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LIVER, BILIARY, AND PANCREAS

Amenorrhoea in women with non-alcoholic chronic liver disease

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Abstract

Amenorrhoea is common in women with non-alcoholic chronic liver disease, but little is known about its causes or consequences. We investigated 12 young women with non-alcoholic chronic liver disease and amenorrhoea and compared them with 11 healthy age matched controls studied in the follicular phase of the menstrual cycle. None of the patients had raised serum concentrations of follicle stimulating hormone suggesting primary gonadal failure, but the variance in serum concentrations of testosterone, oestradiol, prolactin, and luteinising hormone were significantly greater in chronic liver disease patients than control subjects ($p<0.01$). Seven of the 12 chronic liver disease patients had low serum luteinising hormone concentrations, and compared with controls these patients also had significantly reduced median values of oestradiol (64 pmol/l), testosterone (1·1 nmol/l), and follicle stimulating hormone, and were significantly underweight as assessed by skinfold thickness measurements (all comparisons $p<0.025$). In the group with chronic liver disease skinfold thickness was significantly correlated with serum luteinising hormone ($p<0.02$). The five patients with normal serum luteinising hormone had higher median values of both oestradiol (237 pmol/l) and testosterone (3·0 nmol/l) than the control subjects (oestradiol: 113 pmol/l, testosterone: 1·9 nmol/l) but were not more obese or hirsute. Amenorrhoea was unrelated to the duration or severity of liver disease. The metacarpal cortical bone area (an index of bone density) was inversely related to the duration of amenorrhoea ($p<0.02$). We conclude that amenorrhoea in women with non-alcoholic chronic liver disease arises from hypothalamic-pituitary dysfunction and can occur at any stage. The hormonal findings in amenorrhoeic chronic liver disease patients are not uniform. In some, hypogonadotrophic hypogonadism is related to undernutrition whereas others have normal to high values of luteinising hormone and sex steroids. Prolonged oestrogen deficiency can be a risk factor for osteoporosis in women with chronic liver disease.

Hypogonadism and feminisation are well recognised complications of alcoholic cirrhosis of the liver in men.[1] Gonadal dysfunction may also occur, but is less well documented, in alcoholic women.[2,3] There is increasing evidence that many of these endocrine disturbances arise from the effects of alcohol per se rather than the effects of liver disease,[4] but recent studies have confirmed that hypogonadism may nonetheless be a feature of chronic non-alcoholic liver disease in men.[5] Changes in plasma concentrations of sex hormones have also been described in postmenopausal women with non-alcoholic chronic liver disease.[6] There are, however, very few reports examining gonadal dysfunction in younger women with non-alcoholic chronic liver disease.[7]

We have recently shown that a high proportion of women with non-alcoholic chronic liver disease coming to hepatic transplantation have appreciable amenorrhoea,[8] but the causes of this

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TABLE I *Details of patients studied*

Patient	Age (yrs)	Liver disease	Duration of amenorrhoea (yrs)	Child-Pugh score	Serum LH (U/l)†	Drug treatment
1	33	Primary biliary cirrhosis	0·3	8	8·9	
2	33	Chronic active hepatitis – cirrhosis	9·0	10	4·1	Spironolactone
3	32	Chronic active hepatitis – cirrhosis	2·0	9	9·1	
4	32	Chronic active hepatitis – cirrhosis	4·0	11	17·0	
5	29	Chronic active hepatitis – cirrhosis	3·0	12	10·0	Spironolactone, prednisolone, azathioprine, ranitidine
6	33	Chronic active hepatitis – cirrhosis	0·3	11	0·2	Spironolactone, prednisolone, azathioprine
7	33	Budd-Chiari syndrome	1·0	8	0·2	Spironolactone, frusemide, heparin
8	25	Chronic active hepatitis – cirrhosis	0·9	5	0·3	
9	25	Sclerosing cholangitis	0·3	9	2·0	Spironolactone, deoxyurocholic acid
10	21	Cystic fibrosis – cirrhosis	8·0*	10	0·3	Cimetidine
11	19	α_1 antitrypsin deficiency – cirrhosis	6·0*	13	0·2	Spironolactone, frusemide
12	19	Chronic active hepatitis – cirrhosis	6·0*	10	2·9	Spironolactone, prednisolone, cimetidine

*Primary amenorrhoea – duration of amenorrhoea calculated assuming menstruation should have begun at age 13.
†Patients 1–5 were designated normogonadotrophic, and patients 6–12 hypogonadotrophic.
LH=luteinising hormone.

disturbance of gonadal function have not been explored. We have therefore investigated young women with amenorrhoea and chronic liver disease in order to determine the endocrine features and the possible causes and consequences of hypogonadism.

Methods

SUBJECTS

Twelve women aged 19–33 years (mean 27·8) with proved chronic liver disease and amenorrhoea of at least three months' duration were studied. All were studied as inpatients. Eleven of the 12 had been admitted briefly to hospital for diagnostic procedures and were not acutely unwell. Only one patient (patient 11, Table I) had evidence of hepatic decompensation and was admitted to be considered for transplantation. She was studied within three days of admission. Three of the patients, aged 19–21 years, had primary amenorrhoea. In these patients the duration of amenorrhoea was calculated from age 13 (the median age at which menstruation begins in normal women). Only two of these women had ever been pregnant and only one (patient 7, Table I) had had children.

Patients were interviewed and examined by a single observer without knowledge of the biochemical findings. In the physical examination, breast development and genital hair were rated 1–5 according to the method of Tanner,[9] and skinfold thickness (triceps and subscapular sites) were measured with Holtain calipers. The direct measurement of subcutaneous fat thickness was preferred to an indirect measurement (such as body mass index) because of the presence of ascites in some subjects. The nature of the liver disease was determined by standard histological, imaging, and biochemical criteria. The severity of liver dysfunction was graded by the method of Child, as modified by Pugh *et al*,[10] scoring five clinical or laboratory findings (encephalopathy, ascites, bilirubinaemia, serum albumin, and prothrombin ratio) on a scale of 1 (least severe) to 3 (most severe). The maximum total score was thus 15 and the minimum 5 (Table I).

Eleven women aged 20–33 years (mean 26·5) were recruited from hospital staff as healthy controls. None had any history of liver disease. All had regular menstruation and none was using hormonal methods of contraception. Blood samples were drawn from control subjects in the early follicular phase of the cycle.

LABORATORY METHODS

In both chronic liver disease patients and normal controls, serum concentrations of luteinising hormone, follicle stimulating hormone, testosterone (T), oestradiol, prolactin, and sex hormone binding globulin were measured. Luteinising hormone and follicle stimulating hormone were measured by sensitive two site immunoradiometric assays (Sucrosep kits, Boots-Celltech Diagnostics Ltd, Slough) with detection limits of 0·2 U/l and 0·3 U/l and interassay coefficients of variance of 6% and 8%, respectively. Sex hormone binding globulin (SHBG) was measured by the method of Rosner,[11] with interassay coefficient of variance of 9%. The oestradiol/sex hormone binding globulin molar ratio was used as an index of free oestradiol. Derived free testosterone (dfT) concentrations were estimated from the relation:

$$dfT = T(6{\cdot}0–2{\cdot}4 \log_{10} [SHBG])$$

which has been shown to correlate well with measured free testosterone (T) concentrations.[12] Radiographs of the right hand were obtained in 10 of the liver disease patients for measurement of metacarpal bone density. At the mid-point of the second metacarpal the total width (TW) and the medullary width (MW) were measured by dial gauge micrometer. The proportion of the cross sectioned area occupied by cortical bone was calculated from:

$$(TW^2\text{-}MW^2)/TW^2$$

and the results compared with published normal values.[13]

STATISTICAL ANALYSIS

Biochemical measurements in control and chronic liver disease subjects were compared initially by Student's *t* test, but the variance in serum concentrations of luteinising hormone, testosterone, oestradiol, and prolactin was considerably greater in the patients (all $p<0{\cdot}01$) indicating that it was not appropriate to use the *t* test. We therefore elected to subdivide the chronic liver disease patients on the basis of serum luteinising hormone concentrations. Those in whom luteinising hormone concentrations were <3 U/l were designated hypogonadotrophic (n=7) and those in whom the concentration exceeded 4 U/l were designated normogonadotrophic (n=5). Differences among the groups were assessed by analysis of variance using the non-parametric Kruskal-Wallis test. Group values are given as the median, with upper and lower quartiles. The Wilcoxon rank method was used when only two groups were compared. Correlations were calculated by the Spearman method.

Results

The duration of amenorrhoea in the patients ranged from 0·3 to 9 years, and was not related to the known duration of liver disease or the severity as assessed by clinical and laboratory criteria (Table I). The greater variation found in several of the biochemical measurements in chronic liver disease patients (when compared with control subjects), suggested that this group was not uniform in its endocrine features. We therefore divided the patients into two groups on the basis of their serum luteinising hormone concentrations. In control subjects serum luteinising hormone ranged from 4·1 to 9·7 U/l. Seven chronic liver disease patients had serum LH <3 U/l (hypogonadotrophic) and five had values > 4 U/l (normogonadotrophic). When compared with controls these groups showed significant differences in serum concentrations of both gonadotrophins and sex steroids. The hypogonadotrophic patients had median concentrations of luteinising hormone, follicle stimulating

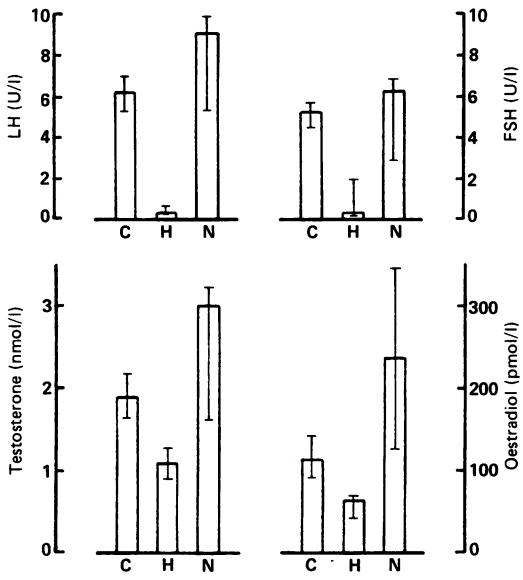

Figure 1: Biochemical measurements in control subjects (C), hypogonadotrophic (H), and normogonadotrophic patients (N). The groups of patients with chronic liver disease were defined on the basis of serum luteinising hormone (LH) concentrations (see Methods). Columns indicate the median values with lower and upper quartiles. See Table II for statistical analysis. FSH=follicle stimulating hormone.

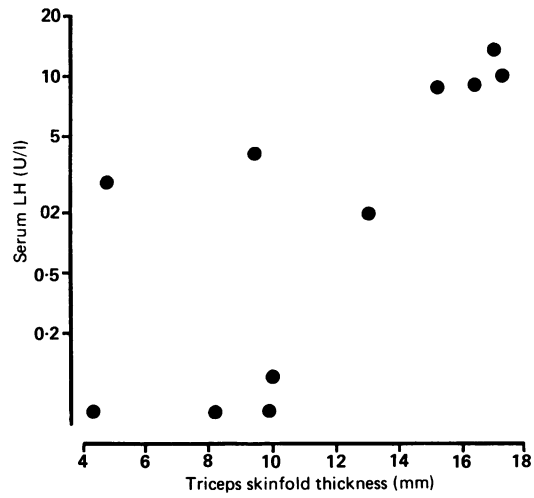

Figure 2: Relation between triceps skinfold thickness and serum luteinising hormone (LH) concentration (logarithmic scale) in patients with chronic liver disease. There is a significant positive correlation (r_s=0·71, p<0·02).

hormone, total testosterone, and total oestradiol that were significantly (p<0·025) below the median values in control subjects, with the median oestradiol concentration (64 pmol/l) within the postmenopausal range. Although the normogonadotrophic patients had median luteinising hormone concentrations 44% greater than control subjects, in none was the value >20 U/l and, moreover, follicle stimulating hormone concentrations were similar, thus excluding primary gonadal failure as a cause of the amenorrhoea. Median values of total testosterone and total oestradiol were 58% and 110% greater respectively in the normogonadotrophic patients than in the control subjects (Fig 1, Table II). Serum sex hormone binding globulin concentrations did not differ significantly between the groups, hence the median values of free sex steroid concentrations parallelled those for total hormone concentrations. In the chronic liver disease patients serum testosterone and oestradiol were significantly correlated (r_s=0·69, p<0·02) but there was no significant relation in control subjects (r_s=−0·20).

Median serum prolactin concentrations were greatest in the normogonadotrophic patients, but the two highest individual values (1290 and 1300 mU/l) were seen in hypogonadotrophic patients. No statistically significant differences were found between either patient group and the control subjects (Table II).

Breast development in the hypogonadotrophic patients was less than that in normogonadotrophic patients (median stage 4 *v* stage 5; p<0·05). Only one patient (from the normogonadotrophic group) was hirsute. Two hypogonadotrophic patients gave a recent history of weight loss (>6 kg in the six months before the study), and as a group the hypogonadotrophic patients had significantly reduced skinfold thickness (p<0·025 at both sites) compared with control subjects and normogonadotrophic patients (Table II). In the chronic liver disease patients, but not control subjects, serum luteinising hormone was positively correlated with skinfold thickness at both the triceps

TABLE II *Biochemical and skinfold thickness measurements in control subjects and patients with chronic liver disease*

		*Chronic liver disease**		*Kruskal-Wallis H statistic*	*p*
	Controls	*Normogonadotrophic*	*Hypogonadotrophic*		
No	11	5	7	–	–
LH (U/l)	6·3 (5·3–7·1)	9·1 (5·3–9·8)	0·3 (0·2–0·7)	14·58	0·001
FSH (U/l)	5·3 (4·4–5·9)	6·3 (2·8–6·9)	0·3 (0·2–2)	11·43	0·001
Total T (nmol/l)	1·90 (1·63–2·18)	3·0 (1·63–3·22)	1·10 (0·88–1·30)	8·05	0·025
dfT (pmol/l)	39 (32–42)	55 (32–66)	21 (19–27)	5·22	0·05
Total E_2 (pmol/l)	113 (93–144)	237 (124–347)	64 (43–70)	9·03	0·025
E_2/SHBG (molar ratio ×10³)	2·60 (1·77–3·72)	3·40 (2·45–4·97)	1·30 (1·02–1·60)	6·21	0·025
SHBG (nmol/l)	43 (40–55)	50 (44–53)	47 (33–54)	1·64	NS
Prolactin (mU/l)	380 (223–495)	690 (260–765)	460 (203–953)	1·98	NS
Triceps skinfold thickness (mm)	15·8 (11·0–17·8)	15·2 (10·5–16·1)	9·1 (4·5–10·0)	7·75	0·025
Subscapular skinfold thickness (mm)	12·1 (8·5–13·0)	14·0 (6·4–14·4)	6·5 (4·3–7·7)	7·86	0·025

LH=luteinising hormone; FSH=follicle stimulating hormone; T=testosterone; dfT=derived free testosterone; E_2=oestradiol; SHBG=sex hormone binding globulin.
Results are given as the median (with lower and upper quartile).
*Chronic liver disease patients were subdivided according to serum LH concentrations: LH <3 U/l=hypogonadotrophic; LH >4 U/l=normogonadotrophic.

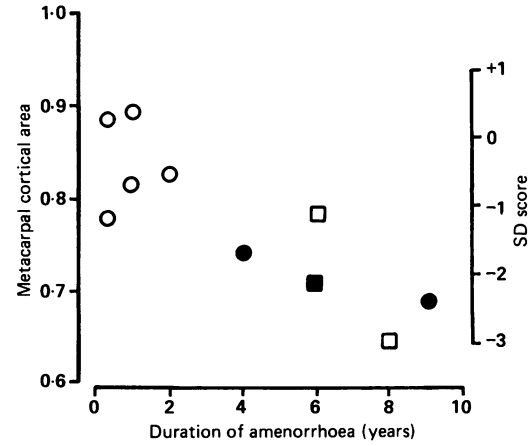

Figure 3: Correlations between metacarpal cortical bone area and the duration of amenorrhoea. The absolute values for metacarpal area are given on the left and the number of SDs from the mean on the right. (Normal ranges from Nordin et al).[13] A significant inverse correlation is present (r_s=−0·73, p<0·02). □=Patients with primary amenorrhoea. ■ ●= steroid treated patients.

(r_s=0·71, p<0·02) and the subscapular (r_s=0·68, p<0·025) sites (Fig 2). The metacarpal cortical bone area was >2 SD below normal in three of the 10 chronic liver disease patients in whom it was measured. All three had serum oestradiol concentrations in the menopausal range (<120 pmol/l). There was an inverse correlation between the duration of amenorrhoea and the metacarpal cortical area (r_s=−0·73, p<0·02; Fig 3).

Discussion

This study shows that no single mechanism causes amenorrhoea in young women with non-alcoholic chronic liver disease. No patient had very high serum gonadotrophin concentrations consistent with primary gonadal failure, thus hypothalamic-pituitary dysfunction was probably common to all. We chose to analyse the results according to luteinising hormone status. Although this distinction was arbitrary and does not take into account changes in luteinising hormone pulsatility, which is certainly a feature of alcoholic liver disease,[14] there were interesting clinical and biochemical differences between the hypogonadotrophic and normogonadotrophic patients. The former, as well as having low serum gonadotrophins, had low testosterone and oestradiol values, were significantly thinner, and had a lower median breast development stage (confirming oestrogen deficiency). The situation in these patients thus closely resembled that of women with weight related amenorrhoea.[15] The relation between serum luteinising hormone and skinfold thickness in the chronic liver disease patients as a whole emphasises the importance of undernutrition as a cause of hypothalamically mediated amenorrhoea. Anorexia can presumably occur as a consequence of ill health at any stage in the evolution of this disorder, hence the occurrence of this type of hypothalamic-pituitary dysfunction was related neither to the severity nor to the known duration of liver disease.

The normogonadotrophic patients were of normal nutritional status and had no clinical evidence of oestrogen deficiency (although we did not undertake more sensitive tests of oestrogen status such as cervical cytology). These patients had median serum testosterone and oestradiol concentrations greater than those found in the follicular phase of the controls. The biochemical findings in these patients were therefore similar to those in patients with polycystic ovary syndrome.[16] Obesity and hirsutism, the clinical associations of the polycystic ovary syndrome, were not a striking feature of our patients, though some were taking drugs such as cimetidine and spironolactone that may have modified hirsutism.[17 18] The origin of the high serum oestradiol and testosterone concentrations is uncertain. The early suggestion that high serum oestrogen values arise because of reduced metabolic clearance is probably incorrect, although considerable controversy still exists as to whether the increased production of oestrogens arises from glandular or extraglandular sources. The observations that are reported have been made almost exclusively on alcoholic men,[19] so there must be doubt as to their relevance to women with non-alcoholic chronic liver disease. Our observation that serum testosterone and oestradiol concentrations were correlated in chronic liver disease patients supports the view that their production is linked, but gives no clue as to the source. Studies in postmenopausal women with non-alcoholic chronic liver disease have also found a higher median plasma oestradiol concentration than in age matched controls, although the median plasma testosterone concentration was not similarly raised.[6]

Serum prolactin concentrations were variably raised in the chronic liver disease patients, but hyperprolactinaemia was not a consistent feature. Considerable variation in serum prolactin has also been noted in alcoholic cirrhotic men.[20] Our data do not suggest a primary role for hyperprolactinaemia in the genesis of amenorrhoea in chronic liver disease. The finding that sex hormone binding globulin concentrations did not differ between controls and cirrhotic subjects is of interest, since in men with cirrhosis of both alcoholic and non-alcoholic aetiology and in postmenopausal women with non-alcoholic chronic liver disease, sex hormone binding globulin concentrations are raised.[6 21 22]

One serious consequence of oestrogen deficiency is a reduction in bone density, and we found evidence that bone density, as assessed by the relatively insensitive technique of metacarpal morphometry, was reduced in the women with the most prolonged amenorrhoea. The aetiology of bone loss in chronic liver disease is complex,[23] but recent evidence suggests an important role of hypogonadism.[24] Not all our patients were obviously oestrogen deficient, but in the absence of longitudinal studies the possibility cannot be excluded that episodes of hypogonadotrophic hypogonadism with oestrogen deficiency occur during the prolonged and fluctuating course of chronic liver disease. Oestrogen deficiency and osteopenia are of more than academic interest in this group of patients, who are likely candidates for hepatic transplantation. Even though regular menstruation usually returns after transplantation[8] the patients are likely to require long term corticosteroid treatment, which is an additional risk factor for bone loss and fracture.[25]

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