Glutathione S-transferases (GST, EC 2.5.1.18) are detoxification enzymes that catalyze the addition of glutathione to a wide variety of xenobiotics. Cytosolic GSTs are dimeric proteins and are divided into four classes: alpha, mu, pi, and theta [1]. More than 2% of the soluble protein in the liver consists of class alpha GST. These enzymes are released in considerable quantity into the bloodstream during hepatocellular damage. Because class alpha GSTs have a short plasma half-life (±1 h), its concentration will follow changes in acute hepatocellular damage more accurately than will concentrations of the aminotransferases [2]. Two class alpha subunits have been identified, and both homodimers (GSTA1-1 and GSTA2-2) and the heterodimer (GSTA1-2) have been purified from human liver [2].

Tiainen and Karhi [3] were the first to report that men had significantly (P <0.02) higher plasma GST-alpha concentrations than women, which was confirmed recently by our group in a much larger study population (median plasma GSTA1-1 concentration in 175 men, 1.92 μg/L, and in 175 women, 1.28 μg/L [4]). However, in women but not in men, a significant increase with age was noted: The median plasma GSTA1-1 concentration in 83 women at ages 20–40 years was 1.08 μg/L, in 57 women at ages 40–60 years, 1.44 μg/L, and in 33 women above age 60 years, 1.84 μg/L (P <0.05 vs women 40–60 and P <0.01 vs women 20–40 [4]). We suggested that a change in hormone concentrations related to menopause may account for the higher concentrations in elderly women [4].

To test the hypothesis that the relatively high plasma GSTA1-1 concentrations in postmenopausal women can be decreased by hormone replacement therapy, these concentrations were measured in 64 healthy postmenopausal women who had received hormone replacement therapy for 1 year in a randomized, double-blind, prospective study. The study was designed to test reduced frequency of progestogen (norethisterone acetate; NETA) use in a 3-month hormone replacement therapy (long cycle) vs the standard monthly replacement therapy (Trisequens®). Long-cycle treatment was given during 4 cycles of 84 days, and Trisequens was given during 12 cycles of 28 days.

Selected subjects were healthy, nonhysterectomized women, ages 45 to 60 years, who were amenorrheic for at least 6 months and who had a serum follicle-stimulating hormone concentration >36 IU/L and a serum estradiol <150 pmol/L. All women had serum concentrations of aspartate and alanine aminotransferases below the upper normal reference limit. The 29 women in the long-cycle group received 2 mg of micronized 17β-estradiol on days 1–7, 1 mg on days 79–84, and 1 mg of NETA on days 69–78. The 35 women in the Trisequens group received 2 mg of 17β-estradiol on days 1–22, 1 mg on days 23–28, and 1 mg of NETA on days 13–22.

Plasma samples (containing K₃EDTA) were collected on three different occasions: (a) at the start of the study (baseline); (b) 1–3 days before the last NETA administration (estradiol only; cycle days 66–68 in the long-cycle group and cycle days 10–12 in the Trisequens group); and (c) on days 8–10 of the last NETA administration (estradiol + NETA; cycle days 76–78 in the long-cycle group and cycle days 20–22 in the Trisequens group).

Plasma GSTA1-1 concentrations were measured by sandwich ELISA as reported recently [4]. To evaluate the significance of differences between or within two groups, the Mann–Whitney U-test and the Wilcoxon matched-pairs signed-rank test were used, respectively. This study was approved by the Medical Ethical Review Committee.

No significant differences in plasma GSTA1-1 concentrations were detected between the long-cycle and the Trisequens group before, during, or at the end of the trial period. When compared with those at baseline, plasma GSTA1-1 concentrations were slightly higher (P =0.20 for the whole group) at the end of the last estradiol-only phase, after almost 1 year of replacement therapy. In contrast, plasma GSTA1-1 concentrations were slightly lower than baseline concentrations at the end of the last estradiol + NETA period (P =0.12 for the whole group). In both the Trisequens group and the long-cycle group, 8–10 days of estradiol + NETA resulted in a significantly lower median plasma GSTA1-1 concentration (P <0.03 and P <0.003, respectively; Table 1). Overall, the last estradiol + NETA phase resulted in a 20% decrease of the median plasma GSTA1-1 concentration (in 48 women, the
Table 1. Plasma GSTA1-1 concentrations in postmenopausal women receiving 17β-estradiol and NETA in a 28-day (Trisequens®) or a 84-day (long-cycle) treatment cycle.

<table>
<thead>
<tr>
<th></th>
<th>Trisequens</th>
<th>Long cycle</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma GSTA1-1 concentration, µg/L (median and range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>29</td>
<td>64</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.84b</td>
<td>2.05b</td>
<td>1.94c</td>
</tr>
<tr>
<td></td>
<td>(0.61–8.27)</td>
<td>(0.53–5.50)</td>
<td>(0.53–8.27)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.99c</td>
<td>2.31c</td>
<td>2.25c</td>
</tr>
<tr>
<td>only</td>
<td>(0.46–9.08)</td>
<td>(0.86–9.35)</td>
<td>(0.46–9.35)</td>
</tr>
<tr>
<td>Estradiol + NETA</td>
<td>1.82a</td>
<td>1.58b</td>
<td>1.81c</td>
</tr>
<tr>
<td></td>
<td>(0.39–4.68)</td>
<td>(0.67–4.84)</td>
<td>(0.39–4.84)</td>
</tr>
</tbody>
</table>

The women in the long-cycle group received 2 mg of micronized 17β-estradiol on days 1–78 and 1 mg on days 79–84 and 1 mg of NETA on days 69–78. The women in the Trisequens group received 2 mg of 17β-estradiol on days 1–22 and 1 mg on days 23–28 and 1 mg of NETA on days 13–22. Plasma samples were collected on three different occasions: (a) at the start of the study (baseline); (b) 1–3 days before the last NETA administration (estradiol only, cycle days 66–68 in the long-cycle group, and cycle days 10–12 in the Trisequens group); and (c) on days 8–10 of the last NETA administration (estradiol + NETA; cycle days 76–78 in the long-cycle group and cycle days 20–22 in the Trisequens group).

| P         | <0.0002 vs estradiol only. | <0.0002 vs estradiol only. | <0.0002 vs estradiol only. |

plasma GSTA1-1 concentration decreased; in 16 women, the plasma GSTA1-1 concentration increased; P <0.0002 for the whole group) when compared with estradiol only.

Sex-related differences in the expression of GST isoenzymes in liver were reported in rats [5] and mice [6] but not in humans. In female mice, ovariectomy resulted in increased liver GST activities that could be normalized by replacement of estrogen and progesterone [6]. In humans, men had higher plasma GST-alpha concentrations than women [3, 4]. In women, but not in men, an increase with age was noted [4]. This study shows that estradiol replacement did not decrease the relatively high plasma GSTA1-1 concentrations detected in elderly women. In contrast, the 8–10 days of replacement with estradiol combined with the progestogen NETA resulted in a 20% decrease of the median plasma GSTA1-1 concentration in postmenopausal women, indicating that the relatively high plasma GSTA1-1 concentrations observed in elderly women may be related to the low progestogen concentrations found in these women.

The effect of hormonal replacement therapy on plasma GSTA1-1 concentrations in women reported in this study is on the same order of magnitude as the influence of gender and age on plasma GSTA1-1 concentrations. Although the effects of these variables are rather small, they should be kept in mind when plasma GSTA1-1 concentrations of groups of patients are compared with reference intervals obtained from the general population.

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References

Serum Cardiac Troponins I and T in Early Posttraumatic Rhabdomyolysis, Alain Lavoinne* and Guy Hue (Lab. de Biochim. Méd., Centre Hosp. Univ., 1 rue de Germont, 76331 Rouen, France; *author for correspondence; fax (33) 2 32 88 87 80; e-mail Biochimie@chu-rouen.fr)

Troponin T was proposed as an aid in the diagnosis of myocardial infarction in 1989 [1]. However, the first generation of commercially available troponin T assays yielded false-positive results in patients with severe skeletal muscle injury because of an unspecific binding of skeletal muscle troponin T to the wall of the test tube. Indeed, the second detection antibody in the test showed marked cross-reactivity with skeletal muscle troponin T. Another protein of the troponin complex, cardiac troponin I (cTnI), recently became commercially available and was shown to be highly specific for cardiac muscle [2].

cTnI can be used for the diagnosis of myocardial damage in patients with posttraumatic rhabdomyolysis [3]. More recently, a cardiac-specific, second-generation assay for troponin T (cTnT) was developed by using a second completely cardiac-specific antibody [4]. We report here the comparison of the results obtained for cTnI and cTnT in patients with early posttraumatic rhabdomyolysis [creatinine kinase (CK) activity >4000 U/L; upper reference limit (URL), 110 U/L].

Total CK activity was assessed at 30 °C according to IFCC recommendations with a Hitachi 704 analyzer (Boehringer Mannheim). The intraassay reproducibility for CK (160 U/L) was 0.9% (n = 10). cTnI (routinely assayed in our laboratory) and cTnT were measured with commercially available immunoassays (Stratus cTnI from Dade and Elecsys cTnT from Boehringer Mannheim). The detection limits for cTnI and cTnT, as assessed by 10 determinations of a sample free of cTnI and cTnT, were 0.10 µg/L and 0.018 µg/L, respectively. Ten determinations of the same serum sample (intraassay reproducibility) showed CVs of 5.4% and 1.3% for cTnI and cTnT, respectively. The mean ± SD concentrations (µg/L) were: 2.79 ± 0.15 and 0.31 ± 0.04, respectively. The reference

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