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MAILIS TÕNISSON

Clinical picture and biochemical changes in blood in children with acute alcohol intoxication

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Clinical picture and biochemical changes in blood in children with acute alcohol intoxication

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Study design, collection of clinical and biological testing materials, participation in data analysis, writing the papers.

ABBREVIATIONS

AAI	acute alcohol intoxication
ACCAL	annual per capita consumption of pure alcohol (100%) in litres
ACTH	adrenocorticotropic hormone
ADH	alcohol dehydrogenase
AILCS	acute alcohol intoxication level by clinical signs
BAC	blood alcohol concentration
Cmax	maximal concentration
CNS	central nervous system
CYP2E1	cytochrome P4502E1
DHEAS	dehydroepiandrosterone-sulphate
DSM	Diagnostic and Statistical Manual of Mental Disorders
ICD	International Statistical Classification of Diseases and Related Health Problems
k_m	Michelis-Menten constant
NAD	nicotinamide adenine dinucleotide oxidized form
NADH	nicotinamide adenine dinucleotide reduced form
PRL	prolactin
SAC	serum alcohol concentration
T	testosterone

1. INTRODUCTION

Ethanol is the most frequently abused drug in the United States and Western countries, including in Estonia (U.S. Department of Health and Human Services, 2007; Falk *et al*., 2008). Increasing alcohol consumption is accompanied by the growing problem of alcohol consumption among children and adolescents (Madu *et al*., 2003; Meyer *et al*., 2003; Meyer *et al.,* 2008; Schöberl *et al*., 2008; Sutherland *et al*., 1998; Woolfenden *et al*., 2002). The International Self-Reported Delinquency Study-2, or ISRD-2, showed that on average 53% of Estonian 15–16-year-old adolescents had had at least one heavy drinking episode in the past 30 days (Markina *et al*., 2007). As per WHO, heavy episodic drinking is defined as the proportion of adults (15+ years) who have had at least 60 gram or more of pure alcohol on at least one occasion in the past 30 days (WHO, 2014). Drinking alcohol at a young age is a risk factor for alcohol addiction later in life (Patrick *et al*., 2013), and is connected with problems in school (Latvala *et al*., 2014), binge drinking (White *et al.*, 2013), tobacco addiction (Hughes *et al*., 2015; Lee *et al*., 2014), illegal drug use (Harris *et al.*, 2014), and risky sexual behaviours (de Looze *et al.*, 2015; Monk *et al.*, 2014; Hagemann *et al*., 2013). Alcohol is also linked to violent crimes (Archimi *et al.*, 2014; Green *et al*., 2011), suicides (Lahti *et al*., 2014; Kaplan *et al.*, 2013; Holmgren and Jones, 2010; Flensborg-Madsen *et al*., 2009), and accidental deaths (Weiss *et al*., 2014; Estonian Institute of Economic Research Yearbook, 2013; Mørland *et al*., 2011; Koski *et al*., 2007).

As the frequency and amount of alcohol consumption by children and adolescents has gradually increased, medical personnel at hospitals have to be prepared for an increased workload due to the medical problems caused by acute alcohol intoxication (AAI). Biochemical tests, such as glucose, lactate and electrolytes levels in plasma or serum are a part of clinical practise to evaluate the clinical condition of patients with AAI. There have been very few studies about the relationships between biochemical and hormonal changes in the blood of children hospitalised with AAI. The aim of the current study was to describe clinical, mental and physical signs in children with different severity of AAI levels. In addition, to investigate the prevalence of changes in plasma levels of glucose, lactate, potassium and sodium in children hospitalized with AAI, and their relationships with plasma cortisol, testosterone, estradiol and progesterone levels.

2. REVIEW OF LITERATURE

2.1. Alcohol consumption

2.1.1. In the world

Alcoholic beverages are available throughout the world. The level of alcohol consumption is conventionally reported in terms of annual per capita consumption of pure alcohol (100%) in litres (ACCAL). The mean worldwide ACCAL in 2005 was 6.13 litres consumed by every person aged 15 years or older, ranging from more than 12.5 litres in the developed world (mostly the Northern Hemisphere, Argentina, Australia and New Zealand) to under 2.5 litres in North and sub-Saharan Africa, the Eastern Mediterranean region, and South-East Asia regions, but worldwide, recorded consumption has been stable at 4.3–4.7 litres of pure alcohol per capita since 1990 (WHO; 2011). There is a gender difference in ACCAL: from 2.3 to 62.1 litres in males and from 0.2 to 33.0 litres in females (WHO, 2011).

According to the Summary Health Statistics for United States, 52% of adults aged 18 and over were regular drinkers, 13% were currently infrequent drinkers, 6% were formerly regular drinkers, and 8% were formerly infrequent drinkers (National Health Interview Survey, 2012). The same study reported that 60% of men and 44% of women were currently regular drinkers.

2.1.2. In Estonia

The total (recorded and unrecorded) consumption of alcohol in Estonia in 2005 was 22.00 litres per capita, 36.1 litres for men and 12.2 litres for women. The alcohol consumed was mostly in the form of spirits and beer in 2005 (WHO, 2011). Over the next eight years, the total consumption of alcohol in Estonia decreased to 11.9 litres per capita in 2013. The largest amount of alcohol consumed in 2013 was beer and light alcoholic drinks, whereas the consumption of strong alcoholic beverages decreased over the period of 2005–2013 (Estonian Institute of Economic Research, 2014). Inhabitants´ assessments of their own alcohol consumption in 2006–2012 showed that 15.3% of respondents did not use alcohol at all, 55.3% drank a little, 26.7% drank moderately and 2.7% drank a lot (Estonian Institute of Economic Research, 2013).

2.1.3. Among children and adolescents

In the European School Survey Project on Alcohol and Other Drugs, 41% of all males between 15 and 16 years of age and 38% of all females at the same age interviewed reported five drinks or more on the same occasion during the past 30 days (European School Survey Project on Alcohol and Other Drugs, 2011). Among a teenage population evaluated in Australia, 29% of the subjects reported drinking to the point of drunkenness (Williams *et al*., 2000). About 86%

of 13 to 16-year-old adolescents in Estonia had consumed alcohol in their lifetime and the average age at initial alcohol consumption was 11.3 years in girls, and 10.7 years in boys (Markina *et al*., 2007). Similar results have been reported in different European countries. For example, 78% of Polish children declared consumption of alcohol in the last 12 months (Kaminska *et al*., 2012). According to the Health Behaviour in School-aged Children (HBSC) study, the prevalence of drunkenness increased significantly between ages 11 and 15 for boys and girls in European countries. Totally, 2% of 11-year-old girls and 5% of boys (1% of girls and 2% of boys in Estonia) and 17% of 15-year-old girls and 25% boys (13% of girls and 20% of boys in Estonia) had drunk alcohol at least once a week. The same study reported that 1% of 11-year-old girls and 3% of boys (1% of girls and 1% of boys in Estonia) and 29% of 15-year-old girls and 34% of boys (42% of girls and 48% of boys in Estonia) had been drunk at least twice (Currie *et al.,* 2012).

2.2. Alcohol pharmacokinetics and pharmacodynamics

Acute alcohol intoxication (AAI) is a clinically harmful condition that follows the ingestion of a large amount of ethanol.

Ethanol (CH3CH2OH) is an easily water-divided compound that rapidly crosses cell membranes. Ethanol is rapidly absorbed in the stomach and in the duodenum, while only a small percentage occurs in the remaining intestinal tract. The consumption of alcohol together with or after a meal leads to a slower rate of absorption because stomach emptying is delayed and the maximal concentration (Cmax) is lower and occurs later compared with a drinking on an empty stomach (Jones *et al.*, 1994; Klockhoff *et al.*, 2002). Blood from the gastrointestinal tract goes to the portal vein, where the alcohol is transported through the liver, and then on to the heart and the systemic circulation. The metabolism of alcohol occurs mainly through the action of enzymes located in the liver, and small amounts are metabolized also in the mucosa of the stomach. After absorption, ethanol is distributed into the water compartment of the body. The concentration of alcohol in the organs and tissues after reaching equilibrium depends primarily on the water content in these tissues (Endres *et al*., 1994). Body fluids such as sweat, saliva and urine, which are almost 100% water, contain a higher concentration of ethanol than whole blood, which is 80% water (Jones, 2006; Jones, 1993). Plasma and serum with a water content of 92% hold a higher concentration of alcohol than blood (Iffland *et al*., 1999). The majority of bloodstream alcohol (95–98%) is eliminated from the body by oxidative metabolism (Lieber *et al.,* 2000), primarily by the liver. Metabolism of ethanol occurs via two pathways. The first pathway comprises two steps. The first step is metabolism to acetaldehyde, catalysed by the enzyme alcohol dehydrogenase (ADH), which takes place in the cytosol of the liver. The second step to acetate, catalysed by the aldehyde dehydrogenase (ALDH), takes place in mitochondria. In ADH-mediated oxidation of alcohol, hydrogen is transferred

from the substrate to the cofactor nicotinamide adenine dinucleotide oxidized form (NAD), converting it to nicotinamide adenine dinucleotide reduced form (NADH), and acetaldehyde is produced. Thus, ethanol consumption leads to an accumulation of NADH. This high concentration of NADH inhibits gluconeogenesis by preventing the oxidation of lactate to pyruvate. The altered redox state, in turn, is responsible for a variety of biochemical and hormonal disturbances. About 3–5% of the alcohol is excreted unchanged in breath, urine, and sweat (Jones, 2006). Less than 1% of the amount of alcohol undergoes conjugation by ethyl glucuronide in the gastric mucosa or in the liver.

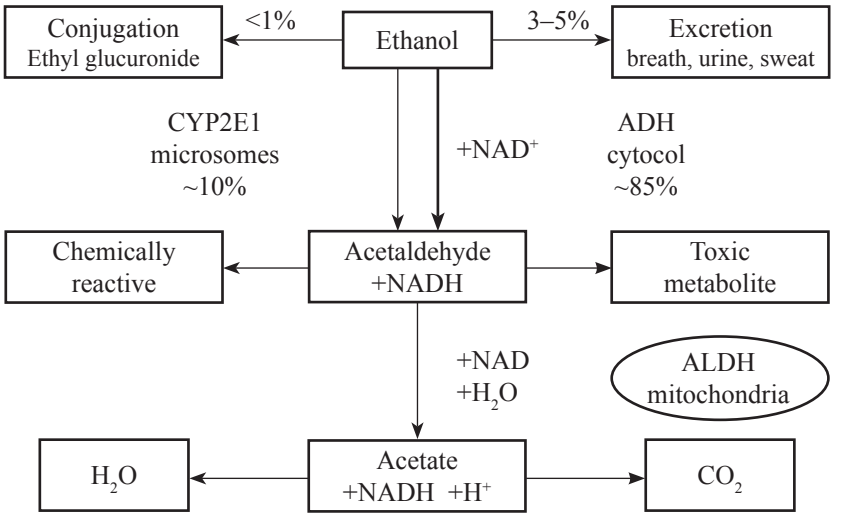


Figure 1. Diagram of alcohol metabolism.

The second pathway for ethanol metabolism is called the ethanol-inducible microsomal ethanol-oxidizing system (MEOS), and about 10% of alcohol is metabolized by the enzyme cytochrome P4502E1 (CYP2E1). This pathway uses oxygen, and therefore generates free radicals that damage tissues (Berg et al., 2002). The CYP2E1 has a higher Michelis-Menten constant (k_m) for oxidation of ethanol, and therefore comes into play when blood alcohol concentration (BAC) reaches higher concentration, as in heavy drinkers and alcoholics (Figure 1). The other effect is that liver mitochondria can convert acetate into acetyl CoA in a reaction requiring adenosine triphosphate (ATP). Further processing of the acetyl CoA by the citric acid cycle is blocked, as NADH inhibits two regulatory enzymes – isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. The result of accumulation of acetyl CoA is forming and releasing of ketone bodies into the blood, exacerbating the acidic condition already resulting from the high lactate concentration. The processing of the acetate in the liver becomes

inefficient, leading to a build-up of acetaldehyde, which forms covalent bonds with many important proteins, impairing protein function (Berg et al., 2002).

2.3. Clinical signs of alcohol intoxication

2.3.1 Clinical features and symptoms of acute alcohol intoxication in adults

The effects of alcohol on the individual depend on the amount of alcohol ingested, the speed of drinking, the rate of increase in BAC, and previous experience with drinking (tolerance).

Clinical signs and symptoms of acute alcohol intoxication have been well studied in adults, and the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-4) published by the American Psychiatric Association for the diagnosis of AAI is used in everyday work (Diagnostic and Statistical Manual of Mental Disorders 4th edition, 1994). Disturbed consciousness or memory, slurred speech, imbalance, aggressiveness, or euphoria may be a sign of AAI. Somnolence, stupor, disorientation, and disturbance of balance are frequent and well-recognised CNS symptoms of alcohol intoxication.

It has been found that the psychomotor disturbances in adults with AAI with similar drinking customs are similar at different age groups (Karch, 1998). Studied men who received ethanol, reliably detected its effects when plasma ethanol levels reached 32 mg/dl, but only the subjects who received the high dose reported episodes of intense well-being or euphoria (Morgan *et al*., 2001). Ethanol-induced euphoria occurred while plasma ethanol levels were rapidly rising (Di Chiara *et al.*, 1996; Lukas *et al.*, 1986).

Measurements of the pulse, frequency of breath, blood pressure, and body temperature are part of the physical examination of a patient, but they are not specific to estimating the level of drunkenness. Some studies have found that the pulse of alcohol intoxicated people is often accelerated (Brunelle *et al*., 2004; Ryan *et al*., 2002), but can also occasionally be slower than normal (Brvar *et al*., 2009). Blood pressure can express a similar tendency – from hypotension in rare cases (Wilson *et al*., 2007) to hypertension in common cases (Puddey and Beilin, 2006; Reims *et al*., 2004; Spencer *et al.*, 1999). However, one study has reported the decrease of blood pressure after acute alcohol consumption and thereafter increase during 24 hours (Barden *et al.*, 2013).

The breathing frequency is related to the intoxication level and lactate level in plasma – an increased lactate level leads to acidosis and this in turn to an increased breathing rate. Body temperature is often influenced by the outside temperature: the hazard to hypothermia is greater in cold weather (Bouthoorn *et al*., 2010; Kornfält and Johansson, 2010; Devaney *et al*., 2003). Many studies describe good correlations between CNS symptoms and the blood alcohol concentration (BAC) in adults (McKnight *et al*., 1997; Zoethout *et al.*, 2011), but there is insufficient information about similar studies in children (Lamminpää, 1994).

2.3.2 Clinical features and symptoms of AAI in children

Although there are a lot of studies related to different aspects of AAI in children, few have looked at the clinical signs and symptoms of AAI (Bouthoorn *et al*., 2011; Weinberg *et al.*, 2006). Bouthoorn *et al*. retrospectively studied that reduced consciousness (45%) and hypothermia (43.1%) were the most common clinical findings of AAI children in the hospital (Bouthoorn *et al*., 2011). The psychomotor disturbances in children varies greatly between different age groups due to the variances in the maturation of the body. Acute alcohol intoxication is worse in younger children (Minera and Robinson, 2014; Fong and Muller, 2014; Palano *et al.*, 2007), because of the faster rise in the blood alcohol concentration and the development of pronounced euphoria.

Children's studies have usually described children with serious alcohol intoxication without the comparable groups of mildly or moderately intoxicated children. Alcohol is distributed throughout the water in the body without binding to plasma proteins. This property causes the gender difference i.e. as females with the same body mass have less water content than males, they develop higher blood alcohol concentration than men (Mirand and Welte, 1994). In younger children, the entire water content of the body exceeds or is similar to the adult man's water content, calculated with Mellits-Cheek formula (http://www.medcalc.com/tbw.html). At the same blood alcohol concentration, the child has much more alcohol per body weight than an adult has and therefore has much more severe alcohol intoxication.

In the emergency department, the paediatrician on-call should diagnose and treat the AAI in the first instance to avoid complications. Assessment of the intoxication level by clinical signs can help to determine treatment tactics, but the clinical assessment of drunken children is not an easy task as there are no objective criteria to determine the alcohol intoxication level: it is often subjective and depends on the experience of the on-call physician.

2.4. Ratio of SAC/BAC

The clinical signs of AAI in adults have been compared with alcohol concentrations in different biological fluids, such as in serum, plasma or blood (Dubowski, 1980; Vonghia *et al*., 2008; Wright, 1991), but there are only a few such studies done involving children (Bouthoorn *et al*., 2011). Alcohol concentration is often measured in serum or plasma in hospitals; however, in legal cases, serum or plasma ethanol concentrations have to be converted into blood alcohol concentration. Therefore, the correlations between the clinical signs of AAI and SAC or BAC are often not comparable, as the SAC is usually slightly higher than the BAC (Shajani *et al*., 1989; Winek et al., 1987). The SAC to BAC ratio in adults is between 1.12:1 and 1.18:1 (Barnhill *et a*l., 2007). To our best knowledge, no such information is available for children.

2.5. Biochemical disturbances in serum of patients with AAI

The different reports have shown that acidosis, including lactic acidosis (Höjer, 1996; Zehtabchi *et al.*, 2005), hypokalaemia (Elisaf et al., 2002; Reid et al., 2012), hypernatraemia (Lamminpää *et al*., 1991; Rauchenzauner *et al*., 2005) and hypoglycaemia (Madsen, 1990; Hammerstedt *et al.*, 2011) or hyperglycaemia (Pach *et al*., 2007) do occur in adults or patients with AAI. There are also studies that have looked at the biochemical changes during AAI in children (Bradford, 1984; Lamminpää *et al*., 1993; Lamminpää, 1994; Lamminpää, 1995; Marchi *et al*., 2003; Roy *et al*., 2003).

2.5.1. Glucose and lactate

The study performed in 4005 non-diabetic intoxicated adults has shown that ethanol was the reason of hypoglycaemia in 20.1 to 40% of patients (Lionte *et al*., 2004). Hypoglycemia is due in part to the block of hepatic gluconeogenesis by ethanol, again as a consequence of the increased NADH/NAD ratio in subjects whose glycogen stores are already depleted by starvation or who have preexisting abnormalities in carbohydrate metabolism.

On the other hand, hyperglycaemia can also be present in patients with AAI . Hyperglycaemia has been associated with decreased insulin levels and elevated levels of counterregulatory hormones as glucagon, cortisol, growth hormone and catecholamines in patients with alcohol-induced ketoacidosis (Umpierrez *et al*., 2000). Indeed, hyperglycemia may also occur in association with alcoholism, as glucose intolerance may be due, at least in part, to decreased peripheral glucose utilization (Lieber, 1992).

Hypoglycaemia has been found to be a severe problem in children with AAI (Beattie *et al.*, 1986; Lamminpää, 1995; Albers *et al.*, 2004). The clinical significance of hypoglycaemia has been highlighted especially in small children, as this may rapidly deteriorate their general condition (Bradford, 1984; Lamminpää *et al*., 1990; Yang *et al*., 1995). Hyperglycaemia can also occur in these children or adolescents, probably as a result of the increased cortisol levels due to the stimulation of the adrenocorticotropic hormone (van Cauter *et al*., 1997; Plat *et al*., 1999; Shavit *et al*., 2012).

The increased glycaemia from the alcohol abuse seems to be the main cause of lactic acidosis in diabetic patients. Krzymien reported that 12 alcohol intoxicated patients from 29 diabetic patients aged 20–87 were in the Intensive Diabetes Care Unit of the Warsaw Medical University in 2007–2012 with the diagnosis of lactic acidosis (lactate level >5 mmol/L) (Krzymien *et al*., 2013). There were some cases of severe lactic acidosis after ethanol ingestion in adults without serious illnesses (Lien *et al*., 1999; Müssig *et al*., 2008), but usually lactate level was in the reference value (Fulop *et al*., 1986; Auzepy *et al*., 1985). In infants or young children with alcohol intoxication, the lactate level can be heighten to a serious level, or >5 mmol/L (Edmunds *et al*., 2014).

2.5.2. Electrolytes

Hypokalaemia has been considered to be an important factor for patients with alcohol withdrawal syndrome or delirium, where hypokalaemia may be a life-threatening factor (Stasiukyniene, 2002). Hypokalaemia was the most common abnormality in the serum electrolytes of AAI children and teenagers (Lamminpää *et al*., 1993). Hyperaldosteronism has been found to be one reason for hypokalaemia, as well as for hypernatraemia in patients with AAI (Hirschl *et al*., 2006). Vomiting and diarrhoea in AAI can lead to dehydration that in turn may cause disturbances in serum potassium or sodium levels (Perkin *et al*., 2007).

There were some studies that reported changing of chloride, calcium, or magnesium levels in AAI patients. Fulop *et al*. described an unexpected and unexplained finding of hyperchloraemia in 10 of the 29 (34.5%) patients, with serum chloride levels of more than 110 mmol/L (Fulop *et al*., 1986). Similarly to adults, hyperchloraemia has been the most frequent electrolyte disturbance (about 33%) in AAI adolescents (Bouthoorn *et al.*, 2011). Rauchenzauner *et al.* reported that in their study the most frequent electrolyte disturbance was hypernatraemia (41%), and that only 21% of patients had hyperchloraemia, followed by hypermagnesaemia (17%) and hypocalcaemia (15%) (Rauchenzauner *et al*., 2005). The depletion of magnesium, phosphate and calcium is frequently found in alcohol-dependent patient. These electrolyte disturbances may be associated with the alcohol-induced hypoparathyroidism and parathyroid hormone resistance of the skeletal muscle, as well as with the decrease of serum osteocalcin (Vamvakas *et al*., 1998).

2.5.3 Hormones

2.5.3.1. Cortisol

The level of cortisol, one of the main stress hormones, is increased by various stress factors, including pain, inflammation, burns and scares (Carlsson *et al*., 2014; Hannibal *et al*., 2014; McBeth et al., 2005). Some researchers have found a positive correlation between heavy drinking and increased serum cortisol concentration (Obasi *et al*., 2015; Husain *et al.*, 2014). Badrick *et al*. found in their study that in 2693 men and 977 women an increased number of alcohol units consumed per week and heavy drinking were associated with increased cortisol levels (Badrick *et al*., 2008). Mennella *et al*. conducted a within-subjects design study and reported that alcohol consumption resulted in significantly higher cortisol in 17 women when compared with the control condition (Mennella *et al*., 2005). Other researchers have found that neither placebo nor the lower dose of alcohol significantly increased cortisol levels, but a relatively high dose of alcohol produced a smaller increase of cortisol in heavy drinkers (N=32) compared to light drinkers (N=23) (King et al., 2006).

2.5.3.2. Sex hormones

Many studies have investigated changing serum sex hormone levels in adults with AAI, finding that a significant relationship exists between alcohol intake and serum sex hormones levels. Serum testosterone concentration has decreased, and serum or plasma concentrations of prolactin, cortisol, 17-hydroxyprogesterone, androstenedione, and dehydroepiandrosterone increased in men with AAI compared to their own without ethanol (Välimäki, 1983; Välimäki *et al.*, 1984). However, serum estradiol concentration in female patients with AAI was not affected by serum ethanol concentration (Holdstock *et al*., 2006). Increased progesterone levels have been found in 12 alcohol-intoxicated women aged 21–35 in the lutheal phase (Holdstock *et al.,* 2006), but ethanol had no effect on progesterone levels in men (N=9) or women in the follicular phase; whereas Pierucci-Lagha *et al.* found decreased serum progesterone concentration in 15 women aged 21–34 in follicular phase (Pierucci-Lagha *et al.*, 2005).

2.5.3.3. Other hormones

Alcohol influences glucose metabolism in several ways in diabetic patients as well as in non-diabetic patients. Since alcohol inhibits both gluconeogenesis and glycogenolysis, its acute intake without food may provoke hypoglycaemia, especially in cases of depleted glycogen stores and in combination with sulphonylurea due to increasing insulin level (Pietraszek, *et al.,* 2010; van de Wiel, 2004; Magis *et al*., 2003). Therefore, chronic alcohol consumption is a major risk factor for the development of type 2 diabetes (Kim *et al*., 2015; Kim *et al*., 2010; Baik *et al*., 2008).

Frias *et al.* reported that AAI produces a decrease in growth hormone levels in the adolescents of both sexes, without significant alteration of either insulin-like growth factor-I or insulin-like growth factor binding protein-3, and an increase in plasma glucose and a decrease in insulin in the female adolescents but not in the males (Frias *et al.*, 2000). The same researchers found that AAI also produces a high increase in plasma prolactin (PRL), adrenocorticotropic hormone (ACTH) and cortisol in adolescents, and a contradictory behaviour with regard to testosterone (T) according to gender: plasma T was increased in females and decreased in males. ACTH and PRL correlated positively with cortisol, dehydroepiandrosterone-sulphate (DHEAS) and T in females, which suggests that ACTH and PRL could synergistically stimulate adrenal androgen production; the decrease in T and increase in beta-endorphin in males suggests that AAI could have an inhibitory effect on testicular T, perhaps mediated by beta-endorphin (Frias et al., 2000).

3. AIMS OF THE STUDY

1. To study the prevalence of clinical signs in different AAI severity groups and the correlation of AILCS with the SAC.
2. To study the diagnostic performance characteristics (sensitivity, specificity, efficiency) of clinical assessment in the diagnosis of AAI in children when the "golden standard" in the determination of AAI levels is SAC.
3. To establish the ratio of SAC to BAC in AAI children.
4. To study the prevalence of biochemical (glucose, lactate, potassium, and sodium) changes in children with AAI, and to establish the impact of SAC on the biochemical changes in AAI children.
5. To describe the hormonal changes (testosterone, estradiol, progesterone and cortisol) in children with AAI, and their relationship with biochemical markers in plasma.

4. METHODS AND SUBJECTS

4.1. Patients

The study included all 8–18-year-old children hospitalised with acute AAI at the two main Estonian children's hospitals – Tartu University Children's Clinic and Tallinn Children's Hospital – over a three-year period (Dec 2005–Dec 2008). No lower age limit was set; that is, all children self-intoxicated by alcohol were included. The upper age limit was 18.0 years due to the law, which establishes the age limit for patients hospitalized at the children's hospital in Estonia. From the given period, 417 children and adolescents hospitalised with suspected AAI were analysed. Twenty children were excluded because their SAC was below 0.20 g/L; according to the International Statistical Classification of Diseases and Related Health Problems 10th (ICD-10) (http://www.who.int/classifications/icd/en/), they were considered not to have used alcohol or all of the blood alcohol had already eliminated by the time of alcohol measurement. Narcotic intoxication was confirmed with a rapid urine test in three children, who were also excluded. One child, a newborn baby with an alcohol concentration of 2.05 g/L, was excluded because she had not consumed alcohol by herself. One hundred and fifty- three children in the hormonal study and one hundred and sixty-one children in the clinical signs study dropped out of the study group due to the following reasons: refused testing, there were mistakes in blood drawing, biochemical tests had not been conducted, or the medical form had not been completed.

Due to the legal criteria, where criminal responsibility starts from the age of 14 years, children were divided into two age groups – 8.0–13.9 years and 14.0–17.9 years. According to the severity of drunkenness, subjects were divided into three groups using two different methods: firstly, according to the clinical signs and symptoms estimated by the on-call paediatrician; and secondly, according to the SAC as the gold standard in hospitals. The children were divided into three groups, based on their serum alcohol concentration: Group 1 ≥ 0.20–1.49 g/L, Group 2 1.50–2.49 g/L, and Group 3 ≥ 2.50 g/L.

The number of subjects increased during the study, so the numbers are therefore different. In the biochemistry study from December 2005 to December 2007, 226 children with suspected AAI were hospitalised at the hospitals (Table 1). The data of 194 children remained for statistical analysis. Hospitalised children were aged 10.0–17.9 years, with the mean age being 14.2 years. There were 94 (48.5%) children in the age group of 10.0–13.9 years and 100 (51.5%) children in the age group of 14.0–17.9 years. In total, there were 119 boys forming a male-to-female ratio of 1.6:1.

In the hormonal study from December 2005 to December 2008, the data of 264 children was used in the analysis. The sample included 81 children from Tartu University Children's Clinic and 183 from Tallinn Children's Hospital. The mean age of children was 14.2 years (range 8.4–17.9 years). There were 154 boys, forming a male-to-female ratio of 1.4:1.

In the clinical signs study 417 children and adolescents were hospitalised with suspected AAI. After excluding children for not meeting the AAI criteria and for other purposes (see above), data from the remaining 256 children were used in the analysis.

Table 1. Number of subjects in different studies.

Study	Hospitalised children	Included as AAI children	Totally excluded from the study	Remained in the study (Tartu:Tallinn)	Ratio of boys to girls
Paper I (Dec 2005–Dec 2007)	226	212	32	194 (78:116)	1.6:1
Paper II (Dec 2005–Dec 2008)	417	394	153	264 (81:183)	1.4:1
Paper III (Dec 2005–Dec 2008)	417	394	164	256 (76:180)	1.4:1

4.2. Reporting of clinical signs of acute alcohol intoxication

Alcohol intoxication was diagnosed by Diagnostic and Statistical Manual of Mental Disorders (DSM IV), and criteria were used for building up a medical assessment form (Addendum 1). Upon hospitalisation, the on-call paediatrician has to confirm the drunkenness of the child by filling in an anonymous encoded medical assessment form. This form included data about the child's mental status (consciousness, balance, speech and behaviour). Consciousness was assessed on a scale from normal to severely aggravated: clear consciousness, disorientation, somnolence, stupor or coma (estimated on the Glasgow Coma Scale); balance on a scale of normal, imbalanced or unable to stand (lying); speech on a scale of normal, rushed, fast, confused (slurred), or unable to speak, and behaviour on a scale of normal, restful, passive, euphoric, aggressive, or impossible to assess. Physical status (muscle tone, body temperature, blood pressure, pulse, and breathing rate), consumption of alcohol (the time passing since the drinking started, the amount of alcohol consumed, and the first or a recurrent episode of AAI) and the time of the assessment were reported. Muscle tone was assessed on a scale of normal, decreased or increased (with or without tremors, spasms, or convulsions). The amount of reported alcohol consumed was registered as the number of standard drinks. If the child consumed different types of alcohol products or consumed these with other subjects, these parameters were taken into consideration in the calculation. According to the clinical signs and the children's general conditions, the on-call paediatrician was asked to subjectively estimate the severity of AAI as mild, moderate, or severe,

using instructions introduced to him/her before the study. For example, mild imbalance, slow speech, or euphoria indicated the mild form; significant imbalance, slurred speech, disturbed consciousness or memory the moderate form; and the inability to stand or severe imbalance, disturbed memory, inability to speak or coma the severe form of AAI.

4.3. Samples and laboratory tests

The venous blood samples were drawn about 10–15 minutes after hospitalisation. The mean time between the ingestion of alcohol and collection of samples was 3.25 hours (range: 0.5 to 9 hours). Three venous blood samples were drawn for the measurements of serum and blood alcohol, plasma cortisol and sex hormones level, glucose, lactate, sodium, and potassium concentrations. Biochemical tests were performed immediately after the collection of samples. Lithium heparin plasma was separated and kept at -20^{o}C until hormone measurements were made within a three-month interval at the laboratory of Tartu University Hospital. Urine sample was also collected to exclude the use of narcotic substances.

At Tartu University Hospital glucose was determined using the glucose oxidase method, lactate with enzymatic colorimetry Cobas Integra 400plus (Roche), and potassium and sodium with the ISE direct method AVL 988-4 (Roche). At Tallinn Children's Hospital, glucose and lactate were determined with enzymatic membranes of amperometric electrodes, and potassium and sodium potentiometrically with an ABL 700 Radiometer (Radiometer Analytical). Serum ethanol concentration was determined using the enzymatic method TDxFLx (Abbott Diagnostics) in both hospitals. Both laboratories participated in the Labquality quality control programme. Cortisol, testosterone, estradiol and progesterone levels were measured by Immulite 2000 (Siemens Healthcare Diagnostics). The hormones laboratory participated in the RIQAS quality control programme.

Blood for the measurement of BAC was drawn into sodium fluoride-potassium oxalate vacutainer tubes. The BAC was measured using headspace gas chromatography (TurboMatrix 40 Headspace Sampler and Clarus 500) with two columns at the Estonian Forensic Science Institute. This laboratory also participates in the Labquality quality control programme. A urine screening test (Multiscreen 10 MTD, Biomedical Diagnostics) for narcotics was used to detect amphetamine, methamphetamine, ecstasy, opioids (morphine/heroine), methadone, cannabis (Δ-tetrahydocannabinole), cocaine, benzodiazepines, barbiturates and tricyclic antidepressants.

Reference values of the local laboratories were used to determine the abnormalities in biochemical results. Thus, hypokalaemia was defined as plasma potassium level < 3.5 mmol/L, hypoglycaemia as plasma glucose level < 3.3 mmol/L and hyponatraemia as plasma sodium level < 132 mmol/L. Biochemical results above the upper reference value were defined as hyperkalaemia

(potassium level > 5.1 mmol/L), hypernatraemia (sodium level > 145 mmol/L), and hyperlactinaemia (lactate level ≥ 2.4 mmol/L). Reference values of estradiol, progesterone and testosterone were used for children at the age of puberty.

Some biochemical results were at clinically critical value. This means that with this value the patient may be in imminent danger unless appropriate therapy is promptly initiated (Dighe *et al.*, 2006).

4.4. Statistical analysis

Statistical analysis was performed using the Statistica 8.0–10.0 statistical programmes. Descriptive statistics were used to analyse the children's characteristics and exposure data. Spearman Rank Order Correlations (r_s) and T-test were used to assess bivariate relationships. A p value < 0.05 was considered statistically significant.

The diagnostic performance characteristics were performed using the calculation´s formulas of sensitivity, specificity and efficiency.

5. RESULTS

5.1. Clinical signs in different AAI severity groups and comparison of AILCS with the SAC

5.1.1. The prevalence of clinical signs in different AAI severity groups

The level of consciousness ranged from normal to coma – somnolence was the most common clinical sign (n=92), followed by a normal conscious level (n=71) and disorientation (n=51). Coma, estimated as 8 points or less (5 to 8) by the Glasgow Coma Scale, was reported in 12 children. Normal balance was reported in 12 children and an imbalanced state in 124 (48.4%); 120 (46.9%) were unable to stand. On the speech scale, rushed speech was a frequent sign (n=103), followed by confused speech (n=64) and an inability to speak (n=60). The most correctly described signs in children in the different SAC groups were consciousness (r_s=0.16) and speech (r_s=0.13). The correlation between estimated drunkenness level and signs was p<0.0001. Passive behaviour was the most frequent sign of the AAI children (n=70; 27.3%), followed by restful behaviour (n=60) and impossible to assess (n=50).

Table 2. The most commonly observed AAI signs for different levels of alcohol intoxication in children.

SAC / SIGNS	0.21–1.49 g/L	1.50–2.49 g/L	≥ 2.50 g/L
Consciousness	Mild disorientation, somnolence	Disorientation or somnolence	Somnolence to coma
Speech	Normal or slow, in some cases rushed speech	Slow and confused speech	Inability to speak or confused speech
Balance	Normal or unbalanced	Unbalanced or not possible to assess due to condition	Impossible to assess due to condition
Behaviour	Apathy, restful	Apathy, aggressiveness or in some cases euphoria	Not possible to assess due to condition
Body temperature (°C)	35.8 (34.2–36.9)	35.6 (33.6–37.2)	35.7 (33.0–37.0)
Pulse (BPM)	85.4 (48–120)	85.6 (47–140)	83.1 (47–129)
Systolic blood pressure (mmHg)	112.1 (76–150)	109.1 (76–153)	109.8 (71–140)
Diastolic blood pressure (mmHg)	66.2 (42–90)	66.5 (30–110)	62.6 (40–90)

Muscle tone had decreased in 119 children and increased in eight. Mean body temperature, pulse, and blood pressure were not statistically different between the different alcohol concentration groups. The mean body temperature was similar from October to March (mean 35.6±0.65 degrees) and from April to September (mean 35.6±0.64). The mean heart rate was 83.9±15.2 beats per minute (in the range of 47–132); systolic blood pressure was 108.7±14.3 mm Hg (in the range of 71–153) and diastolic blood pressure was 65.3 ±12.1 mm Hg (in the range of 35–110). The most commonly observed AAI signs for different levels of alcohol intoxication in children are given in Table 2.

Overall, children had started to consume alcohol 3 hours and 16 minutes (ranging from 30 minutes to 9 hours) before the hospitalisation and the main type of beverage consumed was strong alcohol, such as spirit cocktails, brandy, or vodka (63.2%), followed by light alcohol (21.3%) or both types of beverages. The mean amount of alcohol consumed was four standard drinks, ranging from 0.25 to 16 drinks, and 38.9% of children reported that this AAI was their first. The clinical signs did not differ between boys and girls or between the age groups.

5.1.2. The distribution of subjects by the AILCS and by SAC or BAC

The distribution of subjects by the levels of AAI estimated by the clinical signs (AILCS) and SAC or BAC is given in Table 3.

Table 3. Distribution of children (number and %) by alcohol intoxication estimated by signs (mild, moderate or severe) and serum or blood alcohol concentrations.

SAC; BAC / AILCS	Serum alcohol concentration g/L				Blood alcohol concentration mg/g			
	0.20–1.50 mild	1.51–2.50 moderate	>2.50 severe	Total	0.20–1.50 mild	1.51–2.50 moderate	>2.50 severe	Total
Mild	13 48.2	12 44.4	2 7.4	27 10.5	20 74.1	6 22.2	1 3.7	27 10.5
Moderate	28 23.9	77 65.8	12 10.3	117 45.7	52 44.4	62 53.0	3 2.6	117 45.7
Severe	8 10.2	46 58.2	25 31.6	79 30.9	15 19.0	53 67.1	11 13.9	79 30.9
Not estimated	5 15.2	24 72.7	4 12.1	33 12.9	7 21.2	25 75.8	1 3.0	33 12.9
Total	54 21.1	159 62.1	43 16.8	256 100	94 36.7	146 57.0	16 6.3	256 100

Moderate alcohol intoxication was the most common level assessed by the clinical signs (n=117; 45.7%) followed by severe intoxication (n=79; 30.9%). The concurrency of the assessed signs and the SAC occurred in 48.2% (n=13) in the mild, 65.8% (n=77) in the moderate and 31.6% (n=25) in the severe

alcohol intoxication group. The most noticeable difference was in the severe intoxication by the signs group, with a ≥ 0.20–1.50 g/L (or mild) SAC and a 0.20–1.50 mg/g BAC. The children in this group were seven boys aged 11–14 years and one 16-year-old girl: their balance was disturbed, muscle tone had decreased, they exhibited somnolence and stupor, their memory failed, and body temperature was between 34.8–36.2 degrees Celsius. One sixteen-year-old girl in this group was mistakenly not assessed for narcotic drugs. Additionally, seven boys aged 11–14 years were in the ≥0.20–1.50 g/L SAC group and moved to the mild BAC group. In total, 14 younger children were classified as being severely alcohol intoxicated by the signs.

Two children with frequent experience consuming alcohol (16-year-old boys) were in a mild state of drunkenness by the signs, but in the severe SAC group. One of them was in the moderate alcohol intoxication group by the BAC. The main sign of alcohol intoxication they exhibited was disturbed balance (Figure 7).

Age did not correlate significantly with the AAI stage, BAC, or SAC levels, except in the younger group of children, where the serum alcohol concentration correlated positively with age (r_s=0.23; p=0.013).

5.2. The diagnostic performance characteristics (sensitivity, specificity, efficiency) of clinical assessment in the diagnosis of AAI in children

Comparing the clinical signs of AAI with the SAC (Table 4), the physicians were most accurate in diagnosing the clinical signs in terms of mild intoxication, with a sensitivity of 26.5%, specificity of 92%, and efficiency of 77.6%. Moderate alcohol intoxication was diagnosed with a sensitivity of 57%, specificity of 54.5%, and efficiency of 56.1%; severe drunkenness with a sensitivity of 64.1, specificity of 70.6%%, and efficiency of 69.5%. Thus, the average efficiency to diagnose the right AAI severity group, determined by the SAC and using a physician´s clinical judgement, was 67.7%. These data also show that clinically, doctors estimated the severity of drunkenness to be one level more severe than our definition according to the SAC.

Comparing the clinical signs of AAI with the BAC, mild intoxication was diagnosed with a sensitivity of 23.0%, specificity of 94.9%, and efficiency of 66.8%. Moderate alcohol intoxication was identified with a sensitivity of 51.2%, specificity of 46.1%, and efficiency of 48.9%; severe intoxication with a sensitivity of 73.0%, specificity of 67.3%, and efficiency of 67.7%. Thus, the average efficiency in diagnosing the right AAI severity group, determined by the BAC and using the physician´s clinical judgement, was 61.1%.

Table 4. The sensitivity, specificity and efficiency of clinical assessment in the diagnosis of AAI comparing with the SAC or BAC.

AILCS / Diagnostic performance %	Mild vs SAC	Moderate vs SAC	Severe vs SAC	Mild vs BAC	Moderate vs BAC	Severe vs BAC
Sensitivity	26.5	57.0	64.1	23.0	51.2	73.0
Specificity	92.0	54.5	70.6	94.9	46.1	67.3
Efficiency	77.6	56.1	69.5	66.8	48.9	67.7

5.3. The establishment of SAC/BAC ratio in children

There was the shift from a higher SAC to lower BAC in all AAI groups, and the correlation between the serum and blood alcohol concentrations was very good (r_s=0.93; p<0.0001) (Figure 2).

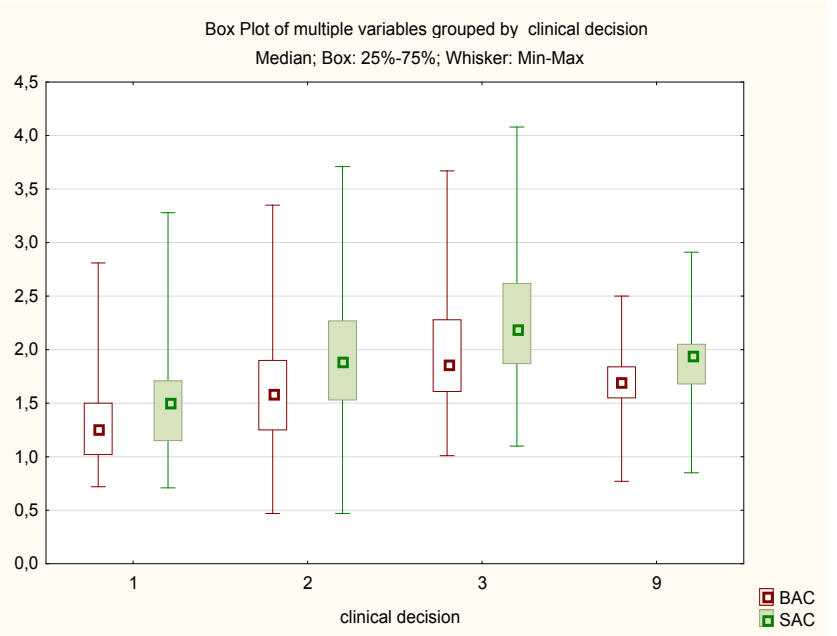


Figure 2. The correlation between blood alcohol concentration (BAC) and serum alcohol concentration (SAC) in alcohol-intoxicated children. X-axis: Alcohol intoxication levels by clinical decision or signs (1: mild; 2: moderate; 3: severe; 9: not estimated). Y-axis: BAC (mg/g) compared with SAC (g/L).

The mean ratio SAC:BAC was 1.19:1±0.13, from 1.17:1 in the mild AAI group to 1.19:1 in the moderate and severe AAI groups. The mean ratio was not significantly different between boys and girls or between the different age groups.

5.4. The prevalence of biochemical (glucose, lactate, potassium and sodium) changes in serum in children with AAI, and the impact of SAC on the biochemical changes in serum

The summary of ethanol, biochemical and hormonal markers concentrations in different AAI groups is given in Table 5.

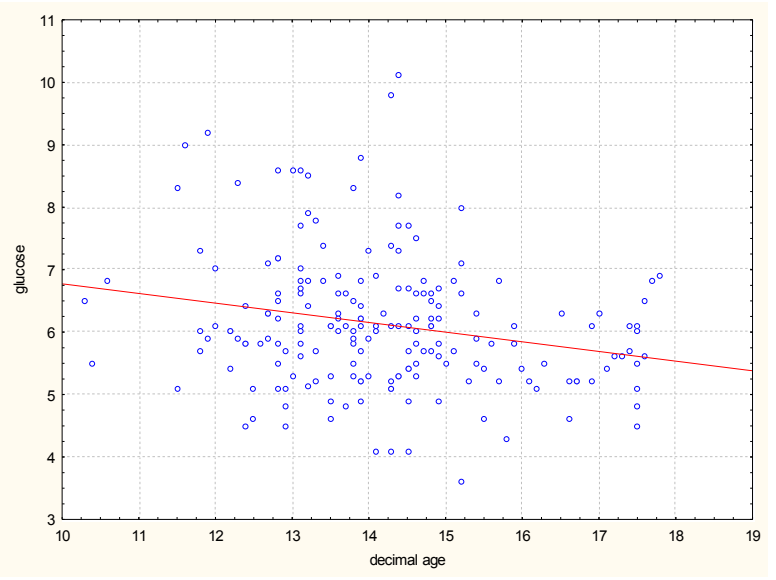
Table 5. Ethanol, glucose, lactate, sodium and cortisol concentrations in all; estradiol and progesterone levels in girls, testosterone level in boys in alcohol-intoxicated children.

Analyte	Mean ± SD range	AAI Gr 1 0.21–1.49 g/L	AAI Gr 2 1.50–2.49 g/L	AAI Gr 3 ≥2.50 g/L	Reference value
Ethanol (g/L) in serum	2.01 ± 0.60 0.47–4.08	1.24 ± 0.24 0.47–1.49	1.99 ± 0.27 1.50–2.48	2.89 ± 0.31 2.52–4.08	<0.20
Glucose (mmol/L)	6.1 ±1.2 3.0–13.1	6.0±1.4 3.5–12.7	6.1±1.2 3.0–13.1	5.9±0.9 4.3–8.3	3.3–-6.1
Lactate (mmol/L)	2.8 ± 0.9 0.5–6.6	2.8±1.0 0.5–5.6	2.8±0.9 1.1–6.6	2.5±0.8 1.0–5.2	<2.4
Sodium (mmol/L)	142.7 ± 3.1 132.0–154.5	142.1±2.9 133.0–148.0	142.6±3.1 132.0–151.0	143.9±3.2 138.0–154.5	132–145
Potassium (mmol/L)	3.6 ± 0.5 2.1–6.9	3.6±0.7 2.1–6.9	3.5±0.4 2.4–5.3	3.6±0.4 2.8–5.0	3.5–5.1
Estradiol (pmol/L)	210.1±185.1 <73.0–910.0	184.3±144.5 <73.0–525.0	209.0±186.3 <73.0–910.0	240.9±218.0 <73.0–903.0	101.0–1468.0
Progesterone (nmol/L)	4.87±5.2 <0.64–32.4	3.3±1.9 0.6–7.3	5.2±5.7 0.6–32.4	4.8±4.5 0.6–22.1	1.5–67.0
Testosterone (nmol/L)	8.5±6.2 <0.7–25.7	6.2±5.7 <0.7–19.4	8.7±6.3 <0.7–23.0	11.3±5.8 0.74–25.7	9.9-52.4
Cortisol (nmol/L)	624.9±242.2 87.5–1294.0	614.2±232 89.4–1013.0	656.6±232.8 139.0–1294.0	520.2±263.0 87.5–1159.0	140.0–600.0 (mornings) ½ from morning level (evenings)

Girls in younger age group (n=3) in the first group of alcohol concentration, SAC ≥ 0.20–1.49 g/L, had significantly higher serum alcohol concentration than boys (n=15; 1.40 vs. 1.21 g/L, p=0.02), whereas opposite tendency was seen in older age group (1.17 vs. 1.26 g/L), however, the latter difference was not statistically significant.

5.4.1. Glucose

In the overall group, there was a statistically significant, but relatively weak, negative correlation between the glucose concentration and age (r=-0.21; p=0.003), i.e. glucose levels decreased with increasing age (Figure 3).



$r = -0.2155$; $p = 0.0025$; $r^2 = 0.0465$

Figure 3. The relationship between plasma glucose concentration (mmol/l) and age (years) (r=-0.21; p=0.003) in acutely alcohol-intoxicated children.

This correlation remained significant after correcting for blood alcohol concentration. In the older age group plasma glucose levels tended to be within reference range, whereas in the younger age group there was a tendency towards increased glucose levels.

As seen in the scatter plot, a glucose level above the reference value (6.1 mmol/L) was evident in 78 (40.2%) of the children. In 29 (15%) children, the glucose level was equal to or higher than 7 mmol/L. In older children glucose values were more stable within reference values, except for four very high values of more than 9 mmol/L that would have necessitated further studies in respect to glucose metabolism. None of our subjects were hypoglycaemic, i.e. had plasma glucose < 3.3 mmol/L.

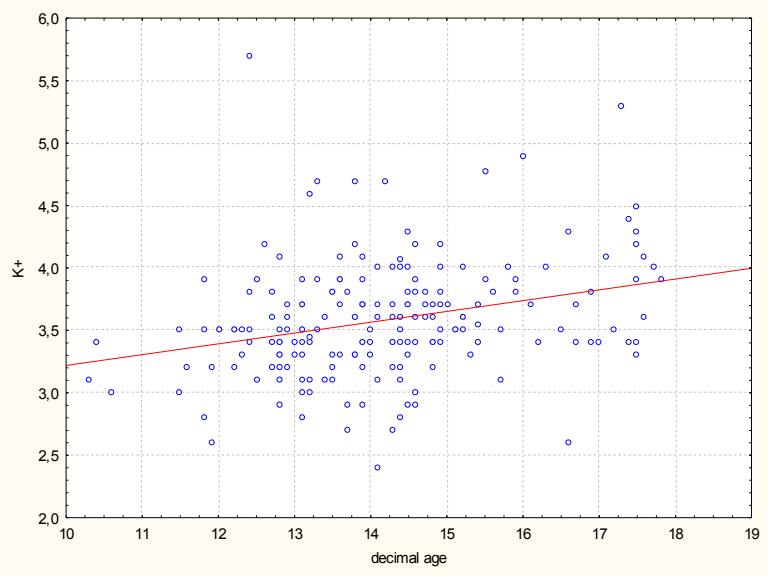
5.4.2. Lactate

Hyperlactinaemia (>2.4 mmol/l) occurred in 66% of the children and there were three children with lactate levels above 5 mmol/L. There was a statistically significant, but relatively weak negative correlation (r=-0.31; p=0.0001) between lactate concentration and age, i.e. lactate values of older children were more within the reference range, whereas increased lactate values were more common in younger children. This correlation remained significant after correcting for blood alcohol concentration.

5.4.3. Potassium

The mean serum potassium concentration (3.6 mmol/L) was very close to the lower reference limit of 3.5 mmol/L, and half of the children (50%) were hypokalaemic (< 3.5 mmol/L). There was a statistically significant but relatively weak positive correlation between plasma potassium concentration and age (r=0.3; p < 0.0001), i.e. the younger children had more hypokalaemia (64%) than older children (Figure 4). This correlation remained significant after correcting for blood alcohol concentration.

Five children (2.6%) had hypokalaemia in the critical value, e.g. under 2.8 mmol/L.



r = 0.2920; p = 0.00004; r^2 = 0.0853

Figure 4. The relationship between plasma potassium concentration (mmol/l) and age (years) (r=0.29; p=0.0004) in acutely alcohol-intoxicated children.

5.4.4. Sodium

In general, plasma sodium concentration was within reference range, although in five children it had relatively high values (above 150.0 mmol/L).

5.4.5. The impact of SAC on the biochemical changes

The plasma glucose concentrations were slightly lower for the girls in each alcohol concentration group compared to boys, but the differences were not statistically significant.

The mean plasma lactate concentration was above the reference value (< 2.4 mmol/L) in each alcohol concentration group, except for the girls in ≥ 0.20–1.49 g/L group.

Plasma potassium concentration did not differ between the different alcohol concentration groups, but in the younger age children group there was a statistically significant but relatively weak negative correlation between the ethanol concentration and plasma potassium concentration values (r=-0.23; p=0.028).

The mean plasma sodium concentration tended to be slightly higher in the ≥ 2.50 g/L alcohol concentration group. The plasma sodium concentration correlation to alcohol concentration was statistically significant, but a relatively weak positive correlation (r=0.21; p=0.023) (Figure 5). There was a strong correlation (p=0.006) between the alcohol levels above 1.50 g/L and sodium concentrations.

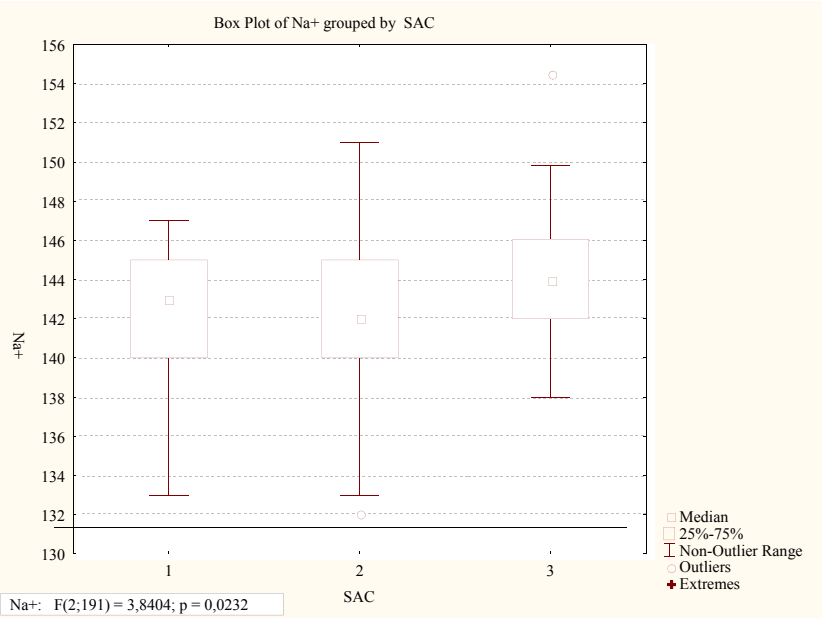


Figure 5. Correlation of sodium (mmol/l) and alcohol (g/L) concentrations. 1: 0.20–1.49 g/L, 2: 1.50–2.49 g/L, 3: ≥2.50 g/L ethanol concentration in serum.

5.5. The hormonal changes (testosterone, estradiol, progesterone and cortisol) in AAI children and their relationship with plasma biochemical markers

In girls, plasma estradiol and progesterone concentration did not significantly correlate to age, but for boys there was a positive correlation between plasma testosterone concentration and age ($r=0.63$; $p<0.0001$). Plasma cortisol concentration in the whole group correlated positively with plasma glucose levels ($r=0.29$; $p<0.05$) (Figure 6).

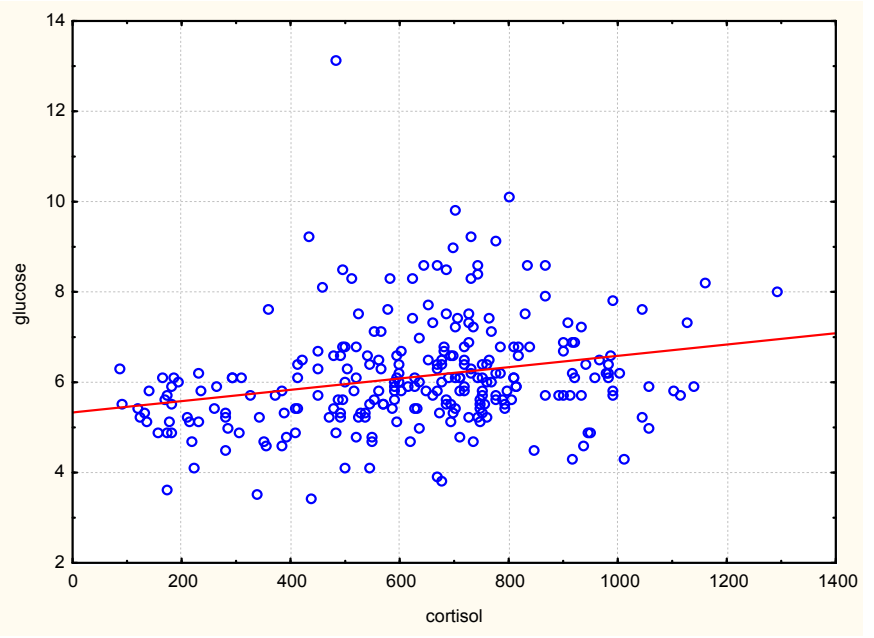


Figure 6. Correlation between plasma cortisol (nmol/l) and glucose (mmol/l) levels ($r=0.29$; $p<0.05$).

Cortisol concentration correlated positively with plasma lactate concentration ($r=0.32$; $p<0.0001$) and negatively with sodium level ($r=-0.25$; $p<0.0001$) (Figure 7).

Figure 7. Correlation between plasma cortisol (nmol/l) and sodium (mmol/l) levels (r=-0.25; p<0.0001).

Plasma progesterone concentration was positively correlated with cortisol (r=0.62; p<0.05), glucose (r=0.30; p<0.05), and lactate (r=0.22; p<0.05), and negatively with sodium levels (r=-0.22; p<0.05).

Plasma testosterone concentration was positively correlated with plasma potassium (r=0.29; p<0.05) and sodium (r=0.23; p<0.05), and negatively with lactate levels (r=-0.17; p<0.05).

For the group of children with serum alcohol concentration ≥0.20–1.49 g/L, testosterone concentration for boys correlated positively with age (r=0.80; p<0.0001) and with sodium concentration (r=0.34; p<0.05). In girls, progesterone concentration correlated positively with glucose (r=0.62; p<0.05) and cortisol (r=0.67; p<0.01) levels.

Among those with a serum alcohol concentration of 1.50–2.49 g/L, testosterone concentration for boys correlated positively with age (<0.0001; r=0.53) and with potassium level (p<0.002; r=0.32). In girls, the progesterone level correlated positively with age (p<0.05; r=0.23) and cortisol (p<0.0001; r=0.65) concentration and negatively with sodium (p<0.05; r=-0.24) level.

Among the group of children with serum alcohol concentration more than 2.50 g/L, testosterone concentration in boys correlated positively with age (p<0.03; r=0.43) and negatively with glucose (p<0.01; r=-0.48) level. Lactate level only correlated positively with estradiol (p<0.02; r=0.62) level in girls with a serum alcohol concentration of more than 2.50 g/L.

6. DISCUSSION

The consumption of alcohol among children is a growing problem in many countries, including Estonia, and therefore our study involved a large number of children: overall, 212 intoxicated minors admitted to 2 hospitals over 2 years, and 394 over 3 years. The ratio of girls and boys, from 1:1.4 to 1:1.6, was similar to other studies (Marchi *et al*., 2003; McIntosh *et al*., 2004), where generally more boys were involved. The mean age of the children in our study (14.2 years) was similar to that in other studies (Schöberl *et al.*, 2008; Weinberg *et al*., 2006), except we encountered significantly fewer 16-year-old adolescents. In our study were three children younger than 10 years (8.4–9.9 year-old; one boy and two girls). We did not ask questions about their families in the medical assessment form, but paediatricians noted, that all these children were from families with social problems. Some studies showed the different pattern of gender and age in AAI children, where the ratio of boys and girls was quite equal and the mean age was over 15 years (Bouthoorn *et al.,* 2011; Kaminska *et al.,* 2012; Kuzelova *et al*., 2009; van Hoof *et al.*, 2010). We did not compare our results with other reports on effects of acute intoxication in young adults. Most of these studies have been done in a controlled situation with volunteers, with mean alcohol concentration in the blood up to 0.80–1.00 g/L (Cameli *et al*., 2009; Davies *et al*., 1999). The mean measured blood serum concentration of alcohol in our study subjects was 2.01 g/L, and we therefore did not consider these two situations comparable. Most frequently, children in our study were hospitalised with alcohol concentration between 1.50–2.50 g/L (N=232). Other studies have found similar results (Schöberl, 2008; Weinberg, 2006). Five children were in potentially lethal SAC or > 3.5 g/L (3.59–4.08 g/L): three of them were boys (one 14 and two 17-years-old) and two girls were 15 years old. The youngest child was in a coma and had a critically low potassium level (2.9 mmol/l). One girl had critically high lactate concentration (> 5.2 mmol/l), but the paediatrician assessed her clinical condition as moderate drunkenness. However, it is quite likely that the majority of children with mild AAI do not go to the hospital because their general condition is not so greatly affected. There were 23 children with previous health problems: the most often were psychiatric and asthmatic diseases (five cases of both), three children were epileptic, two with previous serious trauma, allergic problems, or heart diseases (insufficiency of the mitral valve and ventricular septal defect). The single children had hypothyroidism, diabetes (glucose concentration was 12.7 mmol/L), myxoma, diabetes and epilepsy together (glucose level was 9 mmol/L).

6.1. Clinical signs in different AAI severity groups and comparison of AILCS with the SAC

There have been very few studies about clinical symptoms of acute alcohol intoxication in children; most of these have been in children with a relatively

high level of alcohol concentrations (Madsen, 1990; Lamminpää, 1995). Clinical symptoms of AAI in children have been found similar to those seen in adults, but combining clinical signs and blood alcohol measurements in children will significantly help to asses child's general status. CNS disturbance signs were correlated with the SAC in the children, but the correlation was stronger with the BAC. The disturbed consciousness and balance problems in the children were similar to those seen in adults with AAI, as alcohol induces the inhibition of CNS and the disturbance of coordination, memory, consciousness, and speech. Reduced consciousness was also the most common clinical finding by other studies (Bouthoorn *et al*., 2011; Weinberg *et al.*, 2006), but the second important sign was hypothermia (Madsen, 1990). Ethanol itself enhances the decline in body temperature by the dilatation of the peripheral blood vessels, and therefore body temperature can slightly decrease. However, in our study we did not find statistically significant differences in body temperatures between seasons. Seasonal differences in body temperature can occur in areas with large temperature differences, as in Estonia, but the children in our study were mostly hospitalised from interior rooms. The few who were found outside were discovered and studied in the summer. In our study, slurred speech was not a useful sign in determining the severity of AAI in children, because it was common at every level of AAI. Although slurred speech is not common in chronically intoxicated adults even with a high BAC (Pisoni *et al.*, 1989; Sobell *et al*., 1982), it is common in children with AAI. Change in muscle tone was unpredictable in some children, as alcohol usually produces a decrease in muscle tone, but there were eight children with increased muscle tone, possibly due to aggressiveness.

6.2. The diagnostic performance characteristics of clinical assessment in the diagnosis of AAI in children

Estimation of the clinical signs and BAC of AAI together in the everyday work of hospitals is especially important for the early detection and intervention in the hazardous drinking habit of young people (Touquet *et al*., 2012). One of our major limitations was that we did not use a standardized method to determine the level of AAI by clinical signs and did not perform inter-rater reliability testing. However, all on-call doctors in this study have graduated from the only medical faculty in Estonia and their medical training in paediatrics has been similar. Therefore, we presume that there can be no major differences in the evaluation of a child admitted to the hospital, and the severity of AAI should be comparable. In comparing BAC and estimated AAI levels by the clinical signs, we found that doctors often clinically estimated the severity of intoxication in children as one or even two stages more severe than that indicated by the SAC. Thus, in comparing the diagnostic characteristics of clinical judgement between AAI groups determined biochemically, it is clear that the clinical judgement matched better with AAI determined by the SAC rather than by the BAC.

There were also 15 children for whom BAC was in the mild group (0.20–1.50 mg/g) but who were clinically assessed as severe AAI by the paediatrician i.e. the doctor estimated the severity of AAI as two stages more severe than that determined biochemically. Most of these children were younger than 14 years and the leading sign was strongly disturbed balance. The younger children probably had not used alcohol before and were therefore probably very sensitive to alcohol, or due to the children's organ systems being immature. The body of a younger child contains more water than in older children and adults, and as alcohol disperses easily in water of the body, the SAC in children may even be relatively low, but there are remarkable clinical signs of AAI due to the direct effect of alcohol on CNS. Acute alcohol intoxication signs in younger children can change quickly due to the differences in the alcohol metabolism rate, which in children is twice higher rate than that found in adults (Donovan, 2009; Leung, 1986).

In our study all the children were Caucasians, but generally the ethnicity of the subjects should be taken into consideration, as low activity of aldehyde dehydrogenase enzyme has been found in some Asians (Crabb *et al*., 1989; Thomasson *et al*., 1991).

6.3. The establishment of SAC/BAC ratio in children

Many authors have studied the ratio between the SAC and BAC in adults (Barnhill *et al*., 2007; Labianca, 2002; Rainey, 1993). The results of these studies can be used in legal cases, especially in those related to traffic incidents. Ethanol is spread well in the water of the body, and its concentration in serum is higher than in blood because the water content of the serum is higher than in the whole blood. The ratio between the SAC and BAC in adults has been found from 1.04:1 to 1.26, with an average of 1.14–1.15:1 (Charlebois *et al.,* 1996; Rainey, 1993). We found that the average SAC:BAC ratio in children was a bit higher (1.19:1) than that seen in adults, probably from the lower haematocrit of the blood, with no difference between age groups or gender of the children.

6.4. The prevalence of biochemical changes in children with AAI, and the impact of SAC on the biochemical changes in plasma

Alcohol consumption causes many changes in plasma glucose, lactate, potassium, and sodium concentrations, and is a complex process. For normal ranges, we have used local laboratory reference values that are based on recommended international references (Burtis *et al*., 2006; Heil *et al*., 2004).

Many studies have found that hypoglycaemia is a common problem in AAI (Hart *et al*., 1998; Kerr *et al*., 1990; Wilson *et al*., 1981). A large proportion of scientific articles support the idea that the reason for hypoglycaemia resulting in

AAI is the inhibition of gluconeogenesis and glucogenolysis by alcohol (Marshall *et al*., 2008; Siler *et al*., 1998). However, other opposing articles also exist (Mokuda *et al*., 2004). In our study, we have also seen more of a tendency towards higher glucose levels than hypoglycaemia. Alcohol may increase glucose levels through inhibiting the basal insulin secretion although glucose-stimulated insulin secretion has been found not to be impaired, from increasing cortisol secretion and enhancing gluconeogenesis from lactate (Shin *et al*., 2002). Increased cortisol level in a stressful situation is one of the most important factors in the mechanism of increasing glucose level.

Hyperlactinaemia was a common finding in our children, which was a feature similar to the other studies (McDonald *et al*., 1994; Umpierrez *et al*., 2000). One cause for the lactic acidosis is tissue hypoxia due to mild hypothermia or ethanol-induced central nervous system depression and altered blood vessel tone (Lien *et al*., 1999). Serum lactate concentration above 5 mmol/L is considered to be dangerous, because such lactate level has been associated with increased 3- and 30-day mortality levels in intensive care patients with acute alcohol intoxication (Stacpoole *et al*., 1994). In our study, there were three children with lactate levels above 5 mmol/L. Thus, serum lactate level should be measured in every child with AAI. The other possible reason for lactic acidosis is enhanced anaerobic glucose metabolism, which leads to increased lactate production.

According to the plasma potassium reference values (3.5–5.1 mmol/L), there was a tendency towards hypokalaemia. The mean plasma potassium level was near the lower reference value – 3.6 mmol/L, the lower quartile being 3.3 mmol/L and the upper quartile 3.9 mmol/L. Critically low potassium values, i.e. < 2.8 mmol/L, were present in 2.6% of children (n=5) in the biochemistry study. In the hormonal study, a critically low potassium level was found in 3.4% of children (n=9) and required correction with a potassium infusion. Continuous monitoring of these hypokalaemic patients was also necessary, as they were disorientated, somnolent, and their mean body temperature tended to be lower (35.4^{o}C) than in other children with normal potassium levels (mean temperature 35.8^{o}C). No arrhythmias were reported in any children.

This might result from ethanol-induced lactic acidosis and development of high glucose level, since these two factors may decrease the cellular level of potassium and its increased loss with urine and vomiting.

There was also a tendency towards hypernatraemia. However, in most cases it was mild and the plasma sodium levels exceeded 150 mmol/L only in five children. The most likely cause for hypernatraemia is not the excess of primary sodium, but the water deficit and hyperaldosteronism, which commonly occur in AAI.

Potassium and sodium were positively correlated with age in all groups of drunken children and correlated negatively with glucose and lactate. The possible reason for the differences in the correlations is that younger children were more affected by alcohol than older children were.

Despite the good statistical significance of our findings, the correlation coefficients were relatively low (r=-0.3 lactate concentration and age; r=0.3 potassium concentration and age; r=-0.21 glucose concentration and age; r=-0.23 ethanol and potassium concentrations; r=0.21 ethanol and sodium concentrations).

6.5. The hormonal changes in AAI children and their relationship with plasma biochemical markers

Alcoholism and heavy drinking has been associated with a dysregulation of the hypothalamic-pituitary-adrenal axis, with alcoholics showing higher basal cortisol levels and reduced inhibitory feedback control (Gianoulakis *et al*., 2003; Thayer *et al.*, 2006). Acute alcohol intoxication has also been shown to stimulate the hypothalamic pituitary adrenal axis and to increase plasma cortisol concentration (Owens *et al*., 1992; Patchev *et al*., 1995; Frias *et al*., 2002). For most of the children hospitalised at evening or night (from 5 p.m. to 2.30 a.m.; N=191), when cortisol secretion is normally very low, about half of the amount was secreted in the morning. The majority of patients had plasma cortisol levels above 110–460 nmol/l, the maximum reference value for that time of day (4–7 p.m. 80–460 nmol/L; midnight 30–110 nmol/L) (Marks *et al*., 2002). Pierucci-Lagha *et al*. found no change in cortisol levels in adult volunteers with an alcohol concentration of about 1.00 g/L. Other studies have found increasing cortisol levels in humans acutely intoxicated with ethanol (Välimäki *et al*., 1984; Wirth *et al.,* 2007). In our study, cortisol concentration was increased in all alcohol concentration groups. The most likely cause for rising cortisol levels is the stressful situation of the organism from the central nervous system being depressed by alcohol intoxication.

The study showed a positive correlation between progesterone and cortisol. Most progesterone in males is created during testicular production of testosterone, and most in females by the ovaries; it is also produced in the brain and by the adrenal gland, however, where progesterone is an indirect precursor to cortisol (Baulieu *et al*., 2001). Like cortisol, progesterone is released in response to the ACTH (Wirth *et al*, 2007; Genazzani *et al*., 1998).

Estradiol level was not correlated with alcohol concentration in our study, which was similar to the other studies (Sarkola *et al*., 1999). In our study a lot of girls were at the pre-menarche age or without a regular menstrual cycle. Girls older than 13–14 years were often in medically serious health conditions and we didn't obtain information about their menstrual cycle.

Cortisol elevates the level of plasma glucose (Verberne *et al.*, 2014; Garrel *et al*., 1995), and some studies have shown that the elevation of glucose is more pronounced in the evening than in the morning (Plat *et al*., 1999). Similar cortisol and glucose results were notable in our study.

7. CONCLUSIONS

1. The severity of alteration of consciousness was the leading sign in the clinical evaluation of children with AAI, and correlated well to the SAC. Disturbances in speech and balance were also common in AAI.

2. The variation of AAI signs could be confirmed by measuring the SAC as the "AAI gold standard" in hospitals. The average efficiency in diagnosing the right AAI severity group, determined by the SAC, was 67.7% in AAI children. Clinically, doctors tended to estimate the severity of drunkenness to be one level more severe than that determined by SAC.

3. The SAC/BAC ratio of 1.19 was established, and should be used in children regardless of their gender or age.

4. Glucose levels above the reference value, hyperlactinaemia, and hypokalaemia were common biochemical findings in children hospitalized with AAI. Hypoglycaemia, at least in our study, was rare. Plasma glucose, lactate, potassium, and sodium levels should be measured in all children hospitalized with AAI. Biochemical changes occurred in all SAC levels, but particularly were in SAC above 1.50 g/L.

5. Increased plasma cortisol level above reference values was a common finding in children with AAI. Cortisol correlated positively with plasma glucose level, and in girls, with plasma progesterone concentration.

8. REFERENCES

Albers HM, van der Lely N. (2004) Alcohol intoxication in four adolescents. *Ned Tijdschr Geneeskd* 148:1504–06.

Archimi A, Kuntsche E. (2014) Do offenders and victims drink for different reasons? Testing mediation of drinking motives in the link between bullying subgroups and alcohol use in adolescence. *Addict Behav* 21;39(3):713–6.

Auzepy P, Boutron HF, Richard C, Riou B. (1985) In France, is acute alcoholic intoxication in adults a metabolic emergency? *Rev Med Interne* 6(4):373–9.

Badrick E, Bobak M, Britton A, Kirschbaum C, Marmot M, et al. (2008) The Relationship between Alcohol Consumption and Cortisol Secretion in an Aging Cohort. *The Journal of Clinical Endocrinology & Metabolism* 93(3):750–757.

Baik I, Shin C. (2008) Prospective study of alcohol consumption and metabolic syndrome. *Am J Clin Nutr* 87(5):1455–63.

Barden AE, Croft KD, Beilin LJ, Philips M, Ledowski T, Puddey IB. (2013) Acute effects of red wine on cytochrome P450 eicosanoids and blood pressure in men. *J Hypertens* 31(11):2195–202.

Barnhill MT, Herbert D, Wells DJ. (2007) Comparison of hospital laboratory serum alcohol levels obtained by an enzymatic method with whole blood levels forensically determined by gas chromatography. *J Anal Toxicol* 31:23–30.

Baulieu EE, Robel P, Schumacher M (2001) Neurosteroids: beginning of the story. *Int Rev Neurobiol* 46:1–32.

Beattie JO, Hull D, Cockburn F. (1986) Children intoxicated by alcohol in Nottingham and Glasgow, 1973-84. *Br Med J* 292(6519):519–21.

Berg JM, Tymoczko JL, Stryer L. (2002) *Biochemistry 5th edition*. New York: WH Freeman. Section 30.5 Ethanol Alters Energy Metabolism in the liver.

Bouthoorn SH, van der Ploeg T, van Erkel NE, van der Lely N. (2011) Alcohol intoxication among dutch adolescents: acute medical complications in the years 2000-–2010. *Clin Pediatr* 50:244–51.

Bouthoorn SH, van Hoof JJ, van der Lely N. (2011) Adolescent alcohol intoxication in Dutch hospital centers of pediatrics: characteristics and gender differences. *Eur J Pediatr* 170:1023–30.

Bradford DE. (1984). Alcohol and the young child. *Alcohol Alcohol* 19:173–75.

Brunelle C, Assaad JM, Barrett SP, A Vila C, Conrod PJ, Tremblay RE, Pihl RO. (2004) Heightened heart rate response to alcohol intoxication is associated with a reward-seeking personality profile. *Alcohol Clin Exp Res* 28:394–401.

Brvar M, Bunc M. (2009) High-degree atrioventricular block in acute ethanol poisoning: a case report. *Cases Journal* 2:8559.

Burtis CA, Ashwood ER, Bruns DE. (2006). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th edn USA, Saunders.

Cameli M, Ballo P, Garzia A, Lisi M, Palmerini E, Spinelli T, et al. (2009). Acute Effects of Low Doses of Red Wine on Cardiac Conduction and Repolarization in Young Health Subjects. *Alcohol Clin. Exp Res*. 33:2141–46.

Carlsson E, Frostell A, Ludvigsson J, Faresjö M. (2014) Psychological stress in children may alter the immune response. *J Immunol* 192(5):2071–81.

Charlebois RC, Corbett MR, Wigmore JG. (1996) Comparison of ethanol concentrations in blood, serum, and blood cells for forensic applications. *J Anal Toxicol* 20:171–8.

Crabb DW, Edenberg HJ, Bosron WF, Li TK. (1989) Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. The inactive ALDH2(2) allele is dominant. *J Clin Invest* 83**:**314–6.

Currie C, Zanotti C, Morgan A, Currie D, de Looze M, Roberts C, Samdal O, Smith ORF, Barnekow V., eds. (2012) Social determinants of health and well-being among young people. Health Behaviour in School-aged Children (HBSC) study: international report from the 2009/2010 survey. Copenhagen, WHO Regional Office for Europe. *Health Policy for Children and Adolescents, No 6*.

de Looze M, ter Bogt TF, Raaijmakers QA, Pickett W, Kuntsche E, Vollebergh WA. (2015) Cross-national evidence for the clustering and psychosocial correlates of adolescent risk behaviours in 27 countries. *Eur J Public Health* 25(1):50–6.

Devaney M, Graham D, Greeley J. (2003) Circadian variation of the acute and delayed response to alcohol: investigation of core body temperature variations in humans. *Pharmacol Biochem Behav* 75(4):881–7.

Diagnostic and Statistical Manual of Mental Disorders 4th Ed. (1994) Washington DC: American Psychiatry Association.

Di Chiara G, Acquas E, Tanda G. (1996). Ethanol as a neurochemical surrogate of conventional reinforcers: the dopamine-opioid link. *Alcohol* 13(1):13–7.

Dighe AS, Rao A, Coakley AB, Lewandrowski KB. (2006). Analysis of Laboratory Critical Value Reporting at a Large Academic Medical Center. *Am J Clin Pathol* 125: 758–64.

Domschke S, Domschke W, Lieber CS. (1974). Hepatic redox state: Attenuation of the acute effects of ethanol induced by chronic ethanol consumption. *Life Sci* 15:1327–34.

Donovan JE. (2009) Estimated Blood Alcohol Concentrations for Child and Adolescent Drinking and Their Implications for Screening Instruments. *Pediatrics* 123:975–81.

Dubowski KM. (1980) Alcohol determination in the clinical laboratory. *Am J Clin Pathol* 74:747–50.

Edmunds SM, Ajizian SJ, Liguori A. (2014) Acute obtundation in a 9-month-old patient: ethanol ingestion. *Pediatr Emerg Care* 30(10):739–41.

Elisaf M, Liberopoulos E, Bairaktari E, Siamopoulos K. (2002) Hypokalaemia in alcoholic patients. *Drug Alcohol Rev* 21(1):73–6.

Endres HG and Grüner O. (1994) Comparison of D2O and ethanol dilutions in total body water measurements in humans. *Journal of Clinical Investigation* 72(11):830–7.

Estonian Institute of Economic Research. (2014) Alcohol Market, Consumption and harms in Estonia. *Yearbook, 2014*. Tallinn.

Estonian Institute of Economic Research. (2013) Alcohol Market, Consumption and harms in Estonia. *Yearbook*, *2013*. Tallinn.

European School Survey Project on Alcohol and Other Drugs. (2011) *World Health* Organization (WHO) *Report*.

Falk D, Yi H, Hiller-Sturmhöfel S. (2008) An Epidemiologic Analysis of Co-Occurring Alcohol and Drug Use and Disorders: Findings From the National Epidemiologic Survey of Alcohol and Related Conditions (NESARC). *Alcohol Research Health* 31(2):100–10.

Flensborg-Madsen T, Knop J, Mortensen EL, Becker U, Sher L, Grønbaek M. (2009) Alcohol use disorders increase the risk of completed suicide-irrespective of other psychiatric disorders. A longitudinal cohort study. *Psychiatry Res* 167(1-2):123–30.

Fong HF, Muller AA. (2014) An unexpected clinical course in a 29-day-old infant with ethanol exposure. *Pediatr Emerg Care* 30(2):111–3.

Frank H, Wians J. (2009) Clinical Laboratory Tests: Which, Why, and What Do The Results Mean? *LabMedicine* 40:105–13.

Frias J, Torres JM, Miranda MT, Ruiz E, Ortega E. (2002) Effects of acute alcohol intoxication on pituitary-gonadal axis hormones, pituitary-adrenal axis hormones, β-endorphin and prolactin in human adults of both sexes. *Alcohol Alcoholism* 37(2): 169–73.

Frias J, Rodriguez R, Torres JM, Ruiz E, Ortega E. (2000) Effects of acute alcohol intoxication on pituitary-gonadal axis hormones, pituitary-adrenal axis hormones, beta-endorphin and prolactin in human adolescents of both sexes. *Life Sci* 67(9):1081–6.

Frias J, Torres JM, Rodriguez R, Ruiz E, Ortega E. (2000) Effects of acute alcohol intoxication on growth axis in human adolescents of both sexes. *Life Sci* 67(22):2691–7.

Fulop M, Bock J, Ben-Ezra J, Antony M, Danzig J, Gage JS. (1986) Plasma lactate and 3-hydroxybutyrate levels in patients with acute ethanol intoxication. *Am J Med* 80(2):191–4.

Garrel DR, Moussali R, De Oliveira A, Lesiéqe D, Lariviére F. (1995) RU 486 prevents the acute effects of cortisol on glucose and leucine metabolism. *Clinical Endocrinology & Metabolism* 80(2):379–85.

Genazzani AR, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, et al. (1998) Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. *J Clin Endocrinol Metab* 83(6):2099–2103.

Gianoulakis C, Dai X, Brown T. (2003) Effect of chronic alcohol consumption on the activity of the hypothalamic-pituitary-adrenal axis and pituitary β-endorphin as a function of alcohol intake, age, and gender. *Alcohol Clin Exp Res* 27(3):410–23.

Green KM, Doherty EE, Zebrak KA, Ensminger ME. (2011) Association between adolescent drinking and adult violence: evidence from a longitudinal study of urban African Americans. *J Stud Alcohol Drugs* 72(5):701–10.

Hagemann CT, Helland A, Spigset O, Espnes KA, Ormstad K, Schei B. (2013) Ethanol and drug findings in women consulting a Sexual Assault Center – associations with clinical characteristics and suspicions of drug-facilitated sexual assault. *J Forensic Leg Med* 20(6):777–84.

Hammerstedt H, Chamberlain SL, Nelson SW, Bisanzo MC. (2011) Alcohol-related hypoglycemia in rural Uganda: socioeconomic and physiologic contrasts. *Int J Emerg Med* 4:5; doi: 10.1186/1865-1380-4-5.

Hannibal KE, Bishop MD. (2014) Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation. *Phys Ther* 94(12):1816–25.

Harris SK, Louis-Jacques J, Knight JR. (2014) Screening and brief intervention for alcohol and other abuse. *Adolesc Med State Art Rev* 25(1):126–56.

Hart SP, Frier BM. (1998). Causes, management and morbidity of acute hypoglycaemia in adults requiring hospital admission. *Q J Med* 91:505–10.

Heil W, Koberstein R, Zawta B. (2004). *Reference Ranges for Adults and Children.* Pre-Analytical Considerations (Roche).

Hirschl MM, Derfler K, Bieglmayer C, Röggla H, Zeiner A, Seidler D, Laggner AN. (1994). Hormonal Derangements in Patients with Severe Alcohol Intoxication. *Alcohol Clin Exp Res* 18:761–66.

Holdstock L, Penland SN, Morrow AL, de Wit H. (2006) Moderate doses of ethanol fail to increase plasma levels of neurosteroid 3α-hydroxy-5α-pregnan-20-one-like immunoreactivity in healthy men and women. *Psychopharmacology* 186:442–50.

Holmgren A, Jones AW. (2010) Demographics of suicide victims in Sweden in relation to their blood-alcohol concentration and the circumstances and manner of death. *Forensic Sci Int* 198(1-3):17–22.

Höjer J. (1996) Severe metabolic acidosis in the alcoholic: differential diagnosis and management. *Hum Exp Toxicol* 15(6):482–8.

Hughes K, Bellis MA, Hardcastle KA, McHale P, Bennett A, Ireland R, Pike K. (2015) Associations between e-cigarette access and smoking and drinking behaviours in teenagers. *BMC Public Health* 15(1):1618. doi: 10.1186/s12889-015-1618-4.

Husain K, Ansari RA, Ferder L. (2014) Alcohol-induced hypertension: Mechanism and prevention. *World J Cardiol* 6(5):245-52.

Iffland R, West A, Bilzer N, Schuff A. (1999) Zur Zuverlässigkeit der Blutalkoholbestimmung Das Verteilungsverha¨ltnis des Wassers zwischen Serum und Vollblut. *Rechtsmedizin* 9:123–30.

Jones AW. (1993) Pharmacokinetics of ethanol in saliva: comparison with blood and breath alcohol profiles, subjective feelings on intoxication, and diminished performance. *Clinical Chemistry* 39(9):1837–44.

Jones AW. (2006) Urine as a biological specimen for forensic analysis of alcohol and variability in the urine-to-blood relationship. *Toxicological Reviews* 25(1):15–35.

Jones AW and Jönsson KA. (1994) Food induced lowering of blood-ethanol profiles and increased rate of elimination immediately after a meal. *Journal of Forensic Sciences* 39(4):1084–93.

Kaminska H, Agnieszka ZB, Gawlik A, Malecka-Tendera E. (2012) Acute alcohol intoxication among children and adolescents admitted to the Department of Pediatrics, Pediatric Endocrinology and Diabetes, Medical University of Silesia, Katowice during 2000-2010 – preliminary study. *Przegl Lek* 69(10):777–80.

Kaplan MS, McFarland BH, Huguet N, Conner K, Caetano R, Giesbrecht N, Nolte KB.(2013) Acute alcohol intoxication and suicide: a gender-stratified analysis of the National Violent Death Reporting System. *Inj Prev* 19(1):38–43.

Karch SB. (1998) *Drug Abuse Handbook*. San Francisco, CRC Press LLC p.309.

Kerr D, Mcdonald IA, Heller SR, Tattersall RB. (1990) Alcohol causes hypoglycaemic unawareness in healthy volunteers and patients with type 1 (insulin-dependent) diabetes. *Diabetologia* 33:216–21.

Kim JY, Lee DY, Lee YJ, Park KJ, Kim KH, Kim JW, Kim WH. (2015) Chronic alcohol consumption potentiates the development of diabetes through pancreatic β-cell dysfunction. *World J Biol Chem* 6(1):1–15.

Kim JY, Song EH, Lee HJ, Oh YK, Park YS, Park JW, Kim BJ, Kim DJ, Lee I, Song J, Kim WH. (2010) Chronic ethanol consumption-induced pancreatic beta-cell dysfunction and apoptosis through glucokinase nitration and its down-regulation. *J Biol Chem* 285(48):37251–62.

King A, Munisamy G, de Wit H, Lin S (2006) Attenuated cortisol response to alcohol in heavy social drinkers. *International Journal of Psychophysiology* 59(3):203–209.

Klockhoff H, Naslund I, Jones AW. (2002) Faster absorption of ethanol and higher peak concentration in women after gastric bypass surgery. *British Journal of Clinical Pharmacology* 54:587–91.

Kornfält J, Johansson A. (2010) Occurrence of hypothermia in a prehospital setting, southern Sweden. *Int Emerg Nurs* 18(2):76–9.

Koski A, Sirén R, Vuori E, Poikolainen K. (2007) Alcohol tax cuts and increase in alcohol-positive sudden deaths – a time-series intervention analyses. *Addiction* 102(3):362–8.

Krzymien J, Karnafel W. (2013) Lactic acidosis in patients with diabetes. *Pol Arch Med Wewn* 123(3):91–7.

Kuzelova M, Hararova A, Ondriasova E, Wawruch M, Riedel R, Benedekova M, Kovacs L, Plakova S. (2009) Alcohol intoxication requiring hospital admission in children and adolescents: retrospective analysis at the University Children's Hospital in the Slovak Republic. *Clin Toxicol (Phila)* 47**:**556–61.

Labianca DA. (2002) Conversion of Serum-Alcohol concentrations to corresponding blood-alcohol concentrations. *J Chem Ed* 79:803.

Lahti A, Harju A, Hakko H, Riala K, Räsänen P. (2014) Suicide in children and young adolescents: a 25-year database on suicides from Northern Finland. *J Psychiatr Res* 58:123–8.

Lamminpää A, Vilska J. (1990). Acute Alcohol Intoxications in Children Treated in Hospital. *Acta Paediatr* 79:847–54.

Lamminpää A, Vilska J. (1991). Acid-base balance in alcohol users seen in an emergency room. *Vet Hum Toxicol* 33(5):482–5.

Lamminpää A, Vilska J, Korri UM, Riihimäki V (1993) Alcohol intoxication in hospitalized young teenagers. *Acta Paediatrica* 82(9):783–88.

Lamminpää A. (1994) Acute alcohol intoxication among children and adolescents. *Eur J Pediatr* 153:868–72.

Lamminpää, A. (1995). Alcohol intoxication in childhood and adolescence. *Alcohol Alcsm* 30:5–12.

Latvala A, Rose RJ, Pulkkinen L, Dick DM, Korhonen T, Kaprio J. (2014) Drinking, smoking, and educational achievement: cross-lagged associations from adolescents to adulthood. *Drug Alcohol Depend* 137:106–13.

Lee CK, Corte C, Stein KF, Finnegan L, McCreary LL, Park CG. (2014) Expected problem drinker possible self: predictor of alcohol problems and tobacco use in adolescents. *Subst abus* 31. doi:10.1080/08897077.2014.988323

Leung AK. (1986) Ethyl alcohol ingestion in children. A 15-year review. *Clin Pediatr* 25:617–9.

Lieber CS. Medical and nutritional complications of alcoholism: mechanisms and management. *New York: Plenum Press*, 1992:579.

Lieber CS. (2000). Alcohol: its metabolism and interaction with nutrients. *Annual Review of nutrition*. 20:395–430.

Lien D, Mader TJ. (1999). Survival from Profound Alcohol-Related Lactic Acidosis. *J Emerg Med* 17:841–46.

Lionte C, Sorodoc L, Laba V. (2004) Toxic-induced hypoglycemia in clinical practice. *Rom J Intern Med*. 42:447–55.

Lukas SE, Mendelson JH, Benedikt RA. (1986) Instrumental analysis of ethanol-induced intoxication in human males. *Psychopharmacology.* 89(1):8–13.

Madsen LP. (1990) Acute alcohol intoxication in children. Diagnosis, treatment and complications. *Ugeskr Laeger* 152(33):2362–64.

Madu SN, Matla MP. (2003) Illicit Drug Use, Cigarette Smoking and Alcohol Drinking Behaviour Among a Sample of High School Adolescents in the Pietersburg Area of the Northern Province, South Africa. *J Adolesc* 26:121–36.

Magis DC, Jandrain BJ, Scheen AJ. (2003) Alcohol, insulin sensitivity and diabetes. *Rev Med Liege* 58(7-8):501–7.

Marchi AG, Lamminpää A, Azeredo P, Williamson L, Cabecadas M, Renier S. (2003) Acute alcohol intoxication in adolescents. A multicentre study. *Ital J Pediatr* 29:211–16.

Markina A, Šahverdov-Žarkovski B. (2007) *Eesti alaealiste hälbiv käitumine 2006*. Justiitsministeerium Kriminaalpoliitika osakond, Tallinn: Tartu Ülikooli Õigus-instituut.

Marks V, Cantor T, Mesko D, Pullmann R, Nosalova G. (2002) *Differential Diagnosis by Laboratory Medicine. A Quick Reference for Physicians*. Germany, Springer.

Marshall WJ, Bangert SK, Marshall S. (2008). Alcohol-induced hypoglycaemia. *Clinical Biochemistry*: *Metabolic and Clinical aspects.* 2nd edn. Philadelphia, USA: Elsevier, 2008.

McBeth J, Chiu YH, Silman AJ, Ray D, Morriss R, Dickens C, Gupta A, MacFarlane GJ. (2005) Hypothalamic-pituitary-adrenal stress axis function and the relationship with chronic widespread pain and its antecedents. *Arthritis Res Ther* 7(5):992-1000.

McDonald L, Kruse JA, Levy DB, Marulendra, S, Sweeny PJ. (1994). Lactic acidosis and acute ethanol intoxication. *Am J Emerg Med* 12:32–5.

McIntosh J, MacDonald F, McKeganey N. (2004). Pre-teenage children's experiences of drug use. *Int J Drug Pol*. 16:37–45.

McKnight AJ, Langston EA, Marques PR, Tippetts AS. (1997) Estimating blood alcohol level from observable signs. *Accid Anal Prev* 29:247–55.

Medcalc: *Total Body Water/Urea Volume of Distribution*, Nov 11, 2001. http:// www.medcalc.com/tbw.html

Mennella JA, Pepino MY, Teff KL. (2005) Acute alcohol consumption disrupts the hormonal milieu of lactating women. *Journal of Clinical Endocrinology and Metabolism* 90(4):1979–85.

Meyer S, Steiner M, Mueller H, Nunold H, Gottschling S, Gortner L. (2007). Recent trends in the burden of alcohol intoxication on pediatric in-patient services in Germany. *Klin Padiatr* 220:6–9.

Minera G, Robinson E. (2014) Accidental acute alcohol intoxication in infants: review and case report. *J Emerg Med* 47(5):524–6.

Mirand AL, Welte JW. (1994) Total body water adjustment of mean alcohol intakes. *J Subst Abuse* 6(4):419–25.

Mokuda O, Tanaka H, Hayashi T, Ooka H, Okazaki R, Sakamoto Y. (2004). Ethanol stimulates glycogenolysis and inhibits both glycogenesis via glyconeogenesis and from exogenous glucose in perfused rat liver. *Ann Nutr Metab* 48:276–80.

Monk L, Jones A. (2014) Alcohol consumption as a risk factor for sexual assault: a retrospective analysis. *J Forensic Leg Med* 23:55–61.

Morgan CJ and Badawy AA-B. (2001) Alcohol-induced euphoria: exclusion of serotonin. *Alcohol and Alcoholism* 36(1):22–5.

Mørland J, Steentoft A, Simonsen KW, Ojanperä I, Vuori E, Magnusdottir K, Kristinsson J, Ceder G, Kronstrand R, Christophersen A. (2011) Drugs related to motor vehicle crashes in northern European countries: A study of fatally injured drivers. *Accident analyses and Prevention* 43(6):1920-26. DOI: 10.1016/ j.aap.2011.05.002.

Müssig K, Schleicher ED, Häring HU, Riessen R. (2008) Satisfactory outcome after severe ethanol-induced lactic acidosis and hypoglycaemia. *J Emerg Med* 34(3):337–8.

Nieminen MM, Fyhrquist F, Linkola J, Tikkanen I, Tontti K. (1981) Renin-aldosterone axis in ethanol intoxication. *Pharm Bioch and Behav* 15(6):879–82.

Obasi EM, Shirtcliff EA, Brody GH, MacKillop J, Pittman DM, Cavanagh L, Philibert RA. (2015) The relationship between alcohol consumption, perceived stress, and CRHR1 genotype on the hypothalamic-pituitary-adrenal axis in rural African Americans. *Front Psychol* 6:832.

Owens MJ, Nemeroff CB (1992) Physiology and pharmacology of corticotropin-releasing factor. *Pharm Rev* 43(4):425–73.

Palano GM, Pratico AD, Pratico ER, D´Agata A, Carpinato C, Sottile F, Distefano G. (2007) Accidental ethyl alcohol intoxication in a 30-day-old infant. Clinical findings and neurological follow-up. *Minerva Pediatr* 59(3):275–9.

Patch D, Szurkowska M, Szafraniec K, Targosz D, Sulek M, Kamenczak A, Huszno B. (2007) Carbohydrate metabolism in acute poisoning with xenobiotics. *Przegl Lek* 64(4-5):243–7.

Patchev VK, Hayashi S, Orikasa C, Almeida OF (1995) Implications of estrogen-dependent brain organization for gender differences in hypothalamo-pituitary-adrenal regulation. *FASEB J* 9:419–23.

Paton A. (1999). Reflections on Alcohol and the Young. *Alcohol Alcsm* 34:502–5.

Patrick ME, Schulenberg JE. (2013) Prevalence and predictors of adolescent alcohol use and binge drinking in the United States. *Alcohol Res* 35(2):193–200.

Perkin RM, Swift JD, Newton DA, Anas NG. (2007). *Common Electrolyte Problems in Pediatrics. Pediatric Hospital Medicine, 2nd edn.* Philadelphia, USA: Lippincott Willams @ Wilkins.

Pierucci-Lagha A, Covault J, Feinn R, Khisti RT, Morrow AL, et al. (2005) Subjective effects and changes in steroid hormone concentrations in humans following acute consumption of alcohol. *Psychopharmacology* 186:451–61.

Pietraszek A, Gregersen S, Hermansen K. (2010) Alcohol and type 2 diabetes. A review. *Nutr Metab Cardiovasc Dis* 20(5):366–75.

Pisoni DB, Martin CS. (1989) Effects of alcohol on the acoustic-phonetic properties of speech: perceptual and acoustic analyses. *Alcohol Clin Exp Res* 13:577–87.

Plat L, Leproult R, L´HermiteBaleriaux M, Fery F, Mockel J, et al. (1999) Metabolic Effects of Short-Term Elevations of Plasma Cortisol Are More Pronounced in the Evening Than in the Morning. *Clinical Endocrinology & Metabolism* 84(9):3082–92.

Puddey IB, Beilin LJ. (2006) Alcohol is bad for blood pressure. *Clin Exp Pharmacol Physiol* 33(9):847–52.

Rainey PM. (1993) Relation between serum and whole-blood ethanol concentrations. *Clin Chem* 39:2288–92.

Rauchenzauner M, Kountchev J, Ulmer H, Pechlaner C, Bellmann R, Wiedermann CJ, Joannidis M. (2005) Disturbances of electrolytes and blood chemistry in acute alcohol intoxication. *Wien Klin Wochenschr* 117(3):83–91.

Reid A, Jones G, Isles C. (2012) Hypokalaemia: common things occur commonly – a retrospective survey. *JRSM Short* R*ep* 3*(*11):80.

Reims HM, Kjeldsen SE, Brady WE, Dahlöf B, Devereux RB, Julius S, Beevers G, de Faire U *et al*. (2004) Alcohol consumption and cardiovascular risk in hypertensives with left ventricular hypertrophy: the LIFE study. *J Hum Hypertens* 18:381–9.

Roy M, Bailey B, Chalut D, Senecal P e, Gaudreault P. (2003). What are the adverse effects of ethanol used as an antidote in the treatment of suspected methanol poisoning in children? *J Toxicol Clin Toxicol.* 41:155–61.

Ryan JM, Howes LG. (2002) Relations between alcohol consumption, heart rate, and heart rate variability in men. *Heart* 88:641–2.

Sarkola T, Mäkisalo H, Fukunaga T, Eriksson CJ. (1999) Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. *Alcohol Clin Exp Res* 23(6):976–82.

Schöberl S, Nickel P, Schmutzer G, Siekmeyer W, Kiess W. (2008). Acute ethanol intoxication among children and adolescents. A retrospective analysis of 173 patients admitted to a university children hospital. *Klin Padiatr.* 220:253–58.

Shajani NK, Godolphin W, Image BA. (1989) Blood alcohol analysis: comparison of whole blood analysis by gas chromatography with serum analysis by enzymatic method. *Canadian Forensic Science Journal*. 22(1):317–29.

Shavit I, Konopnicki M, Winkler K, Neuman G, Kozer E, Bentur Y. (2012) Serum glucose and electrolyte levels in alcohol-intoxicated adolescents on admission to the emergency department: an unmatched case-control study. *Eur J Pediatr* 171(9): 1397–400.

Shin JS, Lee JJ, Yang JW, Kim CW. (2002). Ethanol decreases basal insulin secretion from HIT-T15 cells. *Life Sci* 70:1989–97.

Siler SQ, Neese RA, Christiansen MP, Hellerstein MK. (1998). The inhibition of gluconeogenesis following alcohol in humans. *Am J of Physiology* 275:897–907.

Sobell LC, Sobell MB, Coleman RF. (1982) Alcohol-induced dysfluency in nonalcoholics. *Folia Phoniatr* 34:316–23.

Spencer RL, Hutchison KE. (1999) Alcohol, aging and the stress response. *Alcohol Res Health* 23:272–83.

Stacpoole PW, Wright EC, Baumgartner TG, Bersin RM, Buchalter S, Curry SH, Duncan C, Harman EM, Henderson GN, Jenkinson S, et al. (1994). Natural history and course of acquired lactic acidosis in adults. *Am J Med* 97:47–54.

Stasiukyniene V. (2002). Blood plasma potassium, sodium and magnesium levels in chronic alcoholic patients during alcohol withdrawal. *Med* 38:892–95.

Sutherland I, Willner P. (1998). Patterns of Alcohol, Cigarette and Illicit Drug Use in English Adolescents. *Addiction* 93:1199–1208.

Thayer JF, Hall M, Sollers JJ 3rd, Fischer JE. (2006) Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: evidence for impaired inhibitory control of the HPA axis in heavy drinkers. *Int J Psychophysiol* 59(3):244–50.

The Summary Health Statistics for U.S Adults: National Health Interview Survey, 2012. (2012) http://www.cdc.gov/nchs/data/series/sr_10/sr10_260.pdf

Thomasson HR, Edenberg HJ, Crabb DW, Mai XL, Jerome RE, Li TK, Wang SP, Lin YT, Lu RB, Yin SJ. (1991) Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet* 48:677–81.

Touquet R, Harris D. (2012) Alcohol misuse Y91 coding in ICD-11: rational terminology and logical coding specifically to encourage early identification and advice. *Alcohol and Alcoholism* 47:213–15.

Umpierrez GE, DiGirolamo M*, et al*. (2000). Differences in Metabolic and Hormonal Milieu in Diabetic- and Alcohol-Induced Ketoacidosis. *J Crit Care* 15: 52–9.

U.S. Department of Health and Human Services. (2007) *The Surgeon General´s Call to Action to Prevent and Reduce Underage Drinking*. Rockville, MD: U.S. Department of Health and Human Services.

Vamvakas S, Teschner M, Bahner U, Heidland A. (1998) Alcohol abuse: potential role in electrolyte disturbances and kidney diseases. *Clin Nephrol* 49(4):205–13.

van Cauter E, Polonsky KS, Scheen AJ. (1997) Roles of circadian rhythmicity and sleep in human glucose regulation. *Endocr Rev* 18(5):716–38.

van de Wiel A. (2004) Diabetes mellitus and alcohol. *Diabetes Metab Res R* 20(4):263–7.

van Hoof JJ, Lely N, Pereira RR, van Dalen WE. (2010) Adolescent alcohol intoxication in the Dutch hospital departments of Pediatrics. *J Stud Alcohol Drugs* 71**:**366–72.
Verberne AJ, Sabetghadam A, Korim WS. (2014) Neural pathways that control the glucose counterregulatory response. *Front Neurosci* 26(8):38.
Vonghia L, Leggio L, Ferrulli A, Bertini M, Gasbarrini G, Addolorato G. (2008) Acute Alcohol Intoxication. *Europ J Int Med* 19:561–67.
Välimäki MJ, Härkonen M, Ylikahri RH. (1983) Accute effects of alcohol on female sex hormones. *Alcoholism: Clinical and Experimental Research* 7(3):289–93.
Välimäki MJ, Härkönen M, Eriksson CJ, Ylikahri RH. (1984) Sex hormones and adrenocortical steroids in men acutely intoxicated with ethanol. *Alcohol* 1(1):89–93.
Weinberg L, Wyatt JP. (2006) Children presenting to hospital with acute alcohol intoxication. *Emerg Med J* 23(10):774–776.
Weiss HB, Kaplan S, Prato CG. (2014) Analysis of factor associateid with injury severity in crashes involving young New Zealand drivers. *Accid Anal Prev* 65:142–55.
White A, Hingson R. (2013) The burden of alcohol use: excessive alcohol consumption and related consequences among college students. *Alcohol Res* 35(2):201–218.
WHO Indicator Code Book. Global information system on alcohol and health. (2014) *World Health Organization.* Printed 10/14/2014. http://www.who.int/substance_abuse/activities/gisah_indicatorbook.pdf
WHO Global status report on alcohol and health. (2011) *World Health Organization* 2011 ISBN 978 92 4 156415.
Wiel van de A. (2004) Diabetes mellitus and alcohol. *Diabetes Metabolism Research and Reviews* 20(4):263–7.
Williams B, Sanson A, Toumbourou J, Smart D. (2000) Patterns and predictors of teenagers´ use of licit and illicit substances in the Australian Temperament Project Cohort. *Australian Institute of Family Studies.* Melbourne, Australia: University of Melbourne.
Wilson E, Waring WS. (2007) Severe hypotension and hypothermia caused by acute ethanol toxicity. *Emerg Med J* 24:e7 doi:10.1136/emj.2006.041590.
Wilson NM, Brown PM, Juul SM, *et al*. (1981) Glucose turnover and metabolic and hormonal changes in ethanol-induced hypoglycaemia. *Br Med J.* 282: 849–53.
Winek CL, Carfagna M. (1987) Comparison of plasma, serum, and whole blood ethanol concentrations. *Journal of Analytical Toxicology* 11(6):267–8.
Wirth MM, Meier EA, Fredrickson BL, Schultheiss OC. (2007) Relationship between salivary cortisol and progesterone levels in humans. *Biological Psychology* 74:104–107.
Wright JW. (1991) Alcohol and the laboratory in the United Kingdom. *Ann Clin Biochem* 28:212–7.
Woolfenden S, Dossetor D, Williams K. (2002) Children and Adolescents With Acute Alcohol Intoxication/Self-poisoning Presenting to the Emergency Department. *Arch Pediatr Adolesc Med* 56, 345–48.
Zehtabchi S, Sinert R, Baron BJ, Paladino L, Yadav K. (2005) Does ethanol explain the acidosis commonly seen in ethanol-intoxicated patients? *Clin Toxicol* 43(3):161–6.
Zoethout RWM, Wilson LD, Ippel AE, Dahan A, van Gerven JMA. (2011) Functional biomarkers for the acute effects of alcohol on the central nervous system in healthy volunteers. *Br J Clin Pharmacol* 71:331–50.
Yang CC, Yang LY, Deng JF. (1995) Hypoglycemia following ethanol ingestion in children: report of case. *J Formos Med Assoc* 94(5):267–70.
Yki-Jarvinen H, Koivisto KA, Ylikahri R, Taskinen M. (1988) Acute effects of ethanol and acetate on glucose kinetics in normal subjects. *Am J Physiol* 254:175–80.

9. SUMMARY IN ESTONIAN

Etüülalkoholi intoksikatsiooni kliiniline pilt ja olulisemad biokeemilised muutused lastel

Sissejuhatus

Etanool on kõige enam kuritarvitatud aine nii Ameerika Ühendriikides, kui ka Lääneriikides, kaasa arvatud Eestis. Rohke alkoholi tarvitamisega kaasnevad kasvava tendentsiga alkoholist tingitud probleemid ka lastel ja noorukitel (Madu *et al*., 2003; Meyer *et al*., 2003; Meyer *et al.,* 2008; Schöberl *et al*., 2008; Sutherland *et al*., 1998; Woolfenden *et al*., 2002). Laste hälbiva käitumise uuring-2 näitas, et keskmiselt 53% Eesti 15–16 aastastest lastest oli tarvitanud rohkelt alkoholi vähemalt korra viimase 30 päeva jooksul. Umbes 86% 13–16 aastastest noorukitest Eestis oli tarvitanud alkoholi vähemalt korra elus ning esimene alkoholi tarbimine toimus tüdrukutel 11.3 ja poistel 10.7 a vanuselt (Markina *et al*., 2007). Vähemalt 15 aastaste inimeste poolt tarbiti kogu maailmas 2005. aastal keskmiselt 6.13 liitrit puhast etanooli (WHO, 2011). Eestis on alkoholi tarvitamine vähenemistendentsiga: 2005. a tarvitati 22.0 liitrit puhast etanooli inimese kohta (WHO, 2011) ning 2013. a oli kogus vähenenud 11.9 liitrini (Estonian Institute of Economic Research, 2014).

Somnolentsus, stuupor, düsorientatsioon ja tasakaaluhäired on hästituntud alkoholi intoksikatsioonile vihjavad kesknärvisüsteemi (KNS) sümptoomid. Uuringud, mis kirjeldavad head korrelatsiooni KNS sümptoomide ja vere alkoholi kontsentratsiooni vahel, on teostatud täiskasvanutel (McKnight *et al*., 1997; Zoethout *et al.*, 2011), kuid lastel ei ole teadaolevalt läbi viidud. Bouthoorn kaasautoritega (Bouthoorn *et al*., 2011) leidis retrospektiivses uuringus, et teadvushäired (45%) ja hüpotermia (43.1%) olid sagedasemad hospitaliseeritud alkoholijoobes laste kliinilised tunnused.

Juriidilistel juhtudel on vajadus kalkuleerida seerumi ja vere alkoholi kontsentratsiooni suhe, mis täiskasvanutel on 1.12:1–1.18:1 (Barnhill *et a*l., 2007), kuid laste kohta andmed puuduvad.

Alkoholijoobes lastel on kliinilist leidu kirjeldatud väga üldiselt ning pole seostatud alkoholi kontsentratsiooniga seerumis või veres.

Alkoholist tingitud biokeemilisi muutusi, nagu laktatsidoosi, hüpernatreemiat, hüpokaleemiat, hüpoglükeemiat või hüperglükeemiat on kirjeldatud alkoholijoobes täiskasvanutel. Lastel on biokeemilistest muutustest probleemseimaks peetud hüpoglükeemiat ja hüpokaleemiat, kuid kirjeldused on peamiselt juhtumipõhiselt ning tavaliselt on uuritavad lapsed olnud raskes alkoholijoobes. Hüperglükeemiat on seostatud kortisooli taseme tõusuga ning laktatsidoosi tekkega.

Seerumi alkoholi kontsentratsiooni mõju suguhormoonidele on mitmetine: on leitud, et östradiooli tase alkoholi toimest ei muutu, samas progesterooni taseme muutust on käsitletud erinevalt. Holdstock (Holdstock *et al*., 2006) leidis, et follikulaarses faasis progesterooni tase alkoholi toimel ei muutu, kuid Pierucci-Lagha kaasautoritega (Pierucci-Lagha *et al.*, 2005) kirjeldas

progesterooni kontsentratsiooni vähenemist. Testosterooni tase väheneb alkoholi tarvitamisel, kuid enam väljendub alkoholi efekt kroonilisel tarvitamisel.

Uuringu eesmärgid

Uurida kliinilisi tunnuseid ägeda alkoholi intoksikatsiooni erinevatel raskusastmetel ning ägeda alkoholi intoksikatsiooni raskusastmete korrelatsiooni seerumi alkoholi kontsentratsiooniga;

leida lastel esineva ägeda alkoholi intoksikatsiooni kliinilise hindamise diagnostilise kasutuse tõhusus kui kasutada ägeda alkoholi intoksikatsiooni raskuse hindamisel „kuldse standardina" seerumi alkoholi kontsentratsiooni;

tuvastada seerumi alkoholi kontsentratsiooni ja vere alkoholi kontsentratsiooni suhe ägeda alkoholi intoksikatsiooniga lastel;

teha kindlaks ägeda alkoholi intoksikatsiooniga laste biokeemiliste analüütide (glükoos, laktaat, kaalium, naatrium) muutuste esinemine ning tuvastada seerumi alkoholi kontsentratsiooni mõju biokeemilistele muutustele;

kirjeldada ägeda alkoholi intoksikatsiooniga laste hormonaalset (testosteroon, östradiool, progesteroon, kortisool) staatust ning hormoonide suhet plasma biokeemiliste analüütidega.

Meetodid ja patsiendid

Uuringus osalesid Tartu Ülikooli Lastekliinikusse või Tallinna Lastehaiglasse ägeda alkoholi intoksikatsiooniga hospitaliseeritud 8–18 aastased lapsed ajavahemikus detsember 2005 kuni detsember 2008. Uuringu 3 aasta jooksul kogunes teave 417 lapse ja nooruki kohta, kellel kahtlustati ägedat alkoholi intoksikatsiooni. Neist välistati kohe 20, kuna alkoholi kontsentratsioon seerumis oli väiksem kui 0.2 g/L, kolm last, kellel tuvastati narkootilise aine intoksikatsioon, 1 vastsündinud laps, kuna ei olnud ise endale alkoholi manustanud. Erinevatel põhjustel (uuringus osalemisest keeldumine, verevõtu vead, laboriuuringuid ei thetud või kliinilise läbivaatuse protokolli ei täidetud) välistati 153 last hormoonide- ning 161 last kliiniliste tunnuste uuringust. Lapsed jaotati vanuse järgi kahte gruppi: 8.0–13.9 aastat ning 14.0–17.9 aastat. Joobe raskuse järgi jaotati lapsed kolme gruppi kasutades kahte erinevat põhimõtet: kliiniliste tunnuste alusel (kerge, mõõdukas, raske) ja SAC aluseks võttes (1. grupp ≥0.20–1.49 g/L, 2. grupp 1.50–2.49 g/L, 3. grupp ≥2.50 g/L).

Laste arv suurenes uuringu käigus, mistõttu esimeses uuringuetapis detsember 2005 kuni detsember 2007, biokeemiliste muutuste uuringus, osales 226 hospitaliseeritud alkoholijoobe kahtlusega lapsest 194, vanuses 10.0–17.9 (keskmine 14.2) aastat, poiste:tüdrukute suhtega 1.6:1 (Tabel 1).

Teises etapis detsember 2005 kuni detsember 2008, hormonaalsete muutuste uuringus, jäi uuringusse 264 last vanuses 8.4–17.9 (keskmine 14.2) aastat poiste:tüdrukute suhtega 1.4:1 ning kolmandas etapis samast ajavahemikust oli uuringus 256 last.

Kliiniliste tunnuste fikseerimiseks kasutati anonüümset kodeeritud protokolli (Lisa 1), mis sisaldas andmeid lapse kesknärvisüsteemi seisundi (teadvus, tasakaal, kõne, käitumine) kohta astmelise skaalana. Samuti fikseeriti andmed füüsilise seisundi (lihastoonus, kehatemperatuur, vererõhk, pulss, hingamissagedus) ja manustatud alkoholi kohta. Pediaatritelt küsiti lapse kliinilise seisundi alusel hinnangut joobe raskusastme (kerge, mõõdukas, raske) kohta.

Vereproovid koguti 10–15 minutit peale hospitaliseerimist seerumist ja verest alkoholi kontsentratsiooni määramiseks ning plasmast glükoosi, laktaadi, naatriumi, kaaliumi, kortisooli ja suguhormoonide määramiseks. Biokeemilised analüüsid ja alkoholi kontsentratsiooni määramine teostati kohe peale proovide kogumist, hormoonide määramiseks Li-hepariini plasma külmutati –20^{o}C juures määramiseni Tartu Ülikooli Kliinikumis. Uriiniproov võeti narkootiliste ainete kasutamise välistamiseks. Vere etanooli kontsentratsioon määrati Eesti Kohtuekspertiisi Instituudis. Analüütide tulemuste muutusi hinnati labori referentsväärtuste alusel.

Tulemused

Alkoholi intoksikatsiooni kliinilistest tunnustest erinevates intoksikatsiooni raskusgruppides esines sageli teadvusehäire: somnolentsus (N=92), düsorientatsioon (N=51) ja kooma 12 lapsel (GKS 5–8 punkti). Sagedaseim AAI kliiniline leid oli muutunud tasakaal: tasakaaluhäired ja võimetus seista esines 95.3% lastest, millele järgnesid kõnehäired kõnehäiretest ehk „pehme keele“ esinemisest kuni võimetuseni kõneleda 88.7% lastest. Kõne (r_s=0.16) ja teadvuse seisund (r_s=0.13) olid parimad tunnused (p<0.0001) paigutamaks lapsed kliinilise leiu järgi intoksikatsiooni raskusgruppidesse. Käitumisest oli sagedamini esinevaks leiuks rahulikkus, loidus või pole võimalik hinnata 70.3%.

Glükoosi tase oli negatiivses korrelatsioonis AAI lapse eaga, s.t noorematel lastel oli glükoosi tase kõrgem võrreldes vanemate lastega ning üldiselt oli glükoosi tase referentsväärtusest kõrgem 40.2% lastest. Hüperlaktineemia esines 66% lastest ning hüpokaleemia 50% lastest, kusjuures noorematel esines hüpokaleemiat vanematest lastest enam. 5 lapsel (2.6%) esines hüpokaleemia kriitilises väärtuses (<2.8 mmol/l).

Seerumi alkoholi kontsentratsioon oli noorematel tüdrukutel suurem võrreldes poistega, kuid vanemas eas lastel oli suhe vastupidine. Plasma laktaadi kontsentratsioon oli suurem kõigis alkoholi kontsentratsiooni gruppidega võrreldes, väljaarvatud tüdrukutel 0.2–1.49 g/L seerumi alkoholi kontsentratsiooni grupis. Noorematel lastel kerge negatiivne korrelatsioon SAC ja kaaliumi kontsentratsiooni vahel. Naatriumi tase oli veidi kõrgenenud SAC grupis >2.50 g/L ja esines tugev korrelatsioon alkoholi ja naatriumi kontsentratsioonide vahel SAC>1.50 g/L korral.

Östradioolil ja progesteroonil ei esinenud tugevat seost tüdrukute vanusega, kuid poistel esines tugev korrelatsioon testosterooni taseme ning vanuse vahel.

Plasma kortisooli tase korreleerus positiivselt plasma glükoosi ning laktaadi tasemega ja negatiivselt naatriumi tasemega.

Järeldused

1. Alkoholi intoksikatsiooni kliinilises hindamises oli teadvuse häirumise tase peamiseks diagnostiliseks tunnuseks, mis korreleerus hästi seerumi alkoholi kontsentratsiooniga. Kõne- ja tasakaaluhäired on sage leid alkoholi intoksikatsiooniga lastel.

2. Ägeda alkoholi intoksikatsiooni kliinilised leiud peavad olema kinnitatud SAC määramisega, mis on haiglates "AAI kuldseks standardiks". Keskmine tõhusus seerumi alkoholi kontsentratsiooni alusel õige AAI raskusastme diagnoosimisel lastel oli 67.7%. Arstid hindasid kliiniliselt alkoholijoobe raskemaks ühe astme võrra võrreldes seerumi alkoholi kontsentratsiooniga.

3. SAC/BAC suhe lastel soost ja vanusest sõltumata oli 1.19:1.

4. Plasma kaaliumi, naatriumi, glükoosi ja laktaadi taset võiks hinnata kõigil alkoholi intoksikatsiooniga hospitaliseeritud lastel ning seerumi alkoholi kontsentratsiooni >1.50 g/L korral peaks biokeemiliste muutuste hindamine olema teostatud rutiinselt. Lastel esineva alkoholi intoksikatsiooni iseloomulikeks kliinilisteks tunnusteks on teadvuse häirumine ning kõne- ja tasakaaluhäired. Alkoholi intoksikatsiooni kliiniline leid lastel võib olla ühe astme võrra raskem võrreldes seerumi alkoholi kontsentratsioonist lähtuva alkoholijoobe raskusastmega võrreldes. Hüperlaktineemia, hüpokaleemia ja kõrgenenud glükoosi tase on sage biokeemiline leid ägeda alkoholi intoksikatsiooniga hospitaliseeritud lastel. Hüpoglükeemia leid oli harvaesinev. Biokeemilised muutused esinevad paralleelselt kõigi seerumi alkoholi kontsentratsioonide korral, kuid märkimisväärsed muutused on nähtavad SAC üle 1.50 g/L korral.

5. Suurenenud plasma kortisooli tase üle referentsväärtuse on AAI lastel sage leid. Kortisool korreleerus positiivselt plasma glükoosi tasemega ning tüdrukutel progesterooni kontsentratsiooniga.

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Medical Assessment Form No …

1) Medical history no .....
2) Age or date of birth .....
3) Sex: M W (menses not started, last menses .....)
4) Assessment date and time .....
5) Paediatrician´s name .....

Examined child

1) Appearance: clear; dirty; injuries .....
.....
2) Behaviour:
normal; aggressiveness; euphoria; restful; apathy; impossible assess
3) Consciousness:
clear; disorientation; euphoria; somnolence; stupor;
coma (GCS …… points)
4) Memory about last hours: clear; partly; absent
5) Other health problems:
.....
6) Redness of face; redness of conjunctives;
7) Body temperature: …… ^{0}C; sweating: yes; no
8) Heart rate …… beats per minute
9) Blood pressure …… mm Hg
10) Respiration rate: …… per minute
11) Speech: normal; rushed; fast; confused; unable to speak
12) Pupils size: normal; dilated; narrowed
reaction to light: normal; tardy; absent
13) Nystagmus: no; horizontal; vertical; mixed type
14) Balance: normal; imbalanced; unable to stand
15) Muscle tone: normal; decreased; increased
16) Consumed alcohol: amount …… Firstly; repeatedly
Date of consumption .....
Time of consumption .....
17) Results of laboratory tests
Glucose …… mmol/l K^{+} ……. mmol/l
Lactate ……. mmol/l Na^{+} …… mmol/l
Ethanol ……. mg/dl
18) Estimating of the severity of AAI: sober; mild; moderate; severe

Blood! Urine!

Addendum 1. Medical Assessment Form

PROTOKOLL Nr …

1) Haiguslugu / sissekanne nr .....
2) Vanus või sünniaeg .....
3) Sugu: M N (menses puudub, viimane menses .....)
4) Läbivaatuse kuupäev ja kellaaeg .....
5) Läbivaatuse teostanud arst .....

Uuritava

1) välimus: puhas, määrdunud, välised vigastused .....
.....
2) käitumine: tavaline, agressiivne, eufooriline, rahulik, loid, pole hinnatav
3) teadvus: selge, düsorienteeritud, eufooriline, somnolentsus, stuupor, kooma (GKS …… punkti)
4) mälu viimaste tundide kohta: olemas, osaline, puudub
5) kaasnevad haigused:
.....
6) näo punetus; silmade sidekestade punetus;
7) kehatemperatuur: …… ^{0}C; higistamine: esineb, ei esine
8) pulss …… korda minutis
9) vererõhk …… mm Hg
10) hingamissagedus: …… korda minutis
11) kõne: aeglane, kiire, segane, puudub
12) pupillid suurus: normis; laienenud; ahenenud
reaktsioon valgusele: normis; aeglustunud; puudub
13) nüstagm: ei esine; esineb horisontaalne, vertikaalne, segatüüp
14) tasakaal: normis, häirunud, pole hinnatav
15) lihastoonus: normis, alanenud, suurenenud
16) ütlused alkoholi tarvitamise kohta: kogus …… esmakordne, korduv
tarvitamise kuupäev .....
tarvitamise kellaaeg .....
17) laboratoorsete uuringute tulemused
glükoos …… mmol/l K^{+} …….. mmol/l
laktaat …….. mmol/l Na^{+} …… mmol/l
etanool ……. mg/dl
18) arsti otsus joobeastme kohta: joovet ei esine, kerge, keskmine, raske

Veri! Uriin!

Lisa 1. Meditsiinilise läbivaatuse vorm.

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Tõnisson, M., Tillmann, V., Kuudeberg, A., Lepik, D., Väli, M. (2013). Acute alcohol intoxication characteristics in children. Alcohol & Alcoholism, 48(4): 390–5.

Denissov, G., Tuusov, J., Tõnisson, M., Lepik, D., Väli, M. (2012). The impact of changing classification on official fatal poisoning figures. Romanian Journal of Legal Medicine, 20(3): 197–202.

Tuusov, J., Vals, K,Tõnisson, M., Riikoja, A., Denissov, G., Väli, M. (2012). Fatal poisining in Estonia 2000–2009. Trends in illegal drug-related deaths. Journal of Forensic and Legal Medicine, 1–6.

Ringmets, I., Tuusov, J., Lang, K., Väli, M., Pärna, K., Tõnisson, M., Helander, A., McKee, M., Leon, DA. (2012). Alcohol and premature death in Estonian men: a study of forensic autopsies using novel biomarkers and proxy informants. BMC Public Health, 12, 146.

Tõnisson, M., Tillmann, V., Kuudeberg, A., Väli, M. (2011). Plasma cortisol, testosterone, estradiol and progesterone levels in children with acute alcohol intoxication. Journal of Addiction Research and Theraphy, 2(111): 1–16.

Tõnisson, M., Tillmann, V., Kuudeberg, A., Väli, M. (2010). Plasma glucose, lactate, sodium and potassum levels in children hospitalised with acute alcohol intoxication. Alcohol, 44(6): 565–71.

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Tõnisson, M., Tillmann, V., Kuudeberg, A., Lepik, D., Väli, M. (2013). Acute alcohol intoxication characteristics in children. Alcohol & Alcoholism, 48(4): 390–5.

Denissov, G., Tuusov, J., Tõnisson, M., Lepik, D., Väli, M. (2012). The impact of changing classification on official fatal poisoning figures. Romanian Journal of Legal Medicine, 20(3): 197–202.

Tuusov, J., Vals, K,Tõnisson, M., Riikoja, A., Denissov, G., Väli, M. (2012). Fatal poisining in Estonia 2000–2009. Trends in illegal drug-related deaths. Journal of Forensic and Legal Medicine, 1–6.

Ringmets, I., Tuusov, J., Lang, K., Väli, M., Pärna, K., Tõnisson, M., Helander, A., McKee, M., Leon, DA. (2012). Alcohol and premature death in Estonian

men: a study of forensic autopsies using novel biomarkers and proxy informants. BMC Public Health, 12, 146.
Tõnisson, M., Tillmann, V., Kuudeberg, A., Väli, M. (2011). Plasma cortisol, testosterone, estradiol and progesterone levels in children with acute alcohol intoxication. Journal of Addiction Research and Theraphy, 2(111): 1–16.
Tõnisson, M., Tillmann, V., Kuudeberg, A., Väli, M. (2010). Plasma glucose, lactate, sodium and potassum levels in children hospitalised with acute alcohol intoxication. Alcohol, 44(6): 565–71.

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