The correlation coefficient for the thumb was lowest on account of the smaller age related changes—thumb: r=0·52 (n=481); great toe: r=0·74 (n=481); medial malleolus: r=0·71 (n=480).

Discussion and conclusions

No other study of biothesiometry[2-4] has investigated how vibration threshold varies with age. Our results quantify the increase of threshold with age and show that within age decades the logarithms of the readings are normally distributed. In order to ensure that the limits of normality that we have defined were based on the maximum possible number of observations we pooled the results from groups 1 and 2. We showed that there were no detectable differences among machines, among observers, and between the two populations and therefore consider that there was a reasonable case for combining the data.

Whether or not the lower threshold and smaller variance for thumb readings was due to the sensory pathway being longer in the leg was not clear, but our impression is that the threshold in the foot is related to height.

The centile charts may be used in the clinic to help decide whether a patient's vibration threshold is abnormal. Comparing diabetic thresholds with normal ranges will help in quantifying the progress of any peripheral neuropathy. Evidence suggests that vibration sensation may return to normal as control is improved,[1] so possibly we have another method of assessing the benefits of long term diabetic control.

The biothesiometer provides a quick, reliable, and more objective assessment than the time honoured tuning fork.

We thank the 11 student members of the firm who helped collect the data.

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Oral contraceptives, pregnancy, and endogenous oestrogen in gall stone disease—a case-control study

R K R SCRAGG, A J McMICHAEL, R F SEAMARK

Abstract

A case-control study of gall stone disease in women in relation to use of contraceptives, reproductive history, and concentrations of endogenous hormones was undertaken. The study population comprised 200 hospital patients with newly diagnosed gall stone disease, 182 individually matched controls selected from the community, and 234 controls who were patients in hospital. Use of oral contraceptives was associated with an increased risk of developing gall stones among young subjects but a decreased risk among older subjects. The risk of developing gall stone disease increased in association with increasing parity, particularly among younger women. The risk fell with increasing age at first pregnancy, independent of parity. Mean urinary excretion over 24 hours of oestrone, but not of pregnanediol, was significantly ($p<0.05$) greater for postmenopausal patients than controls.

The age dependence of the relative risk associated with exposure to oral contraceptives and pregnancy suggests that there are subpopulations of women susceptible to early formation of gall stones after exposure to either oral contraceptives or pregnancy.

Division of Human Nutrition, Commonwealth Scientific and Industrial Research Organisation, Adelaide, South Australia 5000, Australia

R K R SCRAGG, MB, PHD, research fellow in epidemiology
A J McMICHAEL, MB, PHD, senior principal research scientist

Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia 5000, Australia

R F SEAMARK, BAGSC, PHD, reader in endocrinology

Correspondence and requests for reprints to: Dr R K R Scragg, Department of Community Health, School of Medicine, University of Auckland, Private Bag, Auckland, New Zealand.

Introduction

The greater prevalence of gall stones among women than among men,[1] initially apparent at puberty, is thought to be due to hormonal and reproductive factors such as oral contraception, oestrogens, and pregnancy. Epidemiological studies of living subjects have generally shown a positive association between development of gall stones and prior pregnancy,[2-14] although some studies[15-17] and two series of postmortem examinations did not show any association.[18 19] Exposure to oral contraceptives was initially observed to increase the risk of developing gall bladder disease,[20-22] but subsequent reports have not confirmed this.[13 16 17 23-25] Exogenous oestrogens have also been observed to increase the risk of gall bladder disease developing in women[26] and men,[27] although a study of elderly men showed that oestrogen increased the risk of cholecystectomy but not of gall stones.[28] Urinary excretion of endogenous oestrogen is positively associated with bile cholesterol saturation,[29] but we could find no study that had specifically examined endogenous oestrogens in patients with gall stone disease.

We undertook a case-control study to investigate the role of oral contraceptives, reproductive history, and endogenous hormones in the development of gall stone disease in women of different ages.

Methods

SELECTION OF SUBJECTS

Details of the design of this study have been described elsewhere.[30] Briefly, women presenting for oral cholecystography or ultrasound of the gall bladder at two major public hospitals in Adelaide from December 1978 to September 1980 were entered into the study. Those who had stones visible in their cholecystogram or ultrasound scan and those whose cholecystograms did not show any opacity but in whom stones were confirmed at operation were classified as patients (n=200). Those women (n=234) whose cholecystograms were negative for gall stones were classified as hospital controls. Two hundred and twelve of the hospital controls had abdominal symptoms suggestive of gall stones; of these 166 (78%) had abdominal pain for which no cause could be found. Some of them probably had gall stones that either were not detected because of the imperfect sensitivity of oral cholecystography[31-33] or were passed during an attack of biliary colic before x ray examination.[34-36]

Because most hospital controls had symptoms suggestive of gall stones we recognised that they were not necessarily representative of people without gall stones in the source population. Accordingly, a group of controls from the community (n=182) was randomly selected from the electoral roll after being individually matched with patients for age (within two years), sex, and electoral subdivision as defined by the Australian Bureau of Statistics. Eighteen patients could not be matched with community controls because they either lived outside the Adelaide electoral division or were younger than the youngest controls available from the electoral roll.

COLLECTION OF DATA

The women were questioned in a structured interview by one of us (RKS) about the following hormonal factors: duration and date of each pregnancy; use of oral contraceptives and other exogenous oestrogens; age at menarche and menopause; and date of previous hysterectomy or oophorectomy. Extensive data on recent dietary intake and on current weight, height, and demographic characteristics were also collected from each subject.[30]

Women in hospital were interviewed double blind without knowledge of the result of biliary radiological investigation. In contrast, that community controls did not have symptomatic gall bladder disease was known by them and the interviewer. Community controls were told the purpose of the study, in the same general terms as were hospital subjects, but were not informed of the specific hypotheses behind the study.

URINARY HORMONES

Early morning urine samples were collected from subjects in hospital who were not at the time taking oral contraceptives, oestrogens, or progesterones. For premenopausal subjects urine was collected within the first 10 days of the menstrual cycle. Oestrone was measured by a fluorometric method (R I Cox, unpublished findings) modified from the procedure of Brown *et al*,[37] and pregnanediol by gas chromatography.[38] Ratios of hormone to creatinine concentrations were derived after measurement of creatinine concentrations with a Beckman creatinine analyser II, and converted to urinary excretion over 24 hours.[39]

STATISTICAL ANALYSES

The *t* test for independent samples was used to compare means for patients and controls.[40] The odds ratio was used as an estimate of the relative risk and the test based method used to calculate confidence limits for unmatched analyses.[41] For comparisons of patients with matched community controls the odds ratio was estimated by the matched pairs method with the confidence limits computed from the binomial distribution.[41] The test for linear trend was used to test for dose response relations.[41] Multiple logistic regression was used to estimate the net association between individual variables and the risk of developing gall stones, after controlling for the confounding effects of other variables.[42]

TABLE I—*Age specific distribution of matched pairs of patients and community controls and the associated relative risks for exposure to oral contraceptives and pregnancy (≥5 months' duration)*

Age group (years)	Community control	Oral contraceptives* Patient: Exposed	Oral contraceptives* Patient: Unexposed	Oral contraceptives* Relative risk‡ (95% confidence limit)	Pregnancy† Patient: Exposed	Pregnancy† Patient: Unexposed	Pregnancy† Relative risk (95% confidence limit)
≤29	Exposed	30	4	1·5 (0·4, 7·2)	13	3	4·0 (1·1, 22·1)
	Unexposed	6	0		12	12	
30-39	Exposed	23	9	0·4 (0·1, 1·6)	32	1	4·0 (0·4, 195·1)
	Unexposed	4	1		4	0	
40-49	Exposed	14	10	0·7 (0·2, 2·0)	29	2	1·5 (0·2, 18·0)
	Unexposed	7	3		3	0	
50-59	Exposed	6	18	0·3 (0·1, 0·8)	34	3	1·7 (0·3, 10·7)
	Unexposed	5	13		5	0	
≥60	Exposed	0	2	0·0	21	4	1·0 (0·2, 5·4)
	Unexposed	0	27		4	0	
All ages	Exposed	73	43	0·5 (0·3, 0·9)	129	13	2.2 (1.1, 4.5)
	Unexposed	22	44		28	12	

*Risk for non-users = 1·0.
†Risk for nulliparous women = 1·0.
‡Relative risk = ratio of discordant pairs of patients and controls—for example, for women aged ≤29 the relative risk = 6:4 = 1·5.

Results

ORAL CONTRACEPTIVES AND PREGNANCY

When patients were compared with community controls (table I) or hospital controls (table II) the relative risk associated with previous exposure to oral contraceptives (ever versus never exposed) was found to decrease with increasing age. Only among women aged 29 years or less was the relative risk greater than unity; it was less than unity in all older age groups. Logistic regression analysis showed that the extent of age related variation in the relative risk associated with exposure to oral contraceptives was significant ($p<0·05$). The relation between development of gall stones and previous exposure to one or more pregnancies of five months or more of duration showed a similar age dependency (tables I and II). The risk was greatest for women aged 29 or less and approached unity in the older age groups when patients were compared with community controls (table I), though it was below unity for comparisons of the older patients with hospital controls (table II). These associations of the risk of developing gall stones with exposure to oral contraceptives and with pregnancy were found, with logistic regression analyses, to be independent of each other and of obesity and dietary intake.

There was a dose response relation between the relative risk of developing gall stones and the number of previous pregnancies of five months or more. This relation was stronger among women aged less than 50 than among subjects aged 50 or more (table III).

Among parous women the risk of developing gall stones was negatively related to age at first pregnancy of at least five months' duration. The relation was significant for patients compared with community controls aged less than 50 (mean age at first pregnancy: patients = 21·8 years, controls = 23·1 years ($p<0·05$)). Multivariate analysis showed this effect to be independent of parity.

TABLE II—*Age specific relative risks in patients compared with hospital controls associated with exposure to oral contraceptives and pregnancy (≥5 months' duration)*

Age group (years)	Ever exposed	Oral contraceptives*			Pregnancy†		
		Patients	Hospital controls	Relative risk (95% confidence limit)	Patients	Hospital controls	Relative risk (95% confidence limit)
≤29	Yes	42	56	1·4 (0·2, 9·8)	31	29	2·4 (1·1, 5·5)
	No	6	11		17	38	
30-39	Yes	31	34	0·7 (0·4, 1·5)	40	41	1·0 (0·9, 1·0)
	No	10	8		1	1	
40-49	Yes	21	35	0·5 (0·2, 1·1)	33	45	0·7 (0·3, 1·6)
	No	14	12		2	2	
50-59	Yes	11	19	0·5 (0·2, 1·1)	40	44	0·7 (0·3, 1·6)
	No	33	28		4	3	
≥60	Yes	1	0	∞	28	28	0·8 (0·4, 1·6)
	No	31	31		4	3	
All ages	Yes	106	144	0·7 (0·5, 1·0)	172	187	1·5 (0·9, 2·7)
	No	94	90		28	47	

*Risk for non-users = 1·0.
†Risk for nulliparous women = 1·0.

TABLE III—*Relative risk of developing gall stones associated with the number of pregnancies of at least five months' duration*

No of pregnancies	Patients v community controls						Patients v hospital controls					
	Aged <50 years			Aged ≥50 years			Aged <50 years			Aged ≥50 years		
	Patients	Controls	Relative risk	Patients	Controls	Relative risk	Patients	Controls	Relative risk	Patients	Controls	Relative risk
0	18	31	1·0	7	9	1·0	20	41	1·0	8	6	1·0
1	11	12	1·6	8	10	1·0	15	21	1·5	8	9	0·7
2	28	33	1·5	16	20	1·0	31	37	1·7	16	20	0·6
3	25	20	2·2	12	18	0·9	26	25	2·1	13	18	0·5
4	17	9	3·3	12	8	1·9	18	17	2·2	14	10	1·1
≥5	12	6	3·4	16	6	3·4	14	15	1·9	17	15	0·9
Total	111	111		71	71		124	156		76	78	
Test for linear trend		$\chi^2 = 8·245$ $p < 0·005$			$\chi^2 = 4·243$ $p < 0·05$			$\chi^2 = 13·536$ $p < 0·001$			$\chi^2 = 0·155$ $p > 0·05$	

TABLE IV—*Mean (SE) duration of exposure (months) to pregnancy, lactation, oral contraceptives, oestrogens, and natural ovarian activity*

	Patients v community controls				Patients v hospital controls			
	Aged <50 years		Aged ≥50 years		Aged <50 years		Aged ≥50 years	
	Patients (111 pairs)	Controls (111 pairs)	Patients (71 pairs)	Controls (71 pairs)	Patients (n = 124)	Controls (n = 156)	Patients (n = 76)	Controls (n = 78)
Pregnancy (includes miscarriages)	23·6† (1·5)	17·6 (1·5)	29·8 (2·6)	23·5 (2·0)	23·1 (1·4)	20·7 (1·5)	29·8 (2·5)	28·0 (2·1)
Lactation	5·8 (1·0)	4·2 (0·7)	15·3* (2·3)	8·6 (1·2)§	5·6 (0·9)	5·0 (0·7)	14·5 (2·2)	11·6 (1·5)
Oral contraceptives	33·6 (3·9)	43·3 (4·4)	7·5† (2·7)	23·6 (5·3)	32·9 (3·6)	35·7 (3·4)	7·0 (2·6)	7·2 (2·2)
Oestrogens	0·9 (0·5)	0·2 (0·1)	5·2 (2·8)	8·1 (3·6)	0·8 (0·5)	0·4 (0·3)	5·4 (2·7)	6·2 (2·7)
Ovarian activity	184·5 (9·5)	174·4 (8·7)‡	353·7 (8·8)	362·1 (9·9)‖	178·8 (9·0)	167·8 (8·0)	357·0 (8·5)	344·5 (8·8)¶

Patients significantly different from controls in same age group: *$p < 0·05$. †$p < 0·01$.
‡n = 110. §n = 70. ‖n = 68. ¶n = 76.

Among patients and community controls there was a significantly ($p < 0·01$) increased mean duration of exposure to pregnancy for patients aged under 50 compared with their matched controls, and in both age groups there was a tendency towards a lesser mean period of exposure to oral contraceptives for patients than for controls (table IV), which corroborated the findings in table I. There were no significant differences in the mean period of exposure to exogenous oestrogen. The significant ($p < 0·05$) increase in the mean duration of lactation for patients aged 50 and over compared with matched community controls disappeared after controlling for the number of pregnancies.

ENDOGENOUS HORMONES

The total period of exposure to sex hormones was calculated for each subject by subtracting the age of menarche from the age of either menopause, oophorectomy, or entry into the study. By subtracting the duration of exposure to pregnancy, lactation, oral contraceptives, and oestrogens from this total we calculated the period of natural ovarian activity for each subject. The mean period of natural ovarian activity was similar in patients and community and hospital controls in each age group (table IV).

In contrast with the finding of a similar mean duration in natural ovarian activity for patients and controls, mean 24 hour urinary excretion of oestrone was significantly ($p < 0·05$) greater in patients than in hospital controls among subjects aged 50 or more but not among younger subjects (table V). Mean 24 hour urinary excretion of pregnanediol was, however, similar in patients and hospital controls aged both above and below 50. Furthermore, oestrone and pregnanediol concentrations were not significantly ($p > 0·05$) correlated with the degree of obesity in patients or controls aged above or below 50.

Discussion

The possible biases present in this study have been discussed elsewhere.[30] Briefly, detection bias could, in principle, have occurred in the comparison of patients with community controls as patients and community controls entered the study by different referral pathways.[43] The presence of this bias, however, cannot be determined on a priori grounds.[44]

Interviewer bias and respondent recall bias may also have been present in comparisons of patients with community controls as community controls were not interviewed blindly. The effects of these two biases in misclassification of exposure, if present, would have been minimised by use of a structured questionnaire and by not informing any of our subjects of the specific hormonal hypotheses behind the study. (In fact, the

TABLE V—*Mean (SE) urinary excretion over 24 hours of oestrone and pregnanediol by patients and hospital controls*

	No	Oestrone (pmol)	No	Pregnanediol (μmol)
		Aged <50		
Patients	37	15·5 (2·6)	37	9·4 (0·12)
Controls	40	16·7 (2·2)	41	1·25 (0·44)
		Aged ≥50		
Patients	43	6·7 (0·7)*	44	0·59 (0·06)
Controls	56	4·8 (0·4)*	56	0·63 (0·09)

*Significance of difference: $p < 0.05$.
Conversion: SI to traditional units—Oestrone: 1 pmol ≈ 0·27 μg. Pregnanediol: 1 μmol ≈ 0·32 mg.

age dependence of the effects associated with exposure to oral contraceptives and pregnancy were unexpected.) Bias in misclassification of disease was likely to have been present in comparisons of patients with both groups of controls and to have been greater among hospital controls than community controls.[30]

The consistent direction of the differences between patients and the two groups of controls which were generally greater when patients were compared with community controls than with hospital controls, suggests that the possible effect of any of these biases was slight.

ORAL CONTRACEPTIVES

The age dependent variation in the risk of developing gall stone disease associated with exposure to oral contraceptives (with the risk being above unity for young subjects and below unity for older subjects) suggests that there is a subpopulation of women who are metabolically susceptible to the formation of gall stones. In these women stones may develop soon after initial exposure. This would accord with the observed lower mean duration of oral contraceptive use among women with gall stones than among controls (table IV).

This interpretation is supported by a recent report from a British follow up study, which found, among women taking oral contraceptives, that after an initial rise in the incidence of gall stone development among those taking oral contraceptives for three years or less there was an inverse relation between incidence of gall stone development and duration of use of oral contraceptives among those taking oral contraceptives for more than three years.[25] The authors suggested that "the previously demonstrated short term increase in risk is due to an acceleration of gall bladder disease only in women susceptible to it." Similarly, the relative risk of developing gall stones was increased with short term (6-12 months) use of oral contraceptives but decreased with longer use, according to a study in Boston.[20]

The presence of a group of women susceptible to the formation of gall stones after exposure to oral contraceptives may explain why earlier studies[20-22] found a positive association between risk of developing gall stones and use of oral contraceptives that was not confirmed by later studies.[13 16 17 23-25] The early studies, carried out soon after oral contraceptives became widely used, were likely to have included women who were susceptible to forming gall stones and in whom oral contraceptives accelerated this formation: that subgroup of susceptible women would not, therefore, have been available for inclusion as new cases in later studies.

If this explanation is correct future epidemiological studies will probably find a positive association between use of oral contraceptives and development of gall stone disease only among women recently exposed to oral contraceptives for the first time. Such women are typically teenagers or in their 20s. In our study the smaller increase in the relative risk among women aged 29 or over (tables I and II) compared with a rough doubling in the risk observed in earlier studies[20-22] may have been due to the common use of low dose oestrogen oral contraceptives in Australia after their introduction in the mid-1970s.

PREGNANCY

The age dependence of the risk of developing gall stones associated with pregnancy—the risk being greatest for subjects aged under 29 (tables I and II)—corroborates the findings in other studies. A study in England observed that parous women aged less than 50 showed a greater prevalence of gall stones than did nulliparous women of the same age but that in women aged over 50 the prevalence was greater in nulliparous women.[2] In addition, a positive association between pregnancy and gall stone disease was reported to be strongest for women aged less than 30 among Chippewa Indians[8] and weight conscious women.[9]

The observation of a dose response relation between the relative risk of developing gall stones and exposure to pregnancy, which was maximal among younger women (table III), agrees with previous findings.[2 9] Furthermore, a recent report found a dose response relation among women aged 25-39.[13] Other studies have investigated women over a broad range of ages (20-60 years); some of these studies have described a dose response in the risk associated with pregnancy[4 6 8] and others have not.[3 7]

That pregnancy was significantly ($p < 0.05$) associated with an increased risk of developing gall stones in younger women alone, and not in postmenopausal women (that is, women aged over 50), suggests that the effect of pregnancy on the risk of developing gall stones is immediate and temporary and not long term. This conclusion would be consistent with the known immediate and temporary effect of pregnancy on the contraction of the gall bladder,[45] itself a probable risk factor, and on the cholesterol saturation of bile.[46] There is also, however, evidence of a long term shift in oestrogenic profile of women after completion of the first pregnancy, with an increase in the ratio of oestriol to oestradiol plus oestrone.[47 48] In view of the well documented effect of oestrogens on bile cholesterol saturation[49-51] this oestrogenic shift could partly account for the increase in risk related to parity. The finding that the risk of developing gall stones decreased with increasing age at first pregnancy, independent of parity, could also reflect any such protective effect of the oestrogenic profile associated with nulliparity, although a recent British study found a positive association of the risk of developing gall stones and age at first pregnancy.[13] Further research on this variable is required.

The age dependence of the risk associated with pregnancy was not fully consistent for comparisons of patients with both groups of controls in that, at older ages, the relative risk among patients compared with community controls approached unity but actually fell below unity for patients compared with hospital controls (tables I and II). The first result indicates that all women experiencing pregnancy are susceptible to increased risk of formation of gall stones, and the second suggests that there is a subpopulation of women who are susceptible to gall stones and who are therefore selectively removed at younger ages.

ENDOGENOUS HORMONES

The observation that the mean duration of ovarian activity was similar for patients and controls (table IV), together with the significantly ($p < 0.05$) increased mean excretion of oestrone in patients aged over 50 compared with controls (table V), suggests, at least for older patients, that it is not the period of exposure to ovarian activity but the degree of exposure that is related to formation of gall stones. The finding of raised excretion of oestrone in gall stone disease does not appear to have been reported. Furthermore, there do not appear to have been any studies carried out on the effect of oestrone on biliary cholesterol saturation, although studies in humans and primates have shown that oral contraceptives,[49-51] most of which contain some oestrogen, increase cholesterol saturation. In this respect it is relevant that orally active oestradiol is converted to oestrone by the intestinal mucosa.[52]

Oestrone is the major oestrogen in postmenopausal women,[53] in whom it is metabolised from androstenedione secreted

primarily by the adrenal glands.[54] Obesity is known to increase the conversion of androstenedione to oestrone.[55] The observation in this study, however, that oestrone was not significantly correlated with obesity among postmenopausal women suggests that the raised oestrone concentration in patients was due either to greater production of androstenedione by the adrenals or to greater metabolic conversion of androstenedione to oestrone.[54] The failure to find a raised oestrone concentration in young patients compared with controls may be related to the masking effects of the variable ovarian contribution of steroid precursors.[56]

To conclude, the variation by age in the risk of developing gall stone disease with risk factors related to concentrations of sex hormones suggests that discrete exposures, such as use of oral contraceptives and the occurrence of pregnancy, influence the formation of gall stones predominantly in young women who are metabolically susceptible to them. In postmenopausal women, however, it is endogenous factors, such as raised oestrogen concentrations, that appear primarily to influence the risk of developing gall stone disease.

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