Cysteine–homocysteine mixed disulphide: differing plasma concentrations in normal men and women

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Summary
1. The mixed disulphide of cysteine and homocysteine is known always to be present in the plasma of patients with homocystinuria, an abnormality of methionine metabolism. Recently we have shown that it is also detectable in low concentration in the plasma of normal fasting man. In the present study we measured mixed disulphide concentrations after an overnight fast in 24 normal men and compared the findings with those obtained in 24 normal premenopausal women of similar age.
2. The mean value for men (+SD) of 3.3 ± 0.8 μmol/l was significantly higher than that for women (2.4 ± 0.7 μmol/l; P < 0.001). Of the other neutral and acidic amino acids measured mean values for leucine, isoleucine and valine (P < 0.001) and cystine (P < 0.01) were also higher in the men but methionine concentrations were not significantly different.
3. The higher branched-chain amino acid concentrations in men could be related to larger muscle bulk and protein intake, but the higher cysteine–homocysteine mixed disulphide concentrations are consistent with differences in methionine metabolism between men and women under the age of 50 years.

Key words: amino acids, cysteine–homocysteine, homocystinuria, mixed disulphide, sex difference.

Introduction
Cysteine–homocysteine mixed disulphide is always found in the plasma of patients with homocystinuria (Perry, 1974). Homocystinuria, an abnormality of methionine metabolism, results usually from decreased activity of the enzyme cystathionine β-synthase (EC 4.2.1.22) and as a consequence there is an accumulation of homocysteine. Some of this thiol reacts with cystine to form the mixed disulphide. The oxidized form of homocysteine, homocystine, together with mixed disulphide, is detected in the plasma and cystine concentrations are low and those of methionine elevated (Perry, 1974). Neither homocystine nor mixed disulphide has been detected in the plasma of normal fasting man.

We have shown recently that when a sensitive method for the measurement of sulphur-containing amino acids is used, cysteine–homocysteine mixed disulphide, but not homocystine, can be detected in the plasma of normal fasting adults (Gupta & Wilcken, 1978). In the present study, we have extended our initial findings by reporting a difference in plasma mixed disulphide concentrations between men and women of similar age.

Some of these results were presented at the December 1977 meeting of the Medical Research Society, London (Wilcken & Gupta, 1978).

Methods
Measurements were made in 24 men and 24 women between the ages of 21 and 50 (mean age 35 ± SD 10 years in men, 32 ± 9 years in women).
Most were healthy volunteer subjects but also included were nine men and five women who had been referred for the investigation of chest pain and shown to have normal coronary arteries and cardiovascular function at cardiac catheterization and coronary angiography. Apart from these, all the subjects were asymptomatic and clinically normal; the patients with chest pain were also normal clinically. Investigation included full blood-cell count, determinations of concentrations of fasting serum cholesterol and triglycerides, blood sugar and serum creatinine and urate, and in all subjects the results were within the accepted normal ranges. The patients being investigated for chest pain also had glucose-tolerance tests performed (after 50 g of glucose load) and the results were normal in all. No subject was on any medication at the time of the investigation and, in particular, no woman was taking oral contraceptives; all were eating a normal diet.

A venous blood sample (10 ml) was obtained at approximately 08.00 hours after a 12 h overnight fast. The heparin-treated samples were centrifuged within 5 min and the plasma was immediately deproteinized with sulphosalicylic acid (50 mg/ml of plasma) at 4°C and stored at −20°C until analysis. Plasma neutral and acidic amino acids were measured as in our previous study (Gupta & Wilcken, 1978) by ion-exchange chromatography on a JEOL Amino Acid Analyser (model JLC-6AH) and a buffer system described by Jeppsson & Karlsson (1972). γ-Aminobutyric acid and β-alanine were used as internal standards and these were added before protein precipitation. With the buffer system used (Jeppsson & Karlsson, 1972) no special attempts were made to quantify glutamic acid, asparagine, aspartic acid, glycine and alanine.

The methods we used to establish the identity of cysteine–homocysteine mixed disulphide were described fully in our earlier paper (Gupta & Wilcken, 1978) and will be reviewed only briefly here. Normal plasma was separated chromatographically and the relevant fraction of the column eluates collected by a stream-splitting technique. Several specimens of normal fasting plasma were processed to obtain portions sufficient for chemical characterization. The column eluates were desalted, freeze-dried and oxidized with performic acid (formyl hydroperoxide) (Schram, Moore & Bigwood, 1954). Descending paper chromatography of the oxidized product revealed equimolar amounts of cysteic acid and homocysteic acid. Further confirmation was obtained from co-chromatography of synthetic mixed disulphide and plasma and by demonstrating augmented mixed disulphide peaks after L-methionine loading. We also analysed blood samples from homocystinuric patients, whose plasma was known to contain substantial amounts of mixed disulphide. In these homocystinuric patients, our chromatograms showed a large peak at the elution time for mixed disulphide, identified as outlined above. The peak for the mixed disulphide was situated midway between that of leucine and of tyrosine.

The significance of the differences in amino acid concentrations found between men and women was assessed by using Student's t-test. Values are presented as means ± sd.

Results

Cysteine–homocysteine mixed disulphide was detected in the fasting plasma of all 48 normal subjects, and Fig. 1 shows the individual values. The mean value for men was 3.3 ± 0.8 μmol/l whereas that for women was 2.4 ± 0.7 μmol/l, a significant difference (P < 0.001).

The values obtained for the other neutral and acidic amino acids measured in men and women are shown in Table 1. In addition to cysteine–homocysteine mixed disulphide the values for the branched-chain amino acids valine, isoleucine and leucine are clearly higher in the men, as also is the cystine value, but methionine concentrations are not different.

![Fig. 1. Individual values for cysteine–homocysteine mixed disulphide in fasting men and women. The mean values ± se for each group are shown and are significantly different (P < 0.001).](image-url)
Cysteine–homocysteine mixed disulphide

TABLE 1. Plasma concentrations of neutral and acidic amino acids measured in normal fasting men and women

Mean values ± sd are shown for 24 men and 24 women.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Plasma concn. (µmol/l)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Taurine</td>
<td>69.6 ± 21.5</td>
<td>88.7 ± 37.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>129.1 ± 32.7</td>
<td>132.8 ± 25.1</td>
</tr>
<tr>
<td>Serine</td>
<td>124.0 ± 21.0</td>
<td>120.6 ± 23.9</td>
</tr>
<tr>
<td>α-Aminobutyric acid</td>
<td>22.3 ± 9.0</td>
<td>24.9 ± 11.5</td>
</tr>
<tr>
<td>Valine</td>
<td>210.7 ± 39.7</td>
<td>260.0 ± 48.4</td>
</tr>
<tr>
<td>Cystine</td>
<td>52.8 ± 6.8</td>
<td>64.8 ± 17.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>23.8 ± 4.2</td>
<td>28.9 ± 12.5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>56.7 ± 9.9</td>
<td>77.4 ± 19.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>104.5 ± 16.6</td>
<td>144.1 ± 32.5</td>
</tr>
<tr>
<td>Cysteine–homocysteine mixed disulphide</td>
<td>2.4 ± 0.7</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>55.6 ± 10.6</td>
<td>62.3 ± 12.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>53.0 ± 7.8</td>
<td>56.4 ± 11.4</td>
</tr>
</tbody>
</table>

Discussion

The study confirms our earlier findings (Gupta & Wilcken, 1978) of the presence of cysteine–homocysteine mixed disulphide in the plasma of normal fasting subjects and extends it by demonstrating significantly different concentrations in men and women. There is no question about the identity of the compound. A verification of this formed the basis of our original study (Gupta & Wilcken, 1978) and we refer in the Methods section to the way in which this was done. The data presented in this paper show that the concentrations in plasma of fasting men are significantly higher than those in premenopausal women of the same age (under the age of 50 years). Mechanisms which might be responsible for this difference should be considered.

Mudd & Poole (1975), in a study of labile methyl balances in normal humans on various dietary regimens, found that men and women metabolize methionine differently. They undertook metabolic studies in which methionine and choline intakes were known and cystine was excluded from the diet. When the diet contained a normal amount of methionine, in males each homocysteine moiety formed was remethylated to methionine an average of 1.9 times before condensing with serine to produce cystathionine. But in women the cycling of methionine was reduced to 1.5 times. When methionine intake was restricted, this difference between men and women was preserved. Under these conditions the average homocysteinyl moiety passed through the sulphur conversion cycle at least 3.9 times in the men, but only 3 times in the women.

The transformation of homocysteine to cystathionine is thought to be irreversible (Finkelstein, 1971). The more rapid cycling in women, such that a greater proportion of the available homocysteine is diverted to cystathionine, could be consistent with our findings; for a given methionine intake less homocysteine should be available to combine with cysteine to form the mixed disulphide. As a consequence, concentrations of mixed disulphide might be expected to be lower in women. Differing renal clearances might also contribute (Frimpter, 1963), but we have no data to allow comment on this possibility.

In relation to the other amino acids, from the findings of Oepen & Oepen (1965), Armstrong & Stave (1973) and our own, it appears that large differences occur between men and women in the branched-chain amino acids valine, leucine and isoleucine. The factors regulating the plasma concentrations of the branched-chain amino acids are complex, but their release from muscle is increased and plasma concentrations are known to be elevated in clinical states associated with increased protein catabolism (Felig, Marliss, Pozefsky & Cahill, 1970), glucocorticoid administration (Manchester, 1969) and a brief starvation (Millward, 1970). Furthermore, they are oxidized predominantly in muscle rather than in liver (Young, 1969), and the higher concentrations in men may be related in some way to their larger
muscle bulk, although the mechanisms are not understood.

A larger protein intake may also be a factor. In a recent survey of food consumption patterns of Australian families in a stable community (Busselton, Western Australia), Hitchcock & Gracey (1978) established that not only does the husband of the household eat more than the wife but that this is particularly true of protein. Indeed, in the 31 families surveyed, the parents were aged from 35 to 55 years and the average daily protein intake for men was 98.0 ± 29.8 g and for women 59.7 ± 22.2 g. Thus, by inference, the men included in our own investigation almost certainly had a higher protein intake than the women, although this was not actually measured.

The concentrations of cysteine–homocysteine mixed disulphide measured on the amino acid analyser are thought to represent the plasma concentrations of homocysteine in vivo since the latter is readily oxidized (Perry, Hansen, MacDougall & Warrington, 1967; Perry, 1974). Harker and his associates have shown that prolonged homocysteine infusions produce endothelial damage and atherosclerosis in baboons (Harker, Slichter, Scott & Ross, 1974; Harker, Ross, Slichter & Scott, 1976). There is increasing evidence to indicate that endothelial damage is the primary event in the pathogenesis of atherosclerosis (Haut, More & Movat, 1960; Moore, 1973; Minick & Murphy, 1973; Spaet, 1974; Bjorkerud & Bondjers, 1976) and the precocious vascular disease occurring in homocystinuria is an example of this (McCully, 1969; McKusick, 1972). Although the concentrations of mixed disulphide that we have found in these normal subjects are low and the differences in the concentrations between men and women are small with some overlap, nevertheless it is clear that premenopausal women tend to have lower concentrations than men of similar age. When our findings are considered in conjunction with those of Mudd & Poole (1975), it seems reasonable to infer that homocysteine formed during the metabolism of methionine is removed from the circulation more rapidly in normal premenopausal women. This, operating over many years, might be a possible factor contributing to their unexplained reduced proneness to develop vascular disease. The mean value in men is, however, only 37% higher than that in women, and the average mixed disulphide concentration in normal subjects is only about 10% of the high values found in homocystinuria in this laboratory.

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References


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