0.9 (1.4)	1.3 (2.7)	1.2 (1.5)	1.6 (1.8)	0.3	**<0.001**	**<0.001** / 0.5
Midazolam n=9	1.6 (1.9)	2.6 (4.6)	1.3 (1.6)	0.9 (1.4)			
50% N_2O n=10	0.2 (0.3)	0.3 (0.3)	0.8 (0.9)	1.5 (1.5)			
10% N_2O n=8	1.0 (1.6)	1.3 (1.3)	1.8 (2.0)	2.3 (2.4)			

* ANOVA repeated measurements between Treatments

** ANOVA repeated measurements between Trends

*** ANOVA repeated measurements between OBESE vs GR and Trends regardless treatments

Table 10 a

Comparing samples 1-4 (mean sample) to samples 24-hour after IV access (sample 5) and to a control samples.

	Mean sample	**Sample 5**	**P***	**P****	**Control**	**Mean sample vs control P*****	**Sample 5 vs Control sample P*****
S-cortisol nmol/L							
All children n=83	293 (174)	363 (167)	**0.038**		30 (80)	**0.026**	0.3
Midazolam n=27	221 (142)	297 (131)	**<0.001**	0.06 [1]	305 (80)	**0.002**	
50% N_2O n=29	315 (180)	378 (191)	0.3	0.83 [2]	305 (80)	0.7	
10% N_2O n=27	344 (178)	397 (164)	0.9	**0.026** [3]	305 (80)	0.9	
P-norepinephrine nmol/L							
All children n=83	1.3 (0.5)	1.2 (0.4)	0.08				
Midazolam n=27	1.0 (0.4)	1.2 (0.5)	0.4				
50% N_2O n=29	1.5 (0.4)	1.2 (0.3)	0.08				
10% N_2O n=27	1.5 (0.6)	1.1 (0.3)	0.2				
P-glucose mmol/L							
All children n=83	4.6 (0.3)	5.0 (0.6)	**0.008**		4.9 (0.4)	**<0.001**	0.7
Midazolam n=27	4.5 (0.3)	5.3 (0.8)	**0.02**	0.04 [1]	4.9 (0.4)	**<0.001**	
50% N_2O n=29	4.7 (0.3)	4.9 (0.6)	0.6	0.8 [2]	4.9 (0.4)	0.22	
10% N_20 n=27	4.7 (0.4)	4.9 (0.3)	0.07	0.2 [3]	4.9 (0.4)	0.09	
	mean sample	**Sample 5**	**P***	**P****			
P-insulin pmol/L							
All children n=83	107 (102)	86 (72)	0.09				
Midazolam n=27	114 (80)	109 (88)	0.1				
50% N_2O n=29	122 (142)	89 (71)	0.3				
10% N_2O n=27	83 (54)	64 (57)	0.9				
P-GH mikrog/L							
All children n=83	0.7 (1.1)	0.5 (0.9)	**0.07**				
Midazolam n=27	0.7 (1.4)	0.2 (0.2)	**0.02**	0.9 [1]			
50% N_2O n=29	0.6 (0.7)	0.4 (0.7)		0.9 [2]			
10% N_2O n=27	0.8 (1.0)	1.0 (1.3)	0.6	0.9 [3]			

* T-test for dependent Samples

** Difference ANOVA Scheffe

*** T-test for independent Samples

[1] Midazolam vs 50% N_2O

[2] 50% N_2O vs 10% N_2O

[3] Midazolam vs 10% N_2O

4.9 Work environment

Short-term exposure levels (STEL) were measured during 43 N_2O treatments.
The median value for all STELs measured was 27 [2–515] ppm. Two out of 43 measured STEL values were right outside the set limits (500 ppm), in both cases scavenging had not been active during sampling. The overall STEL median for the sessions in which the scavenging system had been in place was 22 [2 - 319] ppm and for the sessions in which the scavenging had not been properly used it was 324 [158 - 515] ppm: Figure 22.
Eleven TWA samples were analysed after one of the glass vehicles had broken during transport. The median TWA for the 11 samples was 4 [1–30] ppm: Figure 23.
All blood values studied were within normal limits and there were no signs of changes in the levels of homocysteine or other blood status values between those measured before and after a nitrous oxide-free period: Table 9.

Figure 21

Short-term exposure levels illustrated during a Nitrous Oxide procedure

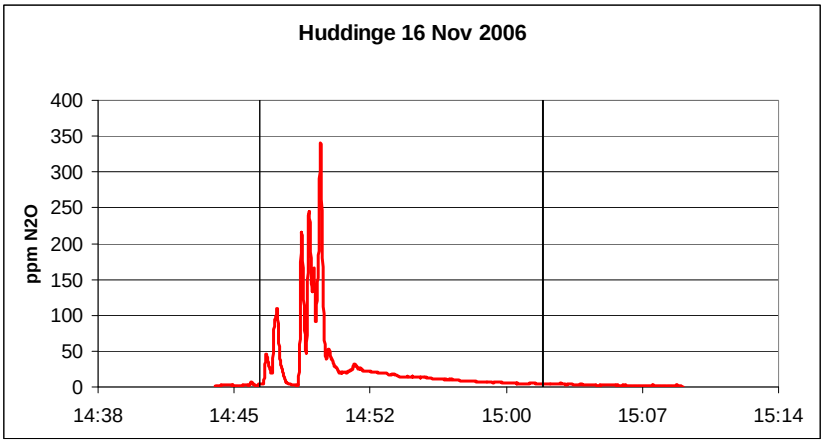


Figure 22

IR measured STEL values, ppm, during procedural pain managed by nitrous oxide inhalation in obese, anxious children. Filled bars indicate measurements when scavenging was not used. The dotted line indicates the upper guideline limit 500 ppm

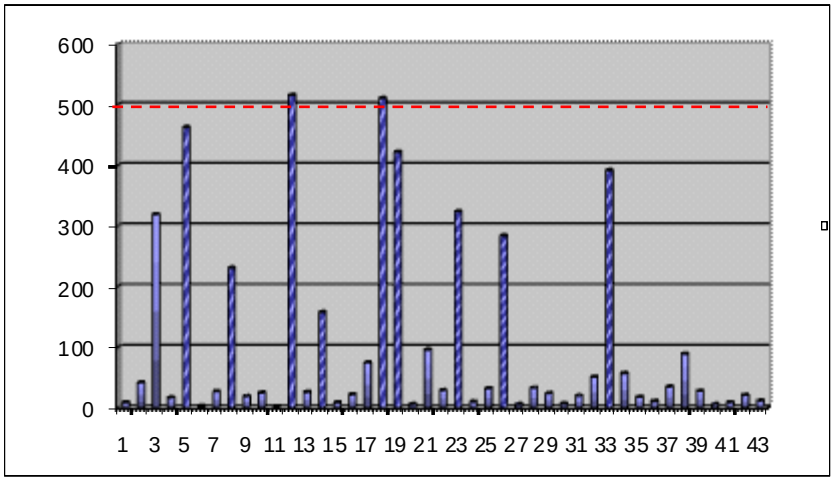


Figure 23

Twelve TWA samplings were done during procedural pain management in the children, however one of the glass vehicles broke during transport and 11 samples were analysed; TWA median 4 (1–30) ppm.

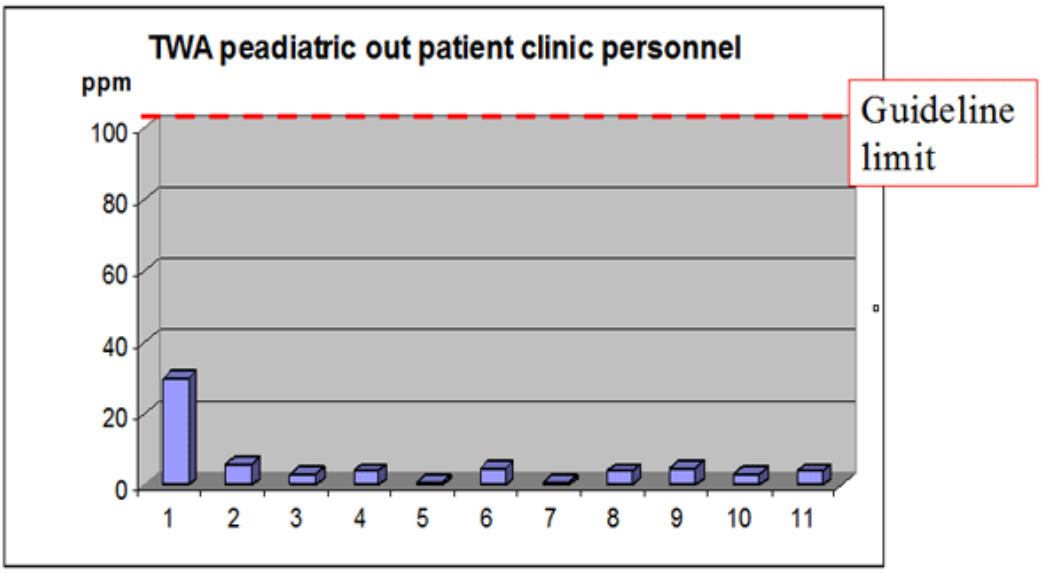


Table 9

Blood status and homocysteine by paediatric nurses before and after vacation.

Nurse 1	Before vacation	After vacation	Before vacation	After vacation
Hb	127	127	124	132
MCH	30	31	32	30
MCV	86	90	88	90
MCHC	329	347	327	332
Homocysteine	11.0	10.2	9.4	7.9
Nurse 2				
Hb	124	127	128	149
MCH	29	29	30	30
MCV	98	90	89	98
MCHC	331	336	326	332
Homocysteine	13.2	12.5	13	13

Main findings

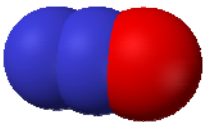
Reassuring results were found in the presented explorative study, with regard to the ambient air quality both when measured as the short-term exposurelevel (STEL) during 15 minutes and as the time-weighted average (TWA) during routine use of N_2O for procedural pain management in children in an outpatient facility, however, appropriate scavenging should be ascertained. No signs of plasma homocysteine or marcocytic blood changes could be seen in connection with the workplace exposure to nitrous oxide.

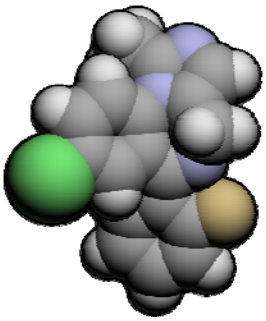
5 DISCUSSION

Problems in connection with achieving IV access in children are common [27]. The treatment of children has shifted more towards paediatric outpatient clinics for economic reasons. Outpatients settings have less resources to handle painful situations than paediatric hospitals and the need for efficacy and speed is high. Therefore, it is important to evaluate the advantages, disadvantages, and the safety of drugs or pain reduction in this specific setting.

5.1 Methods and patients

The study included two groups of patients, obese children and children with endocrine disorders (primarily short children). Both groups are frequently seen at outpatient clinics and they often require blood sampling. Furthermore, short children often have a low BMI and therefore these two groups represent two different problem areas from a body composition perspective in connection with IV access. From a pharmacological perspective it is also important to study subjects with different body composition.
N_2O has been studied extensively in various settings, but there are only a limited number of studies onN_2O in paediatric outpatient clinics [100]. Blinded studies with N_2O are scarce, probably because of the complication involved in comparing, in a double-blinded fashion, an inhalation and an oral drug. Only one such study has been published and it was in children with severe neurological problems [101]. The double-blinded design in Study II made it possible to compare the effect of treatment on subjective parameters.





5.2 Efficiency of IV access with EMLA alone, N_2O or midazolam

Efficiency, defined as the number of attempts, success rate and procedure time, was measured, when EMLA alone, 50% N_2O, 10% N_2O or midazolam was used to facilitating IV access.
The main results demonstrated a significant difference in the number of attempts between the studied treatments groups with 50% N_2O combined with EMLA, making it possible to complete procedures and examinations at a higher rate compared to EMLA alone, 10% N_2O and midazoalm.
A higher number of attempts to obtain IV access was required for children treated with midazolam in the growth-retarded group, but no such difference was found among the obese children. This might be due to the fact that difficulties and technical problems differed between obese, normal-weighed and growth-retarded children [102]. A weak correlation between BMI SDS and the number of attempts was seen, indicating that a higher BMI SDS is associated with increased difficulties in connection with obtaining IV access, which is in accord with previous findings [30]. The low correlation may be due to the fact that all children in Study II received midazolam or N_2O treatment. It can be speculated is that the results might have been different if no treatments had been used to facilitate the IV procedure.

Using 50% N_2O does not prolong the IV procedure time in Study I and II. Rather the reverse in Study I, if the time for induction and completion of N_2O was excluded, the time required was significantly lower. Comparing midazolam, 50% N_2O, 10% N_2O, treatment with 50% N_2O effectively shortened the total procedure time compared to midazolam. Especially in the obese children, midazolam resulted in an unfavourably long procedure time and potential hazardous situation due to sedation. The long recovery time is probably due to the fact that the lipophilicity of the drug, with a slow clearance from the adipose tissue [103], and that the dose is based on weight. Twenty percent of the obese children in the midazolam group reached sedation level 3 on the OAA/S, which requires monitoring not generally available in paediatric outpatient clinics.

A long procedure time consumes working hours for the staff and parents and when an IV access procedure fails it is often regarded as a failure by the children, their parents, and the nursing staff. Furthermore, when cancelled scheduled procedures have to be postponed, it is costly for both the parents and for the medical services.

It made no difference whether a specialist or a general nurse performed the IV access procedure in Study I as well as in Study II with three paediatric nurses involved. These results are confirmed by a prospective observational study, which demonstrated no significant differences on comparing the number of attempts to obtain IV access between trainees and staff [30]. Consequently, and perhaps, surprisingly, the need to facilitate IV access procedures for this group of patients can not be satisfied solely by improving the technical skills of the nurse.

No correlation was seen between the age of the child and the number of attempts to achieve IV access. Thus the number of attempts did not decrease when the children got older. This also indicates that procedural problems might be frequent in all age groups, and most probably also in adults.

It is possible that this is at least to some extent is a hidden problem. It might be more embarrassing to inform caregivers about anxiety associated with IV access when you are an adolescent or adult.

The increased success rate using 50% N_2O indicates that the technical problems are fewer when 50% N_2O is used. This may be surprising since N_2O is known to have a symphaticomimetic effect with peripheral vasoconstriction. However, studies on prepubertal children show a difference between children and adults in sympathetic vascular tonus when using N_2O [104] and the sedative effect of N_2O may decrease the vasoconstriction.

5.3 Effects of treatment on stress hormones, insulin and glucose levels

Children with endocrine disorders often require tests and blood samples for a relevant medication and an optimal growth rate.
It has previously been shown in several randomized controlled studies that cortisol levels are affected by post-surgical pain in children and that this pain response can be modified by analgesia [40, 42, 105].
In Study III, the effects of N_2O and midazolam on glucose and stress hormones immediately after obtaining IV access in distressed children were studied. The main findings were significantly lower and decreasing cortisol levels in children treated with midazolam compared to both 50% N_2O and 10% N_2O. The results are probably due to a pharmacological effect of midazolam on stress hormones rather than that midazolam has a superior effect on stress associated with the IV access procedure. Cortisol levels 24 hours after obtaining IV access were higher, which indicates that pre-treatment midazolam, results in abnormally low cortisol levels [106]. Several authors have studied the effect of midazolam on emotional and/or surgical stress and concluded that the use of midazolam reduces the autonomic and hormonal responses measured as a reduction in catecholamine levels [107]. Midazolam was also associated with a significant decrease in the secretion of cortisol and insulin [108].
In summary, our results together, with those of previous studies, indicate that midazolam should be avoided when correct analyses of cortisol levels are required, for the diagnosis of endocrine and metabolic disorders.
The lack of correlation between pain and mean cortisol levels when using 10% N_2O may be explained by an analgesic effect of 10% N_2O and the lack of a sedative effect could explain the significant correlation between children's evaluations of the procedure and mean values of cortisol (-0.53), GH (-0.52) and norepinephrine (-0.5).
N_2O is known to have a symphaticomimetic effect, which could explained the significantly lower norepinephrine values in the midazolam group as compared to 50% and 10% N_2O [67].
Catecholamines are rarely analysed in children with obesity or endocrine disorders, but they have an impact on glucose homeostasis as well as on the GH IGF-1 axis. Measuring the circulating mediators of the neauroadrenergic response to stress and pain in children may constitute a complement to pain and anxiety scales.
Many of the metabolic and cardiovascular complications of obesity are present during childhood and are related to insulin resistance and hyperinsulinaemia [109]. Hyper

insulinaemia and peripheral insulin resistance are important indicators of alterations of glucose metabolism [49]. When glucose trends are compared in OBESE and GR children (Study III), a significant difference was found with increasing glucose levels in GR children and decreasing levels in OBESE children. As expected significantly higher levels of insulin response were observed among OBESE children regardless of treatment. Stress-induced hyperglycaemia is a common clinical phenomenon [110, 111] and it may also affect the insulin response in obese patients undergoing an IV glucose tolerance test, and thereby affect the calculated values of insulin sensitivity and glucose effectiveness.
A stressful IV access procedure may result in a GH surge and a suppressed GH release afterwards, as GH producing cells are in a refractory stage [46]. As shown previously, the GH levels were lower in the obese children than in the lean children [112]. This combination of low GH levels and the risk of underdetected GH pulses during stressful IV access procedures followed by blunted releases thereafter, may complicate the GH deficiency test in overweight children. The tendency towards higher GH levels after IV access using midazolam is consistent with previous reports [46]. The increased GH response in patients receiving midazolam is due to the fact GABA regulates the GH secretion [108, 113]. Therefore, using midazolam might affect the GH test. This effect might also be prolonged due to the lipophility of midazolam [106].
In summary, in children with obesity and endocrine disorders, there is a risk that a stressful IV access procedure will increase stress hormone release and glucose levels and thereby affect the analyses as fasting insulin and glucose. The evaluation of cortisol and growth hormone levels may also be affected by both stress and drugs used to facilitate IV access.

5.4 Children's, parental and staff evaluations of the IV access procedure

Our findings, as well as previous observational and self-reported studies of children undergoing IV access procedures with high levels of pain and distress [27], indicate that there is a demand for more efficient methods to facilitate painful IV access procedures.

There was a negative correlation between the number of attempts and the children's evaluations of the procedure, i.e., an increased number of attempts correlated with a worse evaluation by the child (Study II). Similar correlations were seen with the parents and staff. This highlights the importance of immediate successful IV access. The best conditions for succeed in establishing a difficult IV access should include a child-friendly atmosphere as well as a well-prepared and flexible staff, The importance of preparation may be obvious, but to take the time needed to choose an optimal vein and to have all equipment within arm's reach are two major perpetuates. The nurses' ability to create a calm and reassuring atmosphere is also an important factor for a successful IV access procedure.

Anaesthetic cream does not induce sufficient analgesia in a considerable number of children treated at outpatient departments, which has been demonstrated in both Study I and II. Among the children in Study 1 who underwent procedures using an anaesthetic cream 60 % found it painful, defined as NRS ≥5 [15, 114]. In Study II, 48% of the children receiving midazolam or 10 % N_2Ofound the IV access procedure painful. The children's, the parents' and the

nurses' evaluations of the IV procedure were rated higher with 50% N_2O compared to EMLA alone as well as to midazolam and 10% N_2O. The children's evaluations of the procedures were similar for 10% N_2O and midazolam. It cannot be ruled out that the result obtained in the midazolam group was affected by the mask and therefore it is possible that, under optimal condition midazolam may be superior to 10% N_2O.

It is possible that children's evaluations affect the parental evaluations. However, when the study was designed it was considered impossible to separate parents and children after the procedure and all evaluations were performed in the same order throughout the study.
The nurses, who established the IV access evaluated the procedures, independently of children's and parents evaluations.

5.5 Safety aspects of N_2O and midazolam

Several randomized controlled trials have compared midazolam and N_2O during procedures with different designs and reached the conclusion that the use of oral midazolam or N_2O provides safe and effective sedation in children [98, 115, 116]. This is in accord with the side effects in Studies I and II, with only three minor complications recorded.
It is a well-known fact that N_2O has a weak emetic effect [90], but only one side effect such as nausea/vomiting was documented in Studies I and II. This can be explained by the fact that obese children, who performed glucose tolerance tests, were not given any solid food or liquid from midnight before the day of treatment and the other children were not given any food 4 hours, and no liquid 2 hours, before the treatment. However, there was no association between the preprocedural fasting state and adverse events in a recent article where 50% of the children receiving procedural sedation in the emergency department were not fasted [117].

No cardio-respiratory adverse events such as hypoxia, bradycardia and hypotension were observed.

These results are applicable to short procedures with no post-procedure pain. Strengthened by the good results with 50% N_2O, in ASA 1 children, there may be no reason why ASA 2 patients could not be included when 50% N_2O is administered in this safety manner.

5.6 Pharmacological and methodological aspects of EMLA, N_2O and midazolam

There are pharmacological pros and cons for the pharmaceuticals used in Studies I and II. EMLA, the most frequently used anaesthetic cream in Scandinavia, is easy to handle as a cream or patch. It is effective for one hour after removal at a depth of 3 mm. Emla may cause local blanching of the skin, followed by redness, which sometimes obstructs the IV access procedure.
Midazoalm, a benzodiazepine, is frequently used for outpatient procedures [71, 118], and provides anxiolysis, sedation and some amnesia [106]. It has been widely used in recent years [71, 74], and been promoted for its good clinical effectiveness and low toxicity [73].

Although effective, the interindividual variation in effect and elimination is high and dose-dependent [30], which may explain the variability in sedation levels observed among children receiving midazolam in Study II. Midazolam causes anterograde and retrograde amnesia in children and this is frequently presented as a specific advantage of midazolam [77]. However, the amnesic effect of midazolam is questioned when it leaves the implicit memory intact [119]. The mechanism of conditioned anxiety in children associated with repeated medical procedures may be explained by the intact implicit memory [75, 76].
Inhalation of 50% N_2O provides analgesia and sedation for minor painful procedures [120]. According to an extensive retrospective French survey, the method works very well in minor surgery [100] and is well established for pain alleviation during emergencies in pre-hospital care, during childbirth and at paediatric hospitals [121, 122]. From this perspective, it is surprising that N_2O is not used more frequently in paediatric outpatient clinics.

5.7 Administration of drugs for facilitation of IV access

The administration of drugs may give rise to various difficulties in distressed children. Topical creams, the easiest form of administration, unfortunately have a relatively long onset of 30–60 minutes, which is a drawback when a rapid onset is desierable in outpatient clinics, where efficiency and the need for speed are two important factors.
Oral or rectal drugs have great interindividual variability and their uptake is difficult to predict. The bioavailability of oral midazolam is about 36% (9–71%). This wide variability is reported to be similar for many other oral drugs in children [75]. Another problems when using oral drugs, such as midazoalm, is the bitter taste even when added to a syrup [75, 123]. Rectal administration is an alternative in infants, but not a good option for adolescents. Inhalational anaesthetics do not show the great inter individual variability in pharmacokinetics as after rectal and oral administration [75].

In Study II only four children (4%) could not participate because they did not understand how to use the face mask. The nurse carefully demonstrated how to use a face mask, which is important for the child and the parents. It is also possible that the widespread use of inhaled drugs for asthma treatment at home has increased the understanding of facemask usage. The study results indicate that the majority of children at paediatric outpatient clinics will be able to use a mask, if it is demonstrated in an illustrative manner.
The most common concentration of N_2O given to children during painful procedures is 50% [100, 122], but according to a recent Japanese open randomized study, the best concentration of N_2O for the best possible effect on reducing IV access pain in children would be 70% [124]. In another open randomized cross-over study the children preferred midazolam to 30% N_2O [115], and in a double-blind randomized study in children with cerebral palsy, no differences were seen in parents' and nurses' satisfaction regarding midazolam and 70% N_2O [101].
In Studies I and II, 50% N_2O was used and it has been speculated that the results might have been better using 70% N_2O.

In Study I, the N_2O concentration was increased in gradual stages in contrast to the procedure in Study II, where the concentration had to be fixed at 50% because of the blinding procedure. The gradual increasing of the N_2O concentration may facilitate the co-operation and participation of the child. However, the results indicate that the co-operation of the children worked out well using both methods. In several studies a fixed 50 % N_2O oxygen

mixture has been studied to assess the use and safety of this mixture with good results [100, 122].
The items required are an anaesthetic block, a suction unit, a scavenging system and a pulse-oximeter. Treatment with N_2O is easy to administer and can easily be performed by a single specially trained nurse if no other concomitant drugs are given apart from EMLA and if local regulations so permit.

5.8 Work environment

The impact of pharmaceutical substances on the environment is a subject that is attracting increasing attention, and personnel health is an important topic and all efforts should be made to secure a good work environment.
Most countries have ambient air limits for gases posing a potential health risk. The no-effect limit for negative health effects due to chronic trace exposure is not known. In the US, as well as in most European nations, all halogenated inhaled anaesthetics, as well as N_2O, have established time-weighted average limits that should not be exceeded in order to assure personnel safety and health [84]. These limits are of the magnitude of 25–100 parts per million (ppm) for N_2O. In Europe, many countries recommend an upper mean exposure for an ordinary 8-hour working day of 100 ppm or 180 mg/m^3.
Study IV is the first one addressing the workplace ambient air quality during routine use of N_2O for procedural pain management in children in an outpatient facility. None of the measured TWA values during procedural pain management in children were the above set Swedish limits, although one was above the set NIOSH (National Institute for Occupational Safety and Health) 25 ppm limit. Also, all the short-term exposures measured were below the set limit when appropriate scavenging was activated. However, in two cases, levels just above the set recommendation were found and, on both occasions, scavenging was not properly activated. This underlines the importance of proper equipment handling if N_2O is to be used more frequently in outpatient settings. N_2O should be administered in rooms with proper ventilation and scavenging equipment in order to satisfy national air quality guidelines and the importance of adequate scavenging has been thoroughly demonstrated [125, 126]. One may argue that, for personnel involved in pain management in children in an outpatient facility, the time weighted average should not be a major concern as the procedures are mostly of short duration and there are frequent N_2O-free pauses. It was therefore reassuring to see that also the short-term exposure levels, the measurements performed during the administration of N_2O by mask to the children, were within the set limits when the given routines were followed.

Another aspect to take into account for a good work environment is the caregivers' evaluations of the effects of different treatment given to the children. The nurses' evaluations of the IV access procedures, demonstrated a significantly better evaluation, when using 50% N_2O combined with EMLA. Caregivers working with well-function methods contribute to job satisfaction, a good work climate and a good quality of care for the patient.

5.9 Strengths and limitations

One strength is the large group of children with IV access problem studied, (n= 140), using a prospective double-blind randomized design in Study II. A dose- dependent effect of N_2O could be demonstrated and strengthened the results of the study.
The strength of Study III is the repeated blood samples in a large group of children comparing analyses with different treatments shortly after (0-30 min) IV access. Samples after 24 hours, representing unstressed IV access, were also compared with the mean value after achieving IV access and with unstressed controls. All samples were collected under similar conditions between 9 and 10 a.m, which is of importance since a number of factors may influence cortisol levels, such as time of day and physical activity [33, 127]
The effectiveness of the blinding procedure used in Studies II and III can always be discussed. To improve the blinding, we used a low concentration of N_2O and nurse 1, who administered N_2O/oxygen, was not the person who evaluated the IV procedure. The fact that all treatments had some pharmacological effect strengthens the validity of the blinding procedure and the great variability in the effect of least midazolam also improved the blinding procedure.
A limitation, when comparing all study children combined is the use of different evaluation and pain measurements scales in Studies I and II. But the results demonstrated significant differences of similar kinds and sizes and the measuring scales have been validated and are in constant development [15, 114].
It is possible that the effect of midazolam was not optimized when using 0.3 mg/kg and a maximum dose of 15 mg midazolam was allowed, despite the fact that the therapeutic range is up to 0.5 mg/kg [128]. However, already with the dose chosen, an extremely long total procedure time was observed in obese children. In all probability, higher doses should therefore be avoided in obese children.
A weakness is that, for ethical reasons (and to strengthen the blinding procedure) a control group used 10% N_2O. Therefore, the study provides no information on the effects of stress without any pain or sedation treatments at all. The use of 10% N_2O was based on the combination of weak analgesic efficacy and pain tolerance previously observed with this dose [129, 130], and report of no depression of the CNS [131].
It may have been of interest to perform a follow-up of the satisfaction of the parents and children after 1–3 days, as was done in a previous study [132], since the amnesic effect of midazolam mainly affects the explicit memory but leaves the implicit memory intact.

A limitation is that the blood samples were only collected for 30 minutes after the IV access and it cannot be ruled out that this period of time was too short. However, when an ACTH stimulation test is performed a marked increase in circulating cortisol levels is observed after 30 minutes [133].

In the explorative environment study, the involved personnel were aware of the fact that ambient air measurements were conducted, which may have enhanced the good results.

6 CONCLUSIONS

- 50% N_2O in combination with EMLA was in all aspects superior to midazolam for the facilitation of IV access in distressed children. The IV access procedure was more efficient, with a shorter total procedure time and an increased number of successful IV line procedures, and the experience of the children, parents and nurses was better

- Nurse controlled self-administered 50% N_2O has all the necessary properties to facilitate procedures and augments the quality of paediatric care for children, parents and the nursing staff

- Midazolam should only be used exceptionally in obese children due to the long recovery time

- N_2O and midazolam influences glucose and hormone sampling. Significantly lower cortisol levels were found when midazolam was used compared to both 50% N_2O and 10% N_2O and unstressed control children. In GR children glucose levels increased the first 30 minutes after obtaining IV access, whereas the opposite was found in OBESE children. The growth hormone levels decreased with time in the midazolam group compared to 50% and 10% N_2O where the effect of time was reversed

- Measurements of workplace ambient air quality during routine use of N_2O for procedural pain management in children in an outpatient facility were well below the set recommendations if appropriate scavenging was activated. This indicates that work environment concerns should not be a major obstacle to the use of N_2O in an outpatient setting

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