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ASSESSMENT AND DETECTION

The Effect of the Binge Drinking Session on the Activity of Salivary, Serum and Urinary β-Hexosaminidase: Preliminary Data

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Abstract — Our report is the first to show that an acute ingestion (6 h) of a relatively large, yet tolerable dose of alcohol (120–160 g), significantly increases activity of total serum β-hexosaminidase (total β-HEX), β-HEX A and β-HEX B isoenzymes, as well as salivary total β-HEX and urinary β-HEX A, in eight infrequent binge drinkers. An increase in the activity of serum and urinary total HEX is mainly due to its secretory isoenzyme β-HEX A.

INTRODUCTION

N-Acetyl-β-hexosaminidase (β-HEX; β-hexosaminidase; E.C.3.2.1.30) is a lysosomal exoglycosidase that releases *N*-acetylhexosamines from non-reducing ends of oligosaccharide chains of glycoconjugates (glycoproteins and glycolipids of the cell membrane and proteoglycans of the extracellular matrix) (Zwierz *et al*., 1999; Markowski *et al*., 2003). β-HEX isoenzyme A (αβ) is heat labile and β-HEX B (ββ) is stable. The activity of β-HEX A in body fluids reflects enzyme loss during cell turnover and the secretory activity of cells. β-HEX B is closely connected with the lysosomal membrane, so its increase in body fluids is an early manifestation of an impaired membrane function, cellular damage and injury progression (Jin *et al*., 1999; Zwierz *et al*., 1999; Sharpe, 2001). The increased activity of urinary β-HEX is a sensitive marker of renal diseases whereas the elevated activity of serum β-HEX has been observed in liver diseases, hypertension, diabetes mellitus, myocardial infarction, thyrotoxicosis and pregnancy (Kärkkäinen *et al*., 1990b; Wehr *et al*., 1991; Sharpe, 2001). Salivary HEX increases during critical illness and diabetes mellitus (Knas *et al*., 2006).

β-HEX, particularly β-HEX B isoenzyme (Hultberg *et al*., 1991; Stowel *et al*., 1997) in serum and total β-HEX in urine, is a very sensitive marker of prolonged alcohol abuse (Kärkkäinen *et al*., 1990a; Nyström *et al*., 1991; Stowell *et al*., 1997; Sharpe, 2001; Taracha *et al*., 2001; Markowski *et al*., 2003; Taracha *et al*., 2006). The increased activity of serum and urinary β-HEX has been reported after drinking >60 g of alcohol daily, for at least 10 successive days (Hultberg *et al*., 1980, 1991; Kärkkäinen *et al*., 1990a; Wehr *et al*., 1991). Less attention has been paid to bingeing-induced toxicity, even though binge drinking is more common than chronic alcoholism. Binge drinking is characterized by the consumption of alcohol leading to intoxication (drinking to get drunk), often measured as having >5–6/4 number of drinks on one occasion or bringing blood alcohol concentration (BAC) above 0.08 gram percent in ∼2 h. Other binge measures include drinking over half the 'sensible' number of units per week (1–14 units per week for women and 1–21 for men), or double the recommended daily guidelines in one session (Kuntsche *et al*., 2004; Savola *et al*., 2004; Cranford *et al*., 2006).

An increasing number of young adults prefer alcohol as a recreational drug, tending to concentrate their drinking at weekends (Waszkiewicz *et al*., 2008). In northern and central Europe, spirit consumption is one of the most important predictors for volume of drinking on a single occasion (Cherpitel *et al*., 2004; Kuntsche *et al*., 2004; Savola *et al*., 2004). The purpose of this study was to evaluate the effect of a single large dose of ethanol on the activity of β-HEX and its isoenzymes in saliva, serum and urine.

SUBJECTS AND METHODS

Subjects and procedure

Eight non-smoking men (aged 22–31 years, 27.0 ± 2.5; BMI 25.0 ± 1.7), not taking medications, participated in the study. Prior to the experiment, all volunteers were verified clinically to be in good oral and general health.

A check-up of the oral cavity was done by one qualified dentist in artificial light, by using a dental mirror and probe, following the World Health Organization criteria. Good oral health was defined: <20 for the DMFS index (Decayed, Missing or Filled Surfaces of teeth; no active caries), <2 for OHI-S (Oral Hygiene Index-Simplified; values from 0 to 6) and <1 for PBI (Papilla Bleeding Index; a score of 0–4) and GI (Gingival Index; a score of 0–3).

All men were infrequent binge drinkers (reported bingeing 1–11 times per year and/or 1–2 episodes in the past month), who had abstained from alcoholic beverages and drugs for 10 days, before the experiment. The participants stayed at home during the drinking session, under the supervision of sober friends and a physician, who helped verify quantities and the time when drinking stopped. During the alcohol session (7 p.m. to 1 a.m.), participants drank 120–160 g of ethanol (12–16 standard drinks) as 40% vodka (2.0 ± 0.3 g/kg of body weight; ranging

from 1.42 to 2.5 g/kg), together with light meals and fruit juice (excluding grapefruit juice). Such amounts of alcohol are common in spirit-drinking countries, including Poland, provoking a tolerable but severe intoxication (Cherpitel *et al*., 2004).

The study was approved by the local Bioethical Committee of the Medical University of Bialystok, Poland. Informed written consent was obtained from all participants after the explanation of the nature, purpose and potential risks of the study.

The subjects were deprived of food and beverages, except water, for 2 h before sample collection. The sets of saliva, blood and urine samples were collected (12 h prior, and 36 and 108 h after acute ethanol consumption), and then centrifuged to remove cells. The supernatants were divided into 200-μL portions, frozen and kept, until analyzed.

Analytical methods

Activities of total β-HEX, β-HEX A and β-HEX B, in supernatants of saliva, serum and urine, were determined in duplicates by Marciniak *et al*.'s method (Marciniak *et al*., 2006), based on colorimetric determination of *p*-nitrophenol released from *p*-nitrophenyl-acetyl-β-D-glucosaminide (Sigma, USA). The mixtures of enzymes and substrates were incubated for 60 min at 37°C. Heat-stable β-HEX B was measured after selective heat denaturation of termolabile β-HEX A (3-h preincubation without a substrate at 50°C).

The protein content in saliva was measured by the method of Lowry *et al*. (1951), and in serum, by the biuret method (Dawson *et al*., 1969). Urinary creatinine was determined colorimetrically according to Jaffe's reaction using a diagnostic test (POCH, Poland).

Statistical analysis

Statistical analysis was performed using Statistica 6.0 (Statsoft, Cracov, Poland). Student's paired *t*-test and Pearson's correlation coefficients were used to study the significance of differences and the associations between variables, respectively. Statistical significance was defined as $P < 0.05$.

RESULTS

As Fig. 1B shows, after the binge drinking session, the specific activity of total β-HEX (pkat/mg protein) in serum at 108 h after intoxication significantly increased (by approximately one-third) (from 33.0 ± 8.4 before to 33.0 ± 0.1 at 36 h and 50.0 ± 11.4 at 108 h after intoxication; mean ± SD) with an accompanying significant increase in the specific activities of β-HEX A (up to 250% at 36 h and up to 350% at 108 h) (from 3.0 ± 0.9 before to 10.5 ± 5.3 at 36 h and 14.3 ± 5.3 at 108 h after intoxication) and β-HEX B (∼30%) at 108 h (from 30.0 ± 8.2 before to 22.4 ± 7.2 at 36 h and 37.5 ± 8.1 at 108 h after intoxication). At 36 h, we noticed a significant rise (∼50%) in the specific activity of total β-HEX (pkat/mg protein) in saliva (Fig. 1A) (from 10.3 ± 3.0 before to 15.6 ± 7.5 at 36 h and 11.0 ± 3.0 at 108 h after intoxication) and a significant rise (∼40%) in the activity of β-HEX A in urine (nkat/g creatinine) (Fig. 1C) (from 2.8 ± 1.6 before to 3.9 ± 1.6 at 36 h and 3.8 ± 1.4 at 108 h after intoxication). The specific activity of salivary β-HEX A and β-HEX B tended to increase after the intoxication (from 6.5 ± 3.5 before to 10.0 ± 6.4 at 36 h and 6.8 ± 3.6 at 108 h for β-HEX A and from 3.7 ± 1.6 before to 5.4 ± 1.7 at 36 h and 4.1 ± 2.1 at 108 h for β-HEX B). The total β-HEX and β-HEX B in urine increased only slightly after the intoxication (from 8.5 ± 3.4 before to 10.1 ± 2.9 at 36 h and 11.6 ± 8.0 at 108 h and from 5.6 ± 2.2 before to 6.2 ± 1.8 at 36 h and 7.8 ± 6.7 at 108 h, for the total β-HEX and β-HEX B, respectively).

In serum, total β-HEX and β-HEX B values over the preconsumption 'norm' (mean ± 2SD) were presented in one to three drinkers at 36 and 108 h. The serum values of β-HEX A were over the 'norm' in seven binge drinkers at 36 and 108 h. In saliva and urine, over the 'norm' values of total β-HEX and its isoenzymes were presented in one to two drinkers at time points after the drinking session.

We have found an inverse correlation ($r = -0.95$, $P < 0.05$) between serum and urinary β-HEX B at 36 h. No correlations were found between serum and salivary β-HEX, β-HEX A and β-HEX B at any time point.

DISCUSSION

After chronic ethanol intoxication, a greater increase in the activity of serum β-HEX B than β-HEX A has been previously reported (Hultberg *et al*., 1995; Stowell *et al*., 1997; Markowski *et al*., 2003), whereas after moderate drinking and in nondrinkers, higher increase in serum and urinary β-HEX A than β-HEX B activity has been reported (Stowell *et al*., 1997). Our results show that after acute ingestion of a large dose of alcohol, the significantly increased activity of serum and urinary total β-HEX is mainly due to the increased activity of the heat-labile A isoform. Although we noticed a similar increase in the mean activity of β-HEX A and β-HEX B in urine, only β-HEX A increased significantly. The minor or lack of 'answer' of alcohol abuse markers in young people has been reported earlier (Kärkkäinen *et al*., 1990a; Nyström *et al*., 1991; Bisson and Milford-Ward, 1994; Taracha *et al*., 2002). The rapid normalization (1 week) of elevated serum β-HEX has been proposed as a reason of false-negative results (Nyström *et al*., 1991). Another reason might be the fact of relatively light drinking in young people (Nyström *et al*., 1991). A significant increase in β-HEX observed in our study may be related to higher doses of daily amounts of ethanol consumed per capita (<60 g in Kärkkäinen *et al*., 1990a; Nyström *et al*., 1991; Bisson and Milford-Ward, 1994, as well as in Taracha *et al*., 2002, studies, and 120–160 g in our study).

Various mechanisms concerning the rate of clearance/elimination or production/release, responsible for the increased activity of total HEX and its isoenzymes in body fluids, have been proposed (Hultberg *et al*., 1991). The change of lysosomal membrane permeability and leakage of the enzyme from lysosomes and subsequently from cells to the body fluids, delayed removal of these enzymes, enhanced synthesis of the enzyme by activated reticuloendothelial cells and leakage from the degenerating cells of various body organs have been described to induce an increase in the activity of β-HEX (Hultberg *et al*., 1980, 1995; Kärkkäinen *et al*., 1990b; Wehr *et al*., 1991 Winchester, 2005).

As the activity of β-HEX A reflects the secretory activity of cells (Jin *et al*., 1999; Zwierz *et al*., 1999), the significant increase in the activity of serum and urinary β-HEX A, after the binge drinking session, suggests its increased production and secretion referred to functional changes. The lower but still

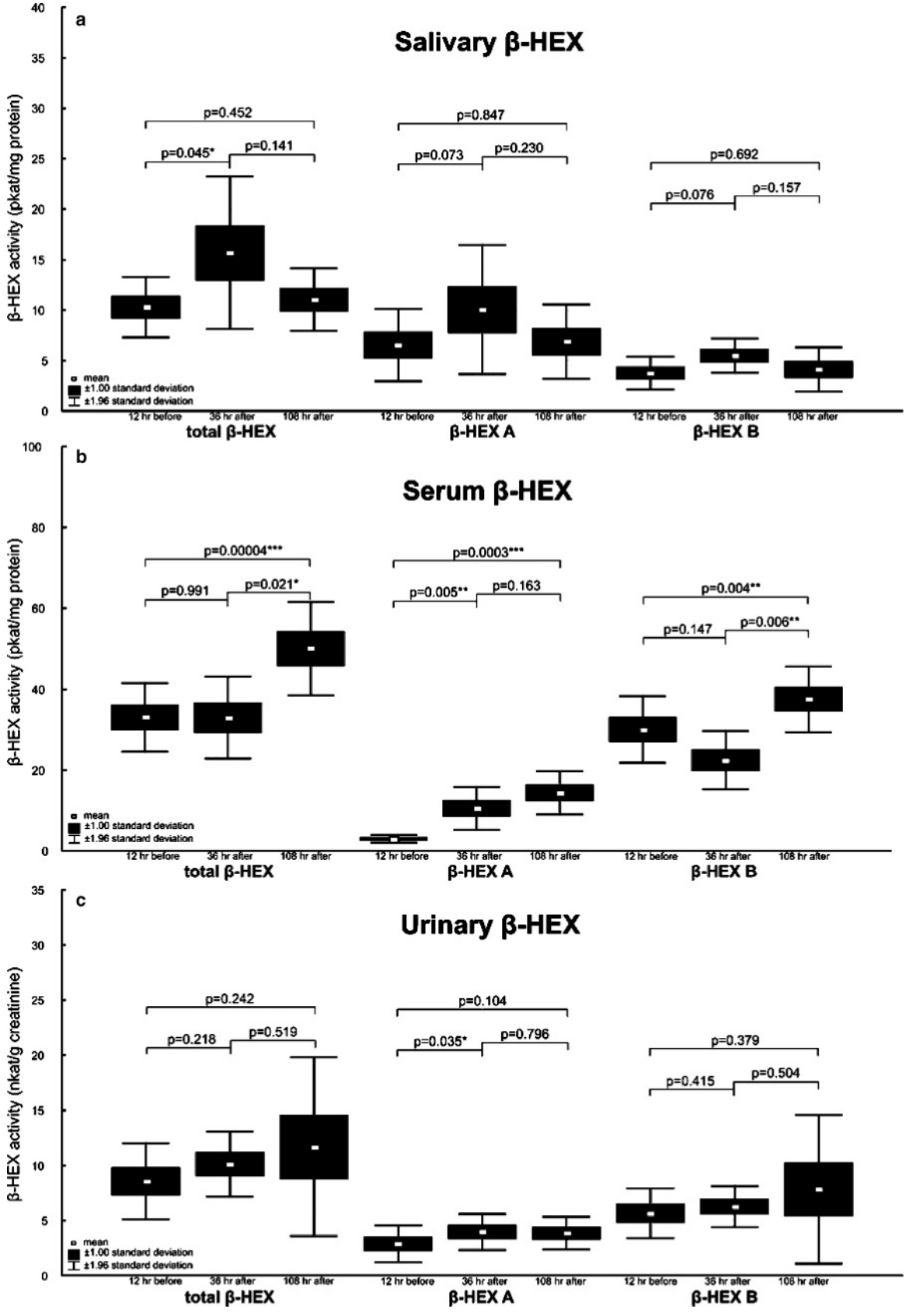

Fig. 1. The activity of total β-HEX, β-HEX A and β-HEX B in saliva (**A**), serum (**B**) and urine (**C**), 12 h before, and 36 and 108 h after the binge drinking session. Statistically significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

significant increase in the serum activity of β-HEX B (1–2:7 of drinkers, respectively for β-HEX B and β-HEX A, had values over the preconsumption 'norm'; mean ± 2SD) might suggest less excessive than chronic, however, still a harmful level of drinking. The increased activity of β-HEX has been found in damaged hepatocytes, and possibly these cells are the source of the increase in circulation, after alcohol intake (Hultberg *et al*., 1991). Although the bulk of the ingested ethanol is metabolized by liver alcohol dehydrogenase (ADH), acetaldehyde can be formed also locally via ADH derived from oral tissues and microbes (Homann *et al*., 2000; Waszkiewicz *et al*., 2006). In saliva, it has been found that the activity of total β-HEX increases during salivary gland dysfunction (Knas *et al*., 2006). Since after drinking, the ethanol concentration in saliva is temporarily much higher than that in plasma, and the level of acetaldehyde in saliva strikingly exceeds the level in systemic blood (Jones, 1995; Homann *et al*., 2000; Waszkiewicz *et al*., 2008), an increase in the activity of total β-HEX in saliva might be related to higher release/production during the salivary glands dysfunction, induced possibly by acetaldehyde and other metabolites of alcohol (Hultberg *et al*., 1991; Knas *et al*., 2006). As ethanol at a concentration of 40% can affect the viability of cells, leading to a local damage of the oral mucosa (Kawashima and Jerzy Glass, 1975; Muller *et al*., 1983; Knoll *et al*., 1998), we cannot also exclude some release of β-HEX isoenzymes from damaged cells of the oral mucosa, even if minimized. Disseminated mucosal ulcerations develop 48 h after ethanol exposition. Healing of the mucosa is rapid; lesions are only barely visible 72 h after alcohol intake (Kawashima and Jerzy Glass, 1975; Knoll *et al*., 1998). Time points chosen (36 and 108 h after intoxication) for saliva collection let us minimize the influence of mucosal tissue damage on the activity of hexosaminidase in saliva. In this study, no significant associations between salivary, serum and urinary isoenzymes of β-HEX were found (except for the inverse correlation between serum and urinary β-HEX B at 36 h), which suggests different mechanisms of the increased activities. In addiction, observed differences in the proportions of β-HEX A and β-HEX B allow us to speculate that the increase in lesional β-HEX B in serum and a tendency to increase in salivary β-HEX B might be due to high levels of toxic alcohol metabolites.

Our results show that even a single but large dose of ethanol can increase the activity of serum total β-HEX, β-HEX A and β-HEX B isoenzymes, as well as salivary total β-HEX and urinary β-HEX A. Since a simple heat treatment can be used to obtain the same results as an immunoassay method to distinguish the activities of the two major isoforms of β-HEX (Stowell *et al*., 1997), we can conclude that the elevated activity of β-HEX in serum and in urine, after the binge drinking session, is mainly due to an increased activity of secretory isoenzyme, β-HEX A. As the activity of β-HEX has been shown to be a very effective marker of harmful drinking, a significant increase in the serum activity of total β-HEX and its isoenzymes as well as in salivary total β-HEX might suggest less excessive than chronic, however, still a harmful level of drinking. An applicability of β-HEX and its isoenzymes as laboratory markers of excessive drinking (binge drinking) needs confirmatory further research, based on a relatively large sample to be sufficiently representative of a vast population.

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