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DIETARY SURVEY AND BILE ANALYSIS OF SUBJECTS WITH AND WITHOUT CHOLELITHIASIS

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Dietary survey and bile analysis of subjects with and without cholelithiasis" submitted by Deborah Ann Smith, B.Sc., in partial fulfilment of the requirements for the degree of Master Of Science in Nutrition.

Margaret Lee
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DATE March 31, 1977

ABSTRACT

Studies were conducted to compare the dietary intakes of subjects with and without cholelithiasis and to examine the relationship between dietary intake, bile composition and bile lithogenicity.

Dietary intake data (48-hour recall method) of 91 cholelithiasis subjects (15 males and 76 females) and 86 control subjects (13 males and 73 females) were compared. The male control group consumed significantly more protein per day than the male cholelithiasis group. These groups were similar in age, weight and height. When subjects with modified intakes (eg. Calorie restricted) were excluded from the sample, this difference in protein intake between the male groups ceased to be significant. The female control group consumed significantly more energy, protein, fat, carbohydrate and crude fibre than the female cholelithiasis group. Although the two female groups were similar in age, the female cholelithiasis group was significantly heavier and shorter than the female control group. When subjects with modified intakes were excluded from the sample, the differences in intake between the two female groups were still significant. Nutrient intakes/1000 Calories were similar for the male cholelithiasis and control groups and for the female chole-

lithiasis and control groups.

When total weekly intakes and food sources of crude fibre were examined, no significant differences were detected between the male cholelithiasis and control groups. However, the total weekly intake of crude fibre and the intake of crude fibre specifically from bread and bakery products was significantly higher for the female control group than for the female cholelithiasis group.

Bile composition and lithogenicity were compared between cholelithiasis (5 males and 10 females) and control (8 males and 5 females) subgroups. For both sexes, the cholelithiasis and control subgroups were similar in age, weight, and height. The percentage of cholesterol in bile was significantly higher for the male cholelithiasis subgroup than for the male control subgroup. The lithogenic index of bile from the male cholelithiasis group exceeded 1.0 (1.17 ± 0.36), which indicated lithogenic bile. This was significantly higher than the lithogenic index of the male control subgroup. No significant differences in bile composition or lithogenicity were noted between the female cholelithiasis and control subgroup. However, for the female subgroups a significant negative correlation was noted between weight and the percentage of bile salt in bile, while a significant positive correlation was noted between weight and both the percentage of cholesterol in bile and lithogenic index.

An examination of the relationship between bile composition, bile lithogenicity, and dietary intake revealed a significant positive correlation between protein intake and the percentage of bile salt in bile for the female subgroups. This was noted when the analysis was performed while controlling for age, weight, height and oral contraceptive use.

The results of these studies suggest a possible relationship between cholelithiasis and a low intake of energy, protein, fat or crude fibre, but more than one of these nutrients could be involved. The positive correlation between protein intake and the percentage of bile salt in bile suggests a possible effect of protein intake on the solubility of cholesterol in bile.

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INTRODUCTION

Although cholelithiasis (gallstones) is a major health problem, little is known about its etiology. It has, however, been suggested that diet may be a factor since the incidence of the disease varies from region to region. This theory is supported by the fact that diet affects the composition of the bile, which in turn is believed to affect gallstone formation.

Although several surveys have been conducted to determine the relationship between diet and gallstone formation, results have been conflicting and no conclusions have been reached. Furthermore, none of these surveys have considered crude fibre intake; although the high incidence of gallstones in developed countries, where fibre intake is low, as compared to that in developing countries where fibre intake is high, would seem to indicate that this may be a factor.

It seems clear that more research is needed to determine the effect of diet on gallstone formation. Therefore the following studies were conducted to compare the dietary intakes of groups of subjects with and without cholelithiasis. In addition, the relationship between dietary intake, bile composition and its lithogenicity was studied.

LITERATURE REVIEW

Cholelithiasis (gallstones) is a major health problem in North America as indicated by the fact that 89,338 cholecystectomies were performed in Canada alone, in 1972 (Information Canada, 1975). Although statistics on cholecystectomies give some indication of the problem, it is actually greater than these would indicate if one takes into account the large number of 'silent' gallstones. The result is a major loss to the economy, both through medical expenses and through decreased productivity.

Gallstones usually produce inflammation in the form of acute or chronic cholecystitis. Frequently gallstones become lodged in the bile ducts, blocking them. (Anderson and Scott, 1972) There is also some evidence that the incidence of cancer of the gallbladder is higher in patients with gallstones. Therefore a search for the causes of gallstone formation is needed.

Since gallstones are composed primarily of cholesterol (Bogren, 1964, Sutor and Wooley, 1970, Womack, 1973) considerable attention has been focused on those factors which affect the solubility of cholesterol in bile . Bile is composed of water (84%), bile salts (11.5%), phospholipids (3%), cholesterol (.5%) and smaller quantities of bile pig-

ment, protein and electrolytes (18) (Holzbach, 1973). While cholesterol is normally highly insoluble in water, it is readily dissolved in bile through the formation of micelles with bile salts and phospholipids (90% lecithin). The precise micellar arrangement is unknown, but the concentration of bile salts and phospholipid present is critical to the solubilization of cholesterol in bile (Wheeler, 1973). Isaksson (1954) was the first investigator to recognize the importance of the ratio of cholesterol to both bile salts and lecithin when determining the solubility of cholesterol in bile. This relationship was illustrated by the construction of a triangular phase diagram which allowed the representation of relative quantities of cholesterol, phospholipid and bile salts as percentages of their total sum. Following early work on lipid phase diagrams by Bourges and co-workers (1968), Admirand and Small (1968) established the boundary zone which indicated the limit of cholesterol solubility in human bile. This was accomplished by the 'in vitro' evaluation of cholesterol solubility in various mixtures of cholesterol, bile salts and phospholipid and the construction of a cholesterol solubility line. These researchers noted that this solubility line on the triangular phase diagram clearly separated cholelithiasis and control subjects. The control group had bile which was unsaturated with cholesterol and therefore fell below the cholesterol solubility line, while the cholelithiasis group exhibited bile which was saturated or supersaturated with cholesterol

and fell on or above the cholesterol solubility line. Bile from this latter group was classified as lithogenic. Other research has confirmed the findings of Admirand and Small (Small and Rapo, 1970) and some investigators have established that even after cholecystectomy, bile remains lithogenic (Almond et al., 1973, Boyer et al., 1974).

However, whether this method of determining bile lithogenicity is a useful method of distinguishing subjects with and without cholelithiasis is a matter of controversy. Several investigators have established that bile may be transiently lithogenic in all healthy subjects (Holzbach et al., 1973, MacKay et al., 1972, Smallwood et al., 1972) particularly after an overnight fast (Metzger et al., 1973, Northfield and Hoffman, 1975). Discrepancies in the results of these studies have been attributed to variations in the methods used for bile analysis (Strasberg, 1975) and the accuracy of the cholesterol solubility line determined by Admirand and Small (Holzbach et al., 1973).

Metzger and co-workers (1972) have suggested that the lithogenic index provides a more useful quantitative method of comparing and contrasting the lithogenic tendency of multiple bile samples. Lithogenic index is the ratio of the actual amount of cholesterol in bile to the maximum amount of cholesterol which could be solubilized by the amounts of phospholipid and bile salts present.

The biochemical and physiological basis of cholesterol

gallstone formation has been the subject of many excellent review articles (Redinger et al., 1972, Brandt and Bernstein, 1976, Swell et al., 1974, Wheeler et al., 1973). Many workers suggest that lithogenic bile formation precedes gallstone formation regardless of the fact that bile may be transiently lithogenic in all individuals. Increased cholesterol saturation of bile, followed by precipitation of cholesterol in the gallbladder, has been attributed to metabolically deranged liver cell function (Small and Rapo, 1970) resulting in an impaired bile salt feedback mechanism and decreased bile salt pool (Bell et al., 1973). Contradictory results have been reported by others (Pomare and Heaton, 1973a, Northfield and Hofmann, 1973, Northfield and Hofmann, 1975). Grundy et al. (1972) observed that increased liver secretion of cholesterol and decreased secretion of bile salts appeared to contribute to lithogenic bile formation in American Indian women. On the other hand, impaired gallbladder function may also contribute significantly to lithogenic bile formation as some researchers have reported that bile appears to become less lithogenic after cholecystectomy (Shaffer et al., 1972, Simmons et al., 1972).

It is apparent that the pre[illegible]iochemical basis of cholesterol gallstone formatio[illegible] remains to be elucidated. It will first be necessary [illegible] solve the inconsistencies present in many of the studi[illegible]orted to date.

Epidemiological surveys have identified many variables which appear to be associated with lithogenic bile and/or cholelithiasis. It is well known that the incidence of cholelithiasis increases with age (Hinkel, 1957, Friedman et al., 1966) however, the incidence appears to peak in the Canadian population between the ages of 35-44 years for the female and 45-64 years for the male (Information Canada, 1975). There is some evidence to suggest that the peak ages may have decreased somewhat in recent years since cholelithiasis appears to have become more common among younger people (Brownrigg, 1969, Goodman, 1976). Better detection procedures may account for the apparent increase however. The predominance of the disease in the female sector of the population is also well-documented (Hinkel, 1957, Lieber, 1952). The cholecystectomy rate per 100,000 population for Canada in 1972 was 190 for males and 629 for females (Information Canada, 1975). Within the female sector, cholelithiasis appears to be related to parity. Comess and co-workers (1967) studied epidemiological data for the Pima Indians of Arizona in comparison to data from the Framingham Heart Disease Study and observed that there was a relationship between a higher mean number of pregnancies and a history of gallbladder disease, particularly in the Indian group. There is also strong evidence to suggest that oral contraceptive use may contribute to lithogenic bile formation and gallstone pathogenesis. Greenblatt and coworkers (1973) noted an association between oral contraceptive use and surgically proven gallbladder disease

among participants of the Boston Collaborative Drug Surveillance Program. Bennion et al., (1976) recently studied the effect of oral contraceptives on the lipid composition of gallbladder bile. They found gallbladder bile to be significantly more saturated with cholesterol during oral contraceptive therapy than during normal cycling although gallstones were not found in any of the study participants. Lithogenic bile also appears to be more common in obese individuals regardless of the presence or absence of gallstones. Freeman et al. (1975) found bile samples from normal obese subjects to fall outside the micellar zone of Admirand and Small. Grundy and colleagues (1974) noted a significant correlation between body weight and cholesterol output when studying a group of cholelithiasis subjects and healthy controls. Recent work by Mabee and co-workers (1976) revealed that the biliary cholesterol secretion of obese subjects was three times that of normal subjects and twice that of gallstone subjects.

The prevalence of cholelithiasis appears to be significantly higher in developed countries than in developing countries. For example, the high prevalence of cholelithiasis in North America contrasts markedly with the Masai tribe of East Africa which exhibits virtually a zero incidence of the disease (Biss et al., 1971). Burkitt (1976) observed that during a recent two year period, only 15 of 84 hospitals in 13 African countries reported a single case of gallstones. Cholelithiasis appears to be associated with

urbanization and a westernized lifestyle rather than any racial factors since the prevalence of the disease among black North Americans is roughly equal to that of whites (Burkitt, 1976). Cholesterol gallstones were once rare among the Japanese (Miyake and Johnston, 1968), but with the increased industrialization and westernization of Japan in the past thirty years, a dramatic increase in the prevalence of this disease has been observed (Nakayama and Miyake, 1970). The Eskimo of Canada has been similarly affected by westernization (Schaefer, 1971). The association between cholelithiasis and a western lifestyle has raised the question as to whether dietary factors may be involved with the pathogenesis of the disease. Although obvious differences in dietary intake exist between peoples of developed and developing countries, differences within specific population groups are less apparent.

Several investigators have examined the dietary intake of groups with and without cholelithiasis within specific populations and also the effect of diet upon bile metabolism and bile lithogenicity.

Sarles and co-workers (1969) compared the dietary intake of a group of 101 French female cholelithiasis subjects to a group of age and sex matched controls. The cholelithiasis group was found to have a significantly greater intake of Calories, irrespective of dietary composition. Body weight, working conditions and physical exercise were not found to

differ between the two groups. Sarles et al., (1970) also examined the effect of various protein, fat, carbohydrate and Calorie intakes upon biliary lipid composition. They reported increases in biliary cholesterol concentration in response to increases in daily protein intakes and also to increases in caloric intake, regardless of dietary composition. This work appeared to be supported by that of Murray et al., (1974) who reported that gallstone patients with supersaturated hepatic bile consumed more calories, protein and fat when compared to a group of gallstone patients who secreted unsaturated bile. It is interesting to note, however, that the group with saturated bile was significantly heavier than the group with unsaturated bile and therefore the differences between the groups, may have been due to obesity rather than dietary differences. Contrary to these findings, an Australian dietary survey of 386 cholelithiasis patients and 397 controls revealed no significant differences in caloric intake although cholelithiasis patients were found to be fatter than controls as shown by anthropometric data (Wheeler et al., 1970). Also, there were no significant differences between the two groups with respect to mean daily intakes of total protein, fat and carbohydrate.

Cholelithiasis is highly prevalent with the Pima Indian population of the United States (Sampliner, 1970). Early investigations by Hesse (1959) using the dietary recall method revealed that Pima Indians consume a diet which is relatively low in fat and animal protein and consists mainly of beans, tortillas, chili peppers and coffee. Reid and co-workers

(1971) used a modification of the dietary interview technique of Burke to compare the dietary intake of a group of Pima Indian women with and without cholelithiasis. No significant differences in nutrient intake (energy, protein, fat, carbohydrate and several vitamins and minerals) were found between the two groups.

Friedman et al. (1966) examined the previously collected dietary intake data of 4469 subjects (age 30-59) who had participated in the Framingham Heart Disease Study and found no relationship between gallbladder disease and the intake of fat, cholesterol and protein. A significant percentage of the intakes examined, however, were of subjects who had radiologically confirmed gallstones but who had not undergone cholecystectomies. The observation of these researchers that cholesterol intake does not appear to be related to cholelithiasis has been supported by the bile studies of Sarles and co-workers (1970a, 1970b). They found the cholesterol content of the diet to have no effect on the cholesterol concentration of T-tube bile collected from a group of patients having cholecystectomy for cholelithiasis and a group of patients having cholecystectomy for other reasons. Contrary to these findings, however, Denbesten and Connor (1971) reported that increasing the cholesterol content of a liquid diet fed to normal subjects resulted in a significant increase in the molar percentage of cholesterol and lecithin, and a significant decrease in the molar percentage of bile salts. Prolonged feeding of a formula diet which contained

1000 mg cholesterol per day for a three month period resulted in progressive increases in the molar percentages of cholesterol eventually producing a bile which was supersaturated with cholesterol. Discrepancies in the results of the studies concerning the effect of cholesterol feeding upon bile composition are possibly due to the method of bile collection employed (T-tube vs duodenal bile sample) and also differences in the length of the study periods used.

Malhotra (1968) observed that the incidence rate of cholelithiasis among railway workers of the northern portion of India was seven times higher than the incidence rate among workers in the southern portion. He also noted that the northern Indians consumed 8-19 times more fat than the southern Indians and that which was consumed was primarily of the saturated type. Somewhat contradictory to these findings, Sturdevant and co-workers (1973) found that a diet high in unsaturated fat, low in saturated fat (P:S ratio $\geq$ 2), low in cholesterol and high in plant sterols appeared to predispose to cholelithiasis in man. In this study, the autopsy records of individuals who participated in the Los Angeles Veterans Administration controlled clinical trial of the dietary prevention of complications of atherosclerosis were examined. It was found that autopsied men who ate more than 33% of the experimental meals served from trial entry until death were more likely to have gallstones than control subjects.

The finding that the type of fat ingested appears to be related to cholelithiasis has not been supported by bile

studies. Dam and co-workers (1967) compared the effects of feeding diets high and low in unsaturated fat on human bile composition in a group of normal subjects. The lithogenicity of duodenal bile samples collected was not found to vary according to the type of fat fed but the percentage of linoleic acid in the total fatty acids of lecithin was found to be proportional to the linoleic acid content of the diet. There are no reports in the literature which explain the significance of this latter finding.

Burkitt (1973) has suggested that a decrease in the crude fibre content of the diet and a decrease in the consumption of foods rich in fibre has contributed to the high prevalence of cholelithiasis in developed countries. He bases this suggestion upon the observation that Africans, who rarely suffer from gallstones, consume a highly unrefined diet which is significantly higher in fibre than the typical North American diet. There are no published reports which indicate that the intake of crude fibre has been compared between cholelithiasis and control subjects within a specific population. However, Pomare and co-workers (1976) recently found that feeding a diet high in fibre (bran) to cholelithiasis subjects for a four to six week period, significantly decreased the cholesterol saturation of bile.

There are no published reports in the literature to suggest that human cholesterol gallstones can be produced through dietary manipulation. It is likely that none have been attempted for ethical reasons. Several studies have

shown however, that gallstones can be produced by altering the normal food intake of several species of animals such as the hamster (Dam, 1971), rabbit (Kyd and Bouchier, 1972), dog (Englert et al., 1969), squirrel monkey (Osuga, et al., 1974) and prairie dog (Denbesten et al., 1974). Generally, the lithogenic response to diets of various composition appears to be dependent upon the species of animal under investigation, so extrapolation of the results to the human would not be valid.

In conclusion, no definite conclusions have been reached on the effect of diet on gallstone formation. The studies which have been conducted on the subject are inadequate and their results are contradictory. The following studies were designed to provide more information on the relationship between dietary intake and gallstone formation.

METHODOLOGY

1. Selection of Subjects

1.1 Cholelithiasis group

The cholelithiasis group was selected from the medical files of the University of Alberta and Royal Alexandra Hospitals, Edmonton, Alberta according to the following criteria:

(a) Caucasian, aged 60 years or less

(b) Resident of the greater Edmonton area

(c) English speaking

(d) No known history of other metabolic disease

(e) Cholecystectomy due to cholesterol gallstones performed from three months to two years previously or current hospitalization for cholecystectomy.

Each subject was contacted by telephone or while hospitalized and informed of the study. It was requested that a dietitian visit the home to conduct a dietary interview. A total of 91 cholelithiasis subjects (15 males and 76 females) agreed to be interviewed.

1.2 Cholelithiasis subgroup

The cholelithiasis subgroup consisted of 15 hospitalized subjects (5 males and 10 females) from the cholelithiasis group who consented to have bile samples taken prior to

cholecystectomy. They also conformed to the following additional criteria:

(a) Functioning gallbladder

(b) No clinical evidence of cholecystitis

A minimum of three months time passed before these subjects were contacted again and interviewed.

1.3 Control group

The criteria for the selection of control subjects were similar to those used for the selection of cholelithiasis subjects, except for criterion (e) which was deleted. These subjects also were to have no medical history of gallstones or gallbladder disease. Control subjects were obtained from two sources:

(a) the gynecology files of the University of Alberta Hospital and

(b) volunteers.

Contact procedures were the same as those used for the cholelithiasis group. A total of 86 control subjects (13 males and 73 females) agreed to be interviewed.

1.4 Control subgroup

The control subgroup consisted of 13 subjects (8 males and 5 females) from the control group who agreed to have bile samples taken by duodenal intubation.

2. Dietary Survey

2.1 Interviewers

Twenty-one dietary interviewers collected the dietary data used in the present study. The author was trained in the Nutrition Canada techniques of dietary interview by two nutritionists who had been trained for the Nutrition Canada National Survey (Canada, 1973). Additional techniques were developed by the author. Interviewers, trained by the author, were eighteen dietetic interns from three city hospitals with B.Sc. (H.Ec.) degrees in Foods and Nutrition, and two registered dietitians who volunteered to assist with the study. Intensive two day training sessions were held by the author at each of the three city hospitals. Each session consisted of a brief outline of study design and methodology, an explanation of the standardized interview techniques to be used, and the demonstration of these techniques (details follow). The techniques of each interviewer were evaluated at the end of each session, and a three-hour refresher session was held just before the study commenced.

2.2 Dietary intake data collection

The dietary survey took place between February and August, 1976. The trained interviewers collected dietary intake data and other pertinent data at the home of each subject. Each interview, which took about two hours to complete, consisted of three parts.

2.2.1 Forty-eight hour recall of food intake

The daily intakes of energy, protein, fat, carbohydrate and crude fibre for each subject were based on a forty-eight hour recall of food intake. This technique was a modification of the twenty-four hour recall used in the Nutrition Canada National Survey (Canada, 1973). Each subject was asked to recall all foods and beverages consumed during the forty-eight hour period which commenced at midnight three days previously and ended at midnight the day before. The form used for recording appears in Appendix 1. For each food item recalled by the subject, the interviewer recorded the time of consumption, an exact description of the food item and the amount consumed. Description of food items included such information as brand name, cost, ingredients and method of food preparation.

Serving sizes were estimated by the use of food portion models, constructed according to Nutrition Canada specifications (Canada, 1973). The models used were:

(a) several sizes of bowls and glasses, etched at various volume levels,

(b) papier maché-filled spoons and mounds,

(c) wooden discs and squares in several sizes, and

(d) special models which were designed to represent specific foods (e.g. pie, cake, fish).

Each food model was coded with a letter which represented a specific amount or volume of food or beverage. Therefore, a letter of the alphabet, representative of a food model, was

usually entered in the 'Amount' column of the recording form (see Appendix 1).

For some food items, the intake was recorded in grams. The densities of some foods (grams/cu. in.) which were supplied by Nutrition Canada (Canada, 1973), made the calculation of intake in grams possible.

One advantage of the home interview was that the dimensions of dishes, utensils and foods could be measured and portion sizes estimated more accurately as a result.

Additional information about food intake was also obtained e.g. whether it was typical of the subject's usual intake, whether it had been modified in any special way and whether any significant changes in food habits had occurred during their lifetime.

The interview techniques used, including communication skills and food model use, were similar to those used for the Nutrition Canada National Survey (Canada, 1973). An outline of the techniques appears in Appendix 2. Attention was paid to standardizing the interview techniques since it was recognized that the interviewer requires considerable skill to obtain and interpret the necessary information.

After the interview, the 'Food Code' column of the food recall form (see Appendix 1) was completed by entering the food code which represented the food or beverage which had been recorded in the 'Food or Drink' column. The food code

numbers used were the item numbers from Composition of Foods Agricultural Handbook, No. 8 (Watt and Merrill, 1963) and additional codes which were created by Nutrition Canada (Canada, 1973) and by the author to represent Canadian foods and new products. The nutrient composition of additional foods appears in Appendix 3.

The food intake data were keypunched, transferred to computer tape and assessed for energy, protein, fat, carbohydrate and crude fibre. Nutrient intakes/1000 Calories were also calculated. Due to the lack of information available regarding the cholesterol, unsaturated fat and saturated fat content of foods, the intake of these nutrients was not assessed.

2.2.2 Weekly intake of crude fibre

A crude fibre weekly intake questionnaire was developed to provide a more detailed picture of crude fibre intake, than that provided by the two day recall of food intake. The recording form used for this purpose appears in Appendix 4. The foods which contribute significant amounts of crude fibre to the diet were listed under six major categories: cereal, fruit, bread and bakery products, soups, nuts, and vegetables. For each food listed, the subject recalled the amount consumed during the previous seven day period. Food models were again used to aid in estimating portion sizes. Additional items not included on the questionnaire could also be listed. Guidelines concerning the recording of additional food items appear in Appendix 4.

Although the fibre questionnaire was designed to determine crude fibre intake during a seven day period, it also served as a valuable cross-check to verify the information given in the forty-eight hour recall.

The crude fibre intake was calculated using the crude fibre values which appear in Composition of Foods (Watt and Merrill, 1963) and in Food Values of Portions Commonly Used (Church and Church, 1975). Crude fibre values for some foods not listed in the two food tables appear in Appendix 3.

2.2.3 General questionnaire

Information about vital statistics (age, weight and height), recent illness or surgery, oral contraceptive use, parity and gravida were obtained from subjects. The questionnaire used appears in Appendix 5.

3. Bile Study

3.1 Bile collection procedures

3.1.1 Cholelithiasis subgroup

Bile samples were obtained from the cholelithiasis subgroup by needle aspiration of the gallbladder prior to excision. The total aspirate was transferred to a stoppered test tube, labelled and packed in ice.

3.1.2 Control subgroup

Radiologically controlled duodenal intubation of fasting subgroup control subjects was performed. The duodenal contents were aspirated until a clear aspirate was obtained. Bile was then collected using one of the following methods:

(a) cholecystokinin - pancreozymin (CCK-PZ)

Forty units (4 cc of reconstituted solution) of cholecystokinin - pancreozymin (Boots Co. Ltd., Nottingham, England) were injected intravenously. Cholecystokinin - pancreozymin is a hormone which is normally secreted by the cells of the duodenal mucosa in response to the products of fat and protein digestion. CCK-PZ causes contraction of the gallbladder resulting in the release and flow of bile to the duodenum. Intravenous administration of this hormone produces a physiological response which is identical to that produced by food stimulation. Approximately two minutes after injection of the CCK-PZ, the duodenal contents were aspirated continuously until clear bile was noted. A 10 ml sample was then drawn, transferred to a stoppered test tube, labelled and packed in ice.

(b) amino acid perfusion

Forty mls of a 10% solution of Travasol (Baxter Laboratories of Canada) was introduced into the duodenum via nasogastric tube. The amino acids present in this solution stimulate the release of endogenous cholecystokinin - pancreozymin which produces gallbladder contraction and emptying. Bile was aspirated and handled as described under method (a).

3.2 Bile analysis

Bile samples were immediately transferred to the biochemistry laboratory of the Department of Laboratory Medicine, University of Alberta Hospital, Edmonton. Duplicate aliquots of each bile sample were immediately analyzed for concentrations of cholesterol and phospholipid in order to minimize enzymatic degradation of the phospholipid fraction and prevent cholesterol precipitation from solution. The remainder of the samples were stored at -10°C and analyzed later for bile salt concentration. The methods of bile analysis employed were as follows:

3.2.1 Cholesterol

The cholesterol concentration of each bile sample was measured by the method of Abell, Levy, Brodie and Kendall (1952). Duplicate aliquots of each bile sample were treated with alcoholic potassium hydroxide to liberate cholesterol from lipoprotein complexes and to saponify cholesterol esters. After dilution of the alcoholic solution with water, the free cholesterol was extracted into petroleum ether. The ether was then evaporated off and the amount of cholesterol present was determined colorimetrically by the Liebermann - Burchard reaction. Standards were also prepared for inclusion in each series of determinations. The concentration of cholesterol in mg per 100 mls of bile was converted to millimoles (mM) of cholesterol (1 mM cholesterol = 387 mg) per 100 ml.

3.2.2 Phospholipid

The method of Bragdon (1960) was used to extract lipids from bile aliquots. The concentration of lipid phosphorus in the extract was then determined according to the method described by Sunderman and Sunderman (1960). Phosphorus was released from the phospholipid complexes by digestion with sulphuric acid and hydrogen peroxide. Standard samples of phosphate were similarly treated. An acid molybdate solution which reacts with the phosphorus to form phosphomolybdic acid, was added to the digested extract. Phosphomolybdic acid was then reduced by amino-napthol sulfonic acid to form a blue color. The concentration of lipid phosphorus present in the aliquots was then determined colorimetrically according to a standard curve. This method gave the concentration of phosphorus in mg per 100 ml of bile. These values were converted to phospholipid concentration (primarily lecithin in bile) by multiplying the values by 25, an average value which describes the amount of phospholipid present per mg of phosphorus. The values were converted to millimoles (mM) of phospholipid (I mM phospholipid [lecithin] = 793 mg) per 100 ml of bile.

3.2.3 Bile acids (salts)

The total bile acid concentration of each bile sample was determined according to Engert and Turner's (1973) modification of the enzymatic method of Talalay (1960). The bile acids present in bile, (chenodeoxycholate, cholate, deoxycholate and lithocholate) are 3α hydroxysteroids. Duplicate

aliquots of each bile sample were incubated with a mixture of the enzyme, 3α hydroxysteroid dehydrogenase, isolated from Pseudomonas testosteroni, and nicotinamide adenine dinucleotide (NAD).

The bile acids were oxidized to steroid ketones and the NAD reduced to NADH in this enzyme system. Hydrazine sulfate was used as the ketone trapping agent. Chenodeoxycholate standards were also prepared for inclusion in each series of determinations. NADH is a colored product at 340 μM, the intensity of which is proportional to the concentration of the compound present. In this reaction, one mole of NADH is formed per mole of bile acid oxidized and therefore the molar concentration of bile acids present of each bile sample could be readily determined. Total bile acid (salt) concentrations were expressed as mM per 100 ml of bile.

3.3 Gallstone analysis

Gallstones were collected from the cholelithiasis sub-group at the time of operation. Stone samples were air dried at room temperature, weighed and ground into a fine powder. Using .5 gm of the powdered stone, the cholesterol content of the sample was determined according to the method of Abell, Levy, Brodie and Kendall (1952). On the basis of this determination, the percentage of cholesterol in the whole stone was calculated.

3.4 Determination of bile lithogenicity

3.4.1 Triangular phase diagram

Percentage of each bile constituent present in each bile

sample was calculated by dividing the concentration (mM/100 ml) of each bile constituent (bile acid, phospholipid or cholesterol) by the sum of the concentrations of all three constituents. These percentages were plotted simultaneously on a triangular phase diagram to represent single points. The position of each bile sample on the triangular diagram, in relation to the cholesterol solubility line of Admirand and Small (1968), was noted. Bile samples which fell on or above this line were lithogenic or supersaturated with cholesterol and those below the line were non-lithogenic or unsaturated.

3.4.2 Lithogenic index

The lithogenic index of each bile sample was calculated according to the method described by Metzger et al. (1972). The lithogenic index is the ratio of the actual amount of cholesterol in the bile sample to the maximum amount of cholesterol which could be dissolved by the amounts of phospholipid and bile acid present. The point of maximum cholesterol solubility was determined by drawing a straight line from the apex of the triangular diagram to the base, through the point which represented a particular bile sample. The point at which this line intersected the cholesterol solubility line gave the maximum amount of cholesterol which could be dissolved. Lithogenic indices greater than or equal to 1.0 represent lithogenic bile and those below 1.0 represent non-lithogenic bile.

4. Data Analysis

Dietary intake, bile analysis and other data collected were keypunched and transferred to computer tape for analysis. Descriptive statistics for each variable were compared between cholelithiasis (CH) and control (CO) groups and subgroups using Student's t-test. Typical day food intakes, weekend day food intakes and questions related to food intake were tested for association by use of the Chi-Square test. For each sex, dietary intake data were compared between CH and CO groups using the analysis of covariance (ANACOVA) adjusting for age, weight and other variables when necessary. This method of analysis was also used to compare bile analysis data between the CH and CO subgroups.

For the subgroups, Pearson correlation coefficients were calculated between dietary intake data and bile analysis data. Partial correlation coefficients were determined between the dietary intake data and bile analysis data, controlling for age, weight, height and oral contraceptive use when necessary.

RESULTS

1. Description of the Groups and Subgroups

1.1 Characteristics

Table 1 shows the characteristics of the cholelithiasis (CH) and control (CO) groups. Statistical analysis (Student's t-test) revealed that the mean age, weight and height of the male CH and CO groups were not significantly different. The age of the female CH and CO group was not significantly different but the female CH group was significantly ($P<0.05$) heavier and shorter.

Table 2 shows the characteristics of the cholelithiasis and control subgroups. No significant differences were detected in the ages, weights or heights of the male subgroups or female subgroups.

1.2 Oral contraceptive use, parity and gravida

Table 3 shows that more female subjects in the CO group than the CH had used oral contraceptives. The reverse was true for the subgroup, but there were only a few subjects considered. Oral contraceptives were used for longer periods of time by subjects in the CO group than by those in the CH group (35.0 ± 43.5 weeks for controls versus 15.9 ± 25.8 weeks for cholelithiasis). The values for the subgroups did not

Table 1: Characteristics of the cholelithiasis and control groups

Group		No. of Subjects	Characteristics		
			Age	Weight	Height
			(yrs)	(lbs)	(in)
Male	Cholelithiasis	15	45.8±9.0[1,a]	174.8±23.8[a]	70.3±2.8[a]
	Control	13	38.4±15.9[a]	174.5±24.0[a]	69.7±1.6[a]
Female	Cholelithiasis	76	38.0±13.3[a]	144.4±35.1[a]	63.1±2.7[2,a]
	Control	73	38.2±10.8[a]	134.1±21.8[b]	64.7±2.5[b]

[1]Values are means ± s.d. Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was Student's t-test.

[2]Value is the mean of 75 female subjects.

Table 2: Characteristics of the cholelithiasis and control subgroups

	Subgroup	No. of Subjects	Characteristics		
			Age (yrs)	Weight (lbs)	Height (in)
Male	Cholelithiasis	5	43.2±13.0[1,a]	165.2±22.7[a]	69.0±2.6[a]
	Control	8	27.1±12.3[a]	165.9±11.3[a]	69.5±1.4[a]
Female	Cholelithiasis	10	31.4±12.8[a]	149.2±42.0[a]	64.1±1.9[a]
	Control	5	30.4±13.6[a]	124.0±13.5[a]	65.2±1.5[a]

[1]Values are means ± s.d. (individual values appear in Appendix 9 and 10). Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was Student's t-test.

Table 3: Oral contraceptive use, parity and gravida for the female cholelithiasis and control groups and subgroups.

	No. of Subjects	Use of Oral Contraceptives		Parity		Gravida[3]
		%	Duration (wks)	%Parous[1]	Degree[2]	
Group						
Female						
Cholelithiasis	76	50	15.9±25.8[4,a]	79	1.7±1.3[a]	2.1±1.6[a]
Control	73	63	35.0±43.5[b]	70	2.3±3.3[a]	2.6±2.7[a]
Subgroup						
Female						
Cholelithiasis	9	66	22.2±32.7[a]	78	1.9±1.4[a]	1.9±1.5[a]
Control	4	25	15.0±30.0[a]	50	2.3±2.2[a]	3.0±4.8[a]

[1]Percent of females with children

[2]Number of children

[3]Number of pregnancies

[4]Values are means ± s.d. Values for females in the group or subgroup for a given parameter, without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was Student's t-test.

differ significantly.

As shown in Table 3, the number of female subjects who had conceived children was slightly greater in the CH group than in the CO groups, but none of the groups or subgroups differed significantly in terms of number of children (parity) or number of pregnancies (gravida).

2. Description of Dietary Intake

Table 4 summarizes the number of typical day and weekend day food intakes recalled by the groups. Analysis of these data (Chi-square) revealed that for both sexes, there were no significant differences between the CH and CO groups. Similar data for the subgroups appear in Appendix 7.

Table 5 describes the food intake data of each group in terms of various dietary intake classifications or whether dietary intake was modified in some way, e.g. restricted in Calories. The male groups were fairly similar with respect to various diet classifications, but more females in the CH group than in the CO group stated that they were restricting the intake of Calories at the time of interview. Similar data for the subgroups appear in Appendix 7.

Table 6 indicates the number of subjects reporting significant changes in food habits during their life. The CH and CO groups did not differ in this respect. Some differences between the groups emerged when the reasons for changing food habits were categorized. It is interesting to note that only ten percent of the subjects in the CH group reported a

Table 4: Number of typical days and weekend days for the cholelithiasis and control groups

Group	No. of Subjects	No. of Typical Days[1]	No. of Weekend Days[2]
Male			
Cholelithiasis	15	1.9[3,a]	.3[a]
Control	13	2.0[a]	.5[a]
Female			
Cholelithiasis	76	1.5[a]	.5[a]
Control	73	1.5[a]	.5[a]

[1]Number of days out of the two days recalled that were typical days.

[2]Number of days out of the two days recalled that were weekend days.

[3]Values are means. Values for males or females, for a given parameter, without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was Chi-square.

Table 5: Classification of dietary intake for the cholelithiasis and control groups

Group	No. of Subjects	Classification of Dietary Intake: No. and (Percent) of Subjects			
		U[1]	CR[2]	SR[3]	MF[4]
Male					
Cholelithiasis	15	14(93.3)	1(6.7)	0	0
Control	13	12(92.3)	1(7.7)	0	0
Female					
Cholelithiasis	76	59(77.6)	16(21.0)	1(1.3)	0
Control	73	65(89.0)	6(8.2)	1(1.4)	1(1.4)

[1]U = unmodified

[2]CR= Calorie-restricted

[3]SR= sodium-restricted

[4]MF= modified fat

Table 6: Changes in food habits for the cholelithiasis and control groups

Group	No. of Subjects[1]	No. and (Percent) of Subjects Reporting a Change in Food Habits[2]	Reasons for Change in Food Habits:[3] No. and (Percent) of Subjects								
			A	B	C	D	E	F	G	H	I
Cholelithiasis	90	40(44.4)[4,a]	4(10)	16(40)	2(5)	4(10)	3(7.5)	1(2.5)	3(7.5)	9(22.5)	0
Control	95	38(44.7)[a]	0	12(31.6)	7(18.4)	2(5.3)	6(15.8)	0	0	5(13.1)	4(10.5)

[1]Data missing for 1 cholelithiasis and 1 control subject

[2]Subjects were asked whether they had experienced any significant changes in their food habits during their lifetime (see Appendix)

[3]A = as a result of recurrent gallbladder trouble
B = frequent restriction of caloric intake
C = medically prescribed diets (not Calorie restricted)
D = marriage
E = cultural change
F = urban - rural shift
G = member of family with a modified diet
H = increased awareness about nutrition
I = other reasons

[4]Values for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was Chi-square.

change in food habits as a result of gallbladder trouble.

3. Nutrient Intake

3.1 Cholelithiasis and control groups

The mean daily intakes of energy, protein, fat, carbohydrate and crude fibre are tabulated in Table 7. The intake of each of these nutrients was higher for the male CO group than for the male CH group. However, analysis of covariance (ANACOVA), adjusting for age and weight indicated that this difference was significant for protein intake only.

The intakes of energy, protein, fat, carbohydrate and crude fibre were also higher for the female CO group than for the female CH group. Analysis of covariance, adjusting for age, weight, height and oral contraceptive use indicated that the difference was significant for each of these nutrients. Analysis of the nutrient intake values for the female group included adjustment for height and oral contraceptive use as well as age and weight because these variables were significantly different as noted in Tables 1 and 3. Nutrient intake data for the subgroups appear in Appendix 8.

3.2 Subjects whose intake was unmodified at the time of interview

When the diet interviews were carried out, some subjects indicated that their food intake was modified in some way (Table 5). In particular, more females in the CH group than the CO group stated that they were following a Calorie restricted diet. It was therefore desirable to consider only

Table 7: Daily intakes of energy, protein, fat, carbohydrate and crude fibre for the cholelithiasis and control groups.

Group	No. of Subjects	Amount Consumed/Day[1]				
		Energy (Cal)	Protein (g)	Fat (g)	Carbohydrate (g)	Crude Fibre (g)
Male						
Cholelithiasis	15	2364±541[2,a]	89.2±24.2[a]	109.1±38.3[a]	253.8±83.7[a]	4.8±1.8[a]
Control	13	2931±1069[a]	124.5±50.1[b]	127.5±56.8[a]	311.0±130.0[a]	6.4±3.2[a]
Female						
Cholelithiasis	76	1508±575[a]	59.6±22.8[a]	65.4±33.9[a]	169.0±73.4[a]	3.3±2.2[a]
Control	73	1806±709[b]	70.5±24.4[b]	83.1±52.3[b]	195.7±74.6[b]	4.0±2.1[b]

[1]Each value is the mean of two days (nutrient intakes for each day appear in the Appendix).

[2]Values are means ± s.d. Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was analysis of covariance, adjusting male values for age and weight and female values for age, weight, height and oral contraceptive use (wks).

Table 8: Daily intakes of energy, protein, fat, carbohydrate and crude fibre for cholelithiasis and control subjects with unmodified dietary intake[1]

Group	No. of Subjects	Amount Consumed/Day[1]				
		Energy (Cal)	Protein (g)	Fat (g)	Carbohydrate (g)	Crude Fibre (g)
Male						
Cholelithiasis	14	2357±494[2,a]	90.7±21.4[a]	108.4±32.8[a]	251.3±80.5[a]	5.0±1.6[a]
Control	12	2967±903[a]	123.7±43.5[a]	127.0±44.0[a]	319.4±121.4[a]	6.1±2.7[a]
Female						
Cholelithiasis	59	1566±511[a]	59.7±18.6[a]	68.8±29.6[a]	174.2±61.2[a]	3.0±1.8[a]
Control	65	1856±560[b]	69.5±18.0[b]	85.6±39.6[b]	203.7±58.6[b]	4.0±1.8[b]

[1]Excludes those individuals with modified dietary intake (eg, Calorie-restricted). (See Table 5)

[2]Values are means ± s.d. Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was analysis of covariance, adjusting male values for age and weight and female values for age, weight, height and oral contraceptive use (wks).

the data from subjects whose intake was unmodified at the time of interview. Table 8 shows that the intakes of all nutrients were higher than those reported for the total group (Table 7). Analysis of covariance, adjusting the values for the male groups for age and weight indicated that these groups were similar with respect to the intake of all nutrients. In comparison, the data for the total male group (Table 7) indicated that the protein intake of the male control group was significantly higher.

Statistical analysis of the data for the females indicated that the female control group consumed significantly more energy, protein, fat, carbohydrate and crude fibre per day than the female CH group. These results are comparable to those observed for the total female group (Table 7).

4. Weekly Crude Fibre Intake

Data illustrating the weekly intake of crude fibre for the groups appear in Table 9. In addition, Table 9 also shows the food sources contributing to the total weekly intake of crude fibre. The control groups, both male and female, consumed more fibre per week than the corresponding CH groups. Cereals, bread and bakery products, fruit and vegetables were found to be the foods which contributed to this difference. Analysis of covariance, adjusting the values for the males for age and weight and the values for the females for age, weight, height and oral contraceptive use was

Table 9: Weekly intakes of crude fibre and breakdown of crude fibre intake as to source for cholelithiasis and control groups.

Group	No. of Subjects	Total Weekly Crude Fibre Intake[2]	Breakdown of Crude Fibre Intake as to Source[1]				
			Cereals	Bread and Bakery Products	Nuts	Fruit	Vegetables
		(g)	(g)	(g)	(g)	(g)	(g)
Male							
Cholelithiasis	15	28.6±11.2[2,a]	1.7±2.5[a]	4.9±4.7[a]	1.1±1.6[a]	6.8±5.9[a]	14.1±45.7[a]
Control	13	44.6±21.0[a]	9.3±12.6[a]	5.8±5.5[a]	1.0±.9[a]	10.1±9.0[a]	18.4±6.1[a]
Female							
Cholelithiasis	76	23.8±11	1.1±3.4[a]	2.4±2.9[a]	.5±.9[a]	8.1±7.4[a]	11.7±4.7[a]
Control	73	30.9±11.0	2.1±3.7[a]	6.0±5.4[b]	.6±1.2[a]	9.1±5.3[a]	13.1±4.5[a]

[1]See Appendix for food items categorized under each source.

[2]Values are means ± s.d. Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was analysis of covariance, adjusting male values for age and weight and female values for age, weight, height and oral contraceptive use.

performed on these data. Comparison of the fibre intakes for the male CH and CO groups revealed no significant differences. However, the differences in the total weekly intake of crude fibre and the intake of crude fibre from bread and bakery products between the female CH and CO groups were found to be significant.

5. Nutrient Intakes/1000 Calories

The data presented in Table 10 indicate the intake of nutrients per 1000 Calories for the CH and CO groups. Analysis of covariance, adjusting the values for the male group for age and weight and female values for age, weight and oral contraceptive use indicated that the intakes of protein, fat, carbohydrate and crude fibre per 1000 Calories were similar for the male CH and CO groups and for the female CH and CO groups.

6. Bile Composition and Lithogenicity

Table 11 indicates the composition of gallbladder bile and lithogenic indices for subgroups. The millimolar concentrations of bile salt, phospholipid and cholesterol in the bile samples appeared to be much lower for the CO subgroup than for the CH subgroup. However, bile samples were collected from the CH subjects by means of gallbladder aspiration and as a result, these samples were more concentrated. The difference between CH and CO subjects disappeared when bile composition was expressed as relative percentages

Table 10: Nutrient intakes/1000 Calories for the cholelithiasis and control groups

Group	No. of Subjects	Nutrient Intake/1000 Calories			
		Protein	Fat	Carbohydrate	Crude Fibre
Male					
Cholelithiasis	15	38.0±8.0[1,a]	46.0±8.0[a]	107.0±23.0[a]	2.0±1.0[a]
Control	13	43.0±8.0[a]	43.0±6.0[a]	105.0±19.0[a]	2.0±1.0[a]
Female					
Cholelithiasis	76	41.0±10.0[a]	43.0±9.0[a]	113.0±23.0[a]	2.0±1.0[a]
Control	73	41.0±12.0[a]	45.0±8.0[a]	109.0±22.0[a]	2.0±1.0[a]

[1]Values are means ± s.d. Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was analysis of covariance, adjusting male values for age and weight and female values for age, weight, height and oral contraceptive use (wks).

Table 11: Composition of bile and lithogenic indices for the cholelithiasis and control subgroup

Subgroup	No. of Subjects	Bile Constituent						Lithogenic Index[3]
		Bile Salt		Phospholipid		Cholesterol		
		mM/l[1]	%[2]	mM/l[1]	%[2]	mM/l[1]	%[2]	
Male								
Cholelit-	5	78.4±44.2	65.4±6.3[4,a]	30.3±23.6	22.6±4.0[a]	15.9±13.2	12.0±3.7[a]	1.17±.36[a]
Control	8	31.1±34.2	69.4±7.8[a]	7.7±4.8	23.0±5.7[a]	2.5±1.5	7.6±2.7[b]	.74±.26[b]
Female								
Cholelithiasis	10	136.2±41.3	68.2±6.3[a]	43.6±18.0	21.6±3.8[a]	19.5±7.3	9.6±2.6[a]	.95±.26[a]
Control	5	39.4±14.1	76.0±5.7[a]	9.0±3.6	17.4±3.2[a]	3.4±2.0	6.6±3.2[a]	.65±.32[a]

[1]Millimoles per liter of bile (duplicate determinations per bile sample).

[2]Percent millimoles of bile constituent per total millimoles of bile salt, phospholipid and cholesterol.

[3]Lithogenic Index = $\frac{\text{Actual amount of cholesterol in bile sample}}{\text{maximum amount of cholesterol which could be dissolved by the amounts of bile salt and phospholipid in bile sample}}$

[4]Values are means ± s.d. (Individual values appear in Appendix 8 and 9) Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was analysis of covariance, adjusting for age and weight.

of biliary lipids (% millimoles of bile constituent per total millimoles of bile salt, phospholipid and cholesterol). When the composition of bile for the male CH and CO subgroups were compared it was found that for the CO subgroups, the percentages of bile salt and phospholipid were higher while the percentage of cholesterol was lower. The lithogenic index of the male CH subgroup exceeded 1.0 and was higher than the CO subgroup. Statistical analysis (ANACOVA) revealed that the differences in the percentages of cholesterol and lithogenic index were significant ($P<0.05$).

Comparing the composition of bile samples of the female CH and CO subgroups revealed that for the CO subgroup, the percentage of bile salt was higher while the percentages of phospholipid and cholesterol were lower. The lithogenic index of the female CH subgroup did not exceed 1.0, but it was higher than the female CO group. Analysis of covariance revealed that these differences were not significant. Gallstone samples from males and females were composed primarily (75.8%) of cholesterol (see Appendix 9).

Figure 1 shows the relative percentages of biliary lipids in the bile of each subject in the male subgroups as plotted on a triangular phase diagram. As the diagram illustrates, three out of five subjects in the male CH subgroup had bile which fell on or above the cholesterol solubility line established by Admirand and Small (1968). In contrast, only one out of eight bile samples from the male CO subgroup fell on or above the cholesterol solubility line. Therefore, a

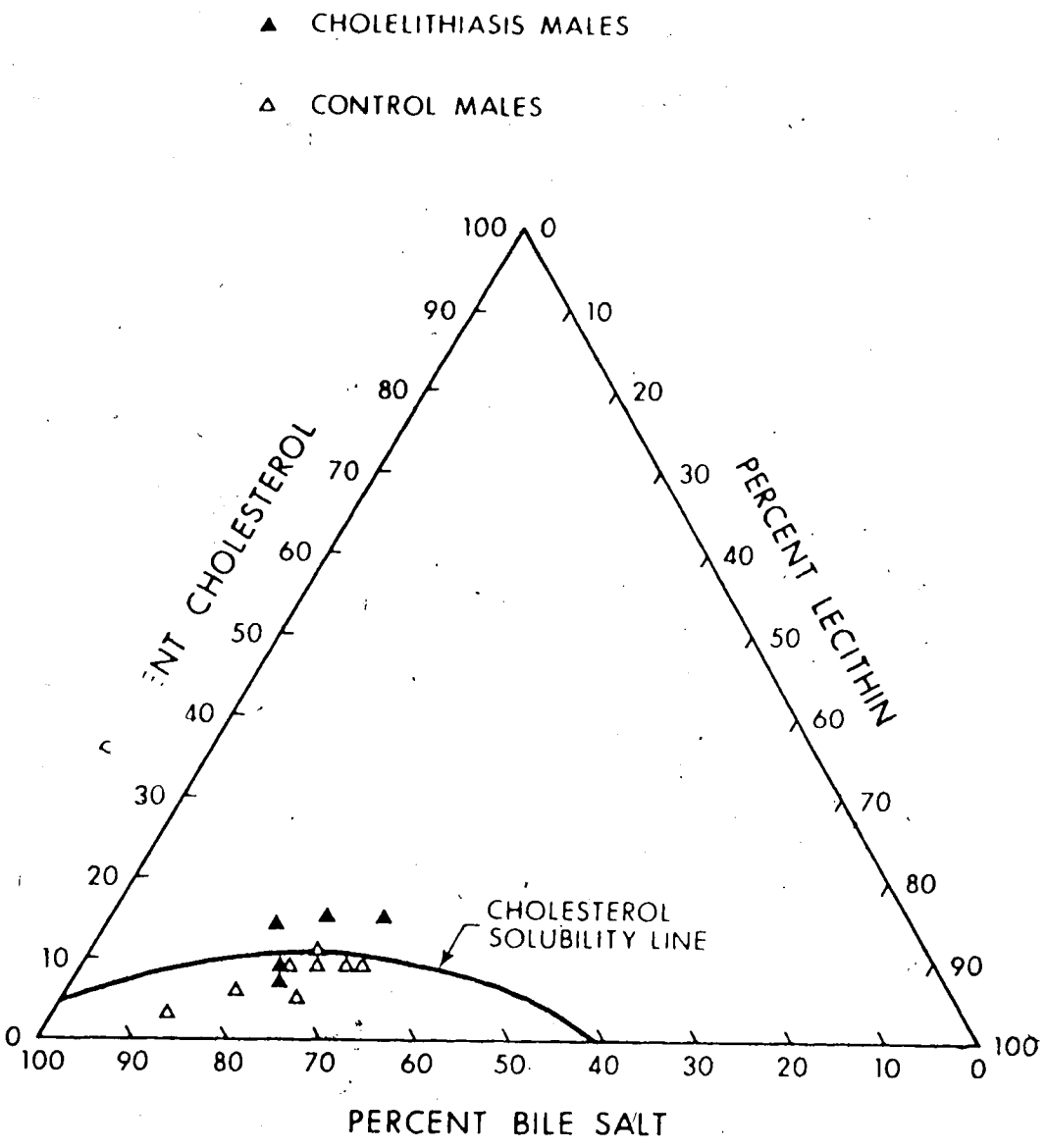

Figure 1. Composition of gallbladder bile from male cholelithiasis and control subjects plotted on the triangular phase diagram of Admirand and Small (1968)

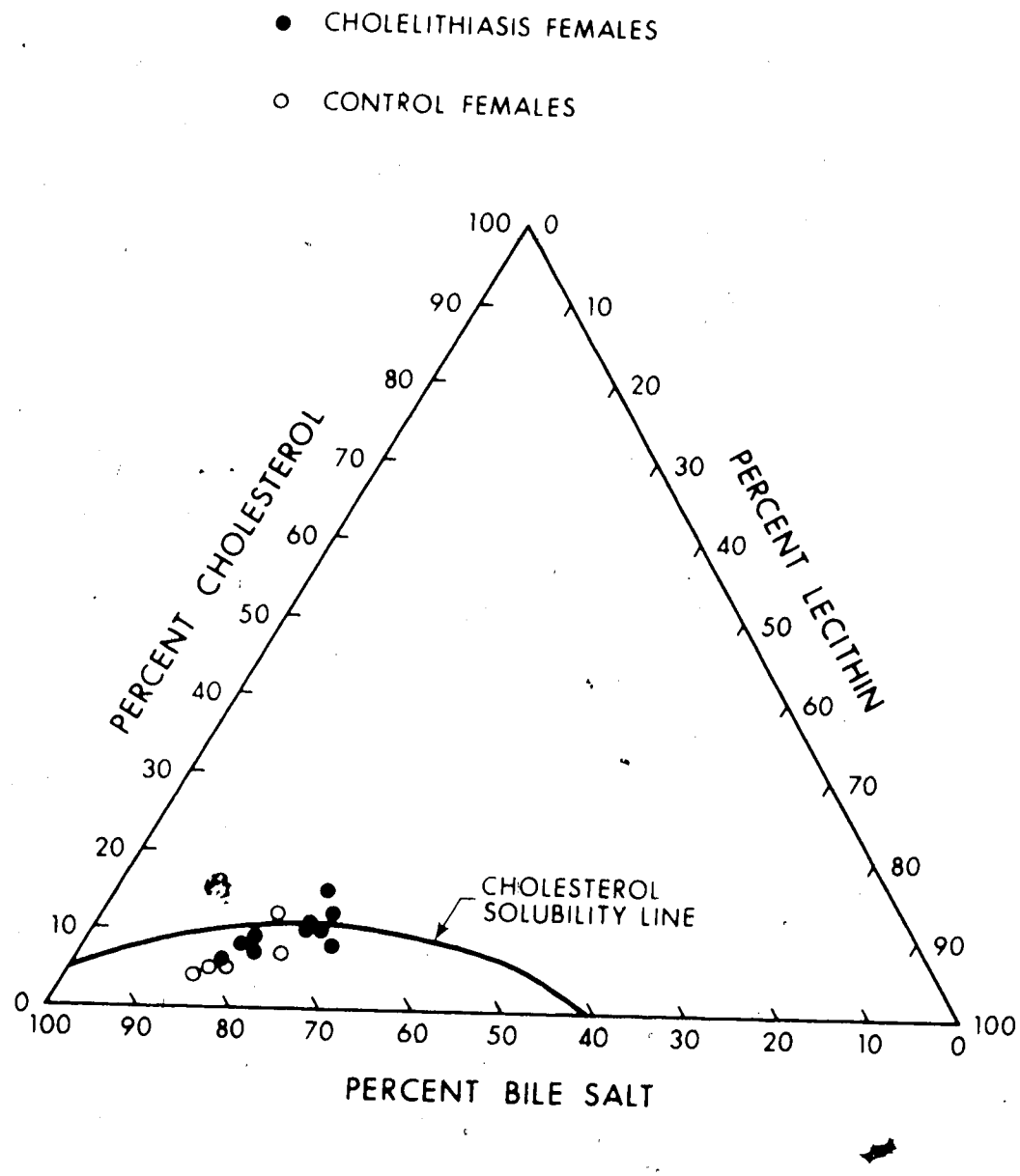

Figure 2. Composition of gallbladder bile from female cholelithiasis and control subjects plotted on the triangular phase diagram of Admirand and Small (1968)

higher proportion of male CH subjects compared to male CO subjects had lithogenic bile at the time of sampling. A similar diagram for the female subgroups appears in Figure 2. Only three out of ten female CH subjects (30%) had bile which fell on or above the cholesterol solubility line. In comparison, one out of five subjects (20%) in the female CO subjects had bile which fell on or above the cholesterol solubility line. This indicated considerable overlap between female CH and CO subjects with respect to bile lithogenicity, although very few subjects were considered.

7. Bile Composition and Lithogenicity as Related to Dietary Intake

Presented in Table 12 are Pearson correlation coefficients comparing bile composition data with the following data for the male subgroups: age, weight, height, and nutrient intake. No significant correlations were noted between these variables.

The data of Table 13 indicate the partial correlation coefficients for the male subgroup calculated by controlling for age, weight and height. Again, no significant correlations were noted between the bile parameters and the dietary factors.

Table 14 indicates the Pearson correlation coefficients comparing bile composition data with the following data for the female subgroup: age, weight, height, oral contraceptive use and nutrient intake. A significant negative correlation was noted between weight and the percentage of bile salt in

Table 12: Pearson correlation coefficients for male subgroups[1]: age, weight, height and daily nutrient intakes vs. bile composition and lithogenic index.

Variable	Bile Salt (%)[2]	Phospholipid (%)[2]	Cholesterol (%)[2]	Lithogenic Index[3]
Age (yrs)	0.0782	-0.1223	0.0291	0.0375
Weight (lbs)	-0.1821	0.2469	-0.0215	-0.0257
Height (in)	0.6554	-0.5156	-0.3760	-0.3685
Energy (Cal)	-0.0065	0.1890	-0.2198	-0.2309
Protein (g)	0.0863	0.1985	-0.3734	-0.3928
Fat (g)	-0.2665	0.3266	0.0108	0.0007
Carbohydrate (g)	0.2278	0.0309	-0.3861	0.0309
Crude Fibre (g)	-0.1272	0.2357	-0.0918	-0.0988

[1]Based on complete data available for 3 cholelithiasis and 6 control male subjects only. No significant correlations were found ($P>0.05$).

[2,3]See footnotes 2 and 3 under Table 11.

Table 13: Partial correlation coefficients for male subgroups[1]: Daily nutrient intakes vs. bile composition and lithogenic index, controlling for age, weight, and height

Variable	Bile Salt (%)[2]	Phospholipid (%)[2]	Cholesterol (%)[2]	Lithogenic Index[3]
Energy (Cal)	-.0065	0.1890	-0.2198	-0.2309
Protein (g)	0.0863	0.1985	-0.3734	-0.398
Fat (g)	-0.2665	0.3266	0.0108	0.0007
Carbohydrate (g)	0.2278	0.0309	-0.3861	-0.3880
Crude Fibre (g)	-0.1272	0.2357	-0.0918	-0.0988

[1-3]See footnotes 1, 2 and 3 under Table 12

Table 14: Pearson correlation coefficients for female subgroups[1]: age, weight, height, oral contraceptive use and daily nutrient intakes vs. bile composition and lithogenic index.

Variable	Bile Salt (%)[2]	Phospholipid (%)[2]	Cholesterol (%)[2]	Lithogenic Index[3]
Age (yrs)	-0.1907	0.0296	0.0684	0.0768
Weight (lbs)	-0.5523[4]	0.3984	0.6850[4]	0.6742[4]
Height (in)	0.1094	-0.0661	-0.2950	-0.3029
Oral Contraceptives (wks)	-0.0708	0.1862	0.0249	0.0061
Energy (Cal)	0.0340	0.1129	-0.0027	-0.0130
Protein (g)	0.3168	-0.1867	-0.2569	-0.2662
Fat (g)	0.0051	0.1329	-0.0011	-0.0113
Carbohydrate (g)	-0.0740	0.2108	0.1369	0.1289
Crude Fibre (g)	0.1028	0.0347	-0.1148	-0.1313

[1]Based on complete data available for 9 cholelithiasis and 4 female control subjects only.

[2,3]See footnotes 2 and 3 under Table 11.

[4]Correlation coefficient significant ($P<0.05$).

bile. A significant positive correlation was noted between weight and both the percentage of cholesterol in bile and lithogenic index.

The data of Table 15 indicates partial correlation coefficients for the female subgroups calculated by controlling for age, weight, height and oral contraceptive use. A significant positive correlation was noted between protein intake and the percentage of bile salt in bile.

Table 15: Partial correlation coefficients for female subgroups[1]: daily nutrient intakes vs. bile composition and lithogenic index, controlling for age, weight, height and oral contraceptive use.

Variable	Bile Salt (%)[2]	Phospholipid (%)[2]	Cholesterol (%)[2]	Lithogenic Index[3]
Energy (Cal)	0.3811	-0.0861	-0.3780	-0.3732
Protein (g)	0.6634[4]	-0.3929	-0.5444	-0.5352
Fat (g)	0.3544	-0.0426	-0.3276	-0.3268
Carbohydrate (g)	0.2616	0.0153	-0.2454	-0.2399
Crude Fibre (g)	0.4078	-0.1943	-0.2997	-0.3019

[1-3]See footnotes 1, 2 and 3 under Table 14

[4]Correlation coefficient significant ($P<0.05$)

DISCUSSION

1. Validity of the Dietary Survey Techniques

A consideration of the validity of the 48 hour recall method used in the present study is necessary. Several factors influenced the choice of this method including:

(a) the relatively large numbers of subjects to be interviewed,

(b) the time and money limitations of the study,

(c) the types of nutrients which were to be evaluated (i.e. vitamin and mineral intake was not a concern),

(d) the decision to conduct home interviews, and

(e) the need to compare the mean nutrient intake of two groups rather than to evaluate the actual nutrient intakes of individuals.

Several reports in the literature support the use of the food recall method in these circumstances. Most of these studies deal with the validity of the 24 hour recall method, but it is felt that the results of these studies may be extrapolated to the 48 hour recall method. Young and co-workers (1952) compared the 24 hour recall method to the seven day record and the dietary history and concluded that

when an estimate of the mean intake of fifty persons or more was desired and when errors of ten percent or more could be tolerated, the 24 hour recall method could be used. Madden et al. (1976) recently investigated the internal validity of the 24 hour recall method by comparing the nutrient intake values derived from weighed dietary intakes to those derived from the 24 hour recall in a group of 76 elderly subjects. Although the recalled intakes were lower, the means of actual intakes and recalled intakes were not significantly different except for Calories. However, the authors noted the tendency of subjects to overestimate actual intake when consumption was low and underestimate intake when consumption was high. Therefore, the actual difference between the two groups would be greater than the recalled difference and given the smaller variance for the actual intake, this difference would be more likely to be significant. It was concluded that the recall would be unlikely to yield false positive results but might indicate false negative, especially for comparisons of Calories, protein and Vitamin A intakes.

In view of the results of these studies, one can be fairly certain that the difference in the mean daily intakes of energy, protein, fat, carbohydrate and crude fibre observed between the female cholelithiasis and control groups in the present study, actually does exist. Differences observed between the male cholelithiasis and control groups (eg. protein intake) are less certain however due to the small sample size.

It should be noted that the accuracy of the present study was made more likely by three factors:

(a) home visits were performed for data collection,

(b) an age restriction was placed on study participants, and

(c) a 48 hour rather than a 24 hour recall of food intake was used.

Beal (1967) has emphasized the value of the home visit for collecting dietary intake data. Although transportation time and cost are disadvantages, these are offset by the fact that home visits allow for the measurement of glasses and serving sizes, the checking of food products and recipes and the evaluation of the home environment. Home visits also reduce the failure rate among subjects since no responsibility is placed on them to travel to an interview site.

One of the shortcomings of the dietary recall is that the acquisition of reliable and accurate data is dependent upon the memory of the respondent. However, as Campbell and Dodds (1967) have illustrated, memory is considerably more of a problem among older subjects (particularly men over 65 years of age), who remember significantly less about food intake than younger subjects. Therefore, in the present study, only those individuals aged 60 years and less were included in the sample. The use of a 48 hour recall taxed the memory of the subject more than if the 24 hour recall had been used. However, the 48 hour recall method was used since it improved

the chance of obtaining at least one typical days intake from each subject.

Little study has been done on the number of days of food intake which must be recalled in order to estimate mean daily intake values representative of typical intake. Young and co-workers (1953) compared the seven day intake of a group of adults to a twenty eight day food record and found the seven day intake to be representative of the twenty eight day period when the average intake of the groups were examined. The authors also noted that the pattern of daily means for the group was so stable that less than a week's record would have provided an estimate of intake with little loss of precision.

One of the major limitations of the present study was the need to use several dietary interviewers for the collection of dietary intake data. Sample size and time limitations made this necessary. The interview techniques were standardized, and the interviewers trained as well as possible, however, the error due to interviewer variation was uncertain.

2. Dietary Survey

The population sample surveyed in this study consisted predominantly of females, and as indicated previously, the results for this group are probably more valid. Therefore, dietary intake comparisons made between the female cholelithiasis and control groups have received more attention in the following discussions than the comparisons made between the

male groups.

The most striking finding of the present study was that the female control group consumed significantly more energy, protein, fat, carbohydrate, and crude fibre per day than the female cholelithiasis group (Table 7). These differences were not due to differences in the number of typical days and/or weekend days recalled by subjects in each group. The weekly intake of crude fibre (Table 9) also showed that the female cholelithiasis group consumed significantly less fibre. It is interesting to note that when nutrient intakes were expressed per 1000 Calories (Table 10), the values for the two groups were remarkably similar, indicating that they consumed foods of essentially the same nutrient composition. The only apparent difference in dietary intake between the groups was that the control group consumed significantly more of the same foods.

These results for the female group contrast markedly with those of Sarles and co-workers (1969) who reported that a group of 101 cholelithiasis females consumed significantly more Calories (energy) than a group of 101 age-matched controls. The results of this latter study should be approached cautiously however, since these researchers disclose few details about the type of dietary interviews performed. They do indicate however, that subjects were questioned about their diet prior to the onset of cholelithiasis symptoms, unlike the present study where patients were interviewed after cholecystectomy. It is therefore possible that the surgery may have

contributed to a drop in caloric intake, but few subjects (10%) claimed to have changed their food habits as a result of recurrent gallbladder problems (See Table 6). Wheeler et al. (1970) performed a dietary survey which was similar to that of Sarles et al. (1969), but they found no significant difference in caloric intake or in the intake of other major nutrients between female cholelithiasis and control groups. These researchers investigated the dietary intake of a much smaller group (36 cholelithiasis versus 44 controls) than that used in the present study. Reid et al. (1971) reached conclusions which were similar to those of Wheeler et al. (1970) in their dietary survey of female Pima Indians. Although the study was well designed and executed, again only small numbers of subjects were investigated (48 cholelithiasis versus 16 controls).

The male control group in the present study also consumed more energy, protein, fat, carbohydrate and crude fibre than the male cholelithiasis group, but this difference was significant for protein intake only (Table 7). Significant differences might have been shown had these groups been larger.

A survey of the literature suggests that the results of the dietary survey may indicate three possible relationships between dietary intake and cholelithiasis. The pathogenesis of cholesterol gallstones could be related to:

(a) a low energy intake irrespective of nutrient intake,

(b) a low protein and/or fat intake as a result of

the low energy intake or,

(c) a low intake of crude fibre as a result of a low energy intake.

The basis of these hypotheses will be considered in detail. It is difficult to separate points (a) and (b) on the basis of the knowledge available on the effect of diet on bile lithogenicity and gallstone formation, so they will be considered together.

2.1 Cholelithiasis as related to a low energy intake and/or low fat and protein intake

Somewhat like the sample surveyed by Wheeler et al. (1970) but unlike that of Sarles et al. (1969), the female cholelithiasis group in the present study was significantly heavier and shorter than the female control group (Table 1). The recommended desirable weights for medium frame women aged 25 years and over with a height of 65.4 inches (as for the cholelithiasis group with 2-inch heels), is approximately 116 to 130 pounds (Metropolitan Life Insurance Co., 1959). Therefore, the female cholelithiasis group was approximately 14 to 28 pounds overweight. The recommended desirable weight for medium frame women aged 25 years and over with a height of 66.7 inches (as for the female control group with 2-inch heels) is approximately 124 to 139 pounds. This indicates that the female control group was approximately 0 to 10 pounds overweight. These data show that although both groups were overweight, the female cholelithiasis group was considerably more overweight that the female control group.

It is a paradox that despite their larger size, the female cholelithiasis group consumed significantly less energy (Calories) than the controls. However, it is well documented that overweight or even obese subjects tend to report lower caloric intakes than do lighter or normal weight subjects. Beaudoin and Mayer (1955) in an early investigation, reported that on the basis of a three-day recall of food intake, obese women consumed significantly fewer Calories than non-obese women. Similarly, Hampton and co-workers (1967) studied the seven-day food intake diaries of a group of teenagers and observed that caloric intake was not correlated with height, lean body weight or total body weight. The trend towards frequent dieting among heavier individuals could result in the reporting of lower caloric intakes by heavier subjects. In an attempt to eliminate this factor in the present study, the intake of subjects whose diet was unmodified was examined (Table 8). It was found that the difference in energy intake between the female cholelithiasis and control groups was still significant.

It is possible that several females in the cholelithiasis group may have adopted a Calorie restricted diet several years previously and may not have considered it a special dietary modification. This group may also experience a greater day to day fluctuation in food intake but this was not noted over the two-day period surveyed in the present study (see Appendix 6). It should be noted that the exercise level of the subjects was not evaluated. It may be that energy expenditure was

higher for the female control group than for the female cholelithiasis group due to a higher exercise level. This would permit the control group to maintain weight on a greater food intake.

How could a low energy intake affect the pathogenesis of cholesterol gallstones? Clues to this relationship lie in a consideration of those studies which have examined the effect on food intake in bile salt metabolism and the concentration of biliary lipids in bile.

Several researchers have investigated the effect of a controlled food intake on biliary lipid concentration and bile kinetics. La Russo and co-workers (1974) observed an increase in serum bile acid concentration representing an increase in bile salt secretion into bile, following the administration of a liquid test meal. They noted that following the meal, four hours passed before serum bile acids returned to basal levels. When Soloway et al. (1975) gave 30% of individual daily energy requirements to subjects at breakfast, they noted a significant increase in bile flow, an increase in bile salt secretion and an increase in the bile salt and phospholipid/cholesterol ratio in bile. A significant finding of this study was that bile salt output peaked 1-2 hours after the meal and remained high for 2 - 4 hours. This implies that bile remains less saturated with cholesterol while food is entering the duodenum, when bile flow and secretion as well as gallbladder contraction is stimulated. Many investigators have made similar observations

(Metzger et al., 1973, Northfield and Hofman, 1975).

It appears that during periods of fasting, the bile salt pool is sequestered in the gallbladder, resulting in a significant decrease in bile salt secretion from the liver. Animal studies have shown that at high bile salt secretion rates, a linear relationship exists between bile salt secretion and phospholipid and cholesterol secretion (Dowling et al., 1971, Strasberg et al., 1976). However, when bile salt secretion approaches zero, this relationship between bile salt and cholesterol secretion no longer exists and a fraction of cholesterol is secreted independently. Therefore, the concentration of cholesterol in bile relative to the concentration of bile salt and phospholipid is higher during these periods. These observations have been found to be similar for both cholelithiasis and healthy control groups (Northfield and Hofman, 1975), indicating that the liver functions normally in cholelithiasis subjects.

Although some investigators have postulated that bile salt secretion rates may be depressed in cholelithiasis subjects (Grundy et al., 1972) due to a diminished bile salt pool (Bell et al., 1973), there is more convincing evidence that pool size and bile salt secretion rates do not differ between normal subjects and those with cholelithiasis (Northfield and Hofman, 1973, Northfield and Hofman, 1975).

In the studies cited thus far, dietary intake has been carefully controlled. Unfortunately, few studies have

examined the response of biliary lipid concentrations to variations in caloric intake. Sarles et al. (1969) examined the response of T-tube bile to changes in energy (caloric) intake. They noted an increase in biliary cholesterol concentration in response to increases in caloric intake irrespective of dietary composition and also to increases in protein intake. However, these researchers interrupted the enterohepatic circulation of bile salts several times each day while collecting T-tube bile samples, which probably accounts for their results.

Although little research has been performed in the area, it is conceivable that the length of time that bile is saturated each day is closely related to food intake or more precisely to the quantity of energy, fat and protein consumed. The flow rate of chyme through the gastrointestinal tract is not well documented, but it is known that a continuous flow through the duodenum may occur for up to fifteen hours per day (Luckey, 1974). The flow of the products of fat and protein digestion particularly, stimulates the release of several gastrointestinal hormones including secretin and cholecystokinin - pancreozymin, which have significant effects on bile flow and gallbladder contraction. The effects of gastrointestinal hormones on bile flow and composition has been the subject of some limited research. Using the rhesus monkey as the experimental animal, Gardiner and Small (1972) found that increasing doses of cholecystokinin and secretin produced a decrease in cholesterol and

phospholipid secretion and an increase in bile salt secretion. In other words, gastrointestinal hormones improve the solubility of cholesterol in bile thereby making it less lithogenic. The authors note that the increased secretion of bile salts was due to a decrease in the circulation time of the bile salt pool rather than to an increase in pool size or synthetic rate of bile salts. Other researchers (Jablonski et al., 1975) have reported similar results.

There are no reports in the literature to suggest that the hormonal response to diets of various composition, has been measured in man. However, Thompson et al. (1975) recently measured the cholecystokinin response (by radioimmunoassay) to a high energy, high protein, liquid meal (59 g protein, 166 g carbohydrate and 9 g fat). They noted that serum levels of cholecystokinin remained elevated above base line values from 30 - 240 minutes after the test meal. It is possible that the solubility of cholesterol in bile was improved during this time period. These investigations provide convincing evidence that bile lithogenicity may be controlled by various gastrointestinal hormones.

In the present study, it appears that the reduced energy intake of the female cholelithiasis group, perhaps as a result of dieting, contributed to a decrease in the length of time per day that chyme was flowing through the gastrointestinal tract and stimulating the release of hormones. This would have favored the sequestration of bile salts in the gallbladder and contributed to the secretion of lithogenic

bile and gallstone formation. Much research is required to confirm or negate this theory.

2.2 Cholelithiasis as related to a low crude fibre intake

The low energy intake of the female cholelithiasis group as compared to the female control group resulted in a low intake of crude fibre (Tables 7 and 9). Crude fibre intake was also lower in the male cholelithiasis group as compared to the male control group but the difference was not significant (Tables 7 and 9). In view of the fact that the decreased consumption of fibre has been suggested as an important factor in the pathogenesis of cholelithiasis (Burkitt, 1976), this observation deserves careful consideration.

Crude fibre is that portion of a food stuff which remains after acid and alkali digestion and consists primarily of cellulose and lignin (Cummings, 1973). Unfortunately, crude fibre values do not give an accurate estimate of the part of plant material in the diet which is resistant to digestion in the human gastrointestinal tract. This latter material is dietary fibre which includes hemicellulose and pectic substances as well as cellulose and lignin. Tables of dietary fibre content of food stuffs are not available, so in the present study, the crude fibre content of the diet was calculated.

Most researchers have considered the dietary fibre content

of the diet in relation to bile salt metabolism and biliary constituent concentrations. This work is applicable to the results of the present study, but it should be kept in mind that the dietary fibre content of the diet may be five to ten times higher than the crude fibre content which was calculated in the present study (Southgate, 1973).

Several investigators have studied the effect of the fibre content of the diet on bile salt and cholesterol metabolism. In an early study, Portman (1960), observed that rats fed a high fibre commercial diet excreted significantly more total bile acids in their feces than rats fed a low fibre purified diet. Gustafsson (1969) reported similar findings.

Unfortunately, few studies have examined the effects of dietary fibre on bile salt metabolism and the concentrations of biliary lipids in the human. Antonis and Bersohn (1962) reported that increasing the fibre content of the diet of South African White and Bantu subjects, resulted in bulkier stools which contained appreciably greater amounts of fatty acids, bile acids and sterols. Similarly, Jenkins et al. (1975) noted that the addition of dietary fibre in the form of wheat bran, to the controlled diets of six young males, resulted in increased stool weights and the increased fecal excretion of bile acids and neutral steroids. Contrary to these findings, Eastwood et al. (1973) did not observe any increase in bile acid excretion in subjects consuming a normal diet to which wheat bran was added. They did note

however, that fecal bile acid excretion increased during the control period following the consumption of the high fibre diet.

In the studies cited thus far, the response of biliary lipid concentrations to the dietary fibre content of the diet was not examined. It appears that only one group of researchers has examined the effect of increasing dietary fibre on bile lithogenicity. Pomare et al. (1976) noted that the solubility of cholesterol in bile was improved by feeding wheat bran ad libitum to six subjects for a four to six week period. Fecal bile salt excretion was not increased by bran feeding since the half lives of the primary bile salts were not affected. One would expect the half live to increase if bile salt loss was increased.

With few exceptions, most investigators have deduced that dietary fibre binds bile salts in the gastrointestinal tract, prevents their absorption and increases their excretion. After reviewing the literature, Story (1976) speculated that this effect might result in the prevention of micelle formation and the reduced absorption of cholesterol, thus promoting the loss of this latter compound in the feces. Also, the increase in bile salt loss in the feces would reduce the return of bile salts to the liver, thus stimulating the conversion of cholesterol to bile salts in order to replace fecal losses. The increased synthesis of bile salts from cholesterol could possibly cause a reduction in the

total body pool of cholesterol which could conceivably affect the secretion of cholesterol into bile. This last effect was actually noted by Pomare and co-workers (1976), but as noted earlier they did not cite it as a result of increased fecal bile salt loss. They cite the increase in the primary bile salt pool (chenodeoxycholate) and decrease in the secondary bile salt pool (deoxycholate) as factors which influence the synthesis of cholesterol. Previous studies by Pomare and Heaton (1973b) showed that deoxycholate feeding favored the formation of a lithogenic bile. The biochemical mechanisms operating here are not clear due to a lack of research in the area.

The results of many of these investigations should be approached cautiously since wheat bran was favored as the extra source of dietary fibre to be added to the diet. Recent studies have shown that the physical properties of dietary fibre may vary according to source (anatomy of the food) and age, especially in the case of fruit and vegetables (Eastwood, 1973). Eastwood and Hamilton (1968) studied the 'in vitro' adsorption of bile salts to fractions of dietary fibre and found that bile salts were more avidly bound by lignin than by hemicellulose or pectic substances. Similarly, Story and co-workers (1976) found that lignin bound bile salts more than alfalfa, bran or cellulose, with cellulose binding only negligible amounts. Research of this nature suggests that not only the presence of fibre in the diet but also the source and type of fibre affects bile salt

metabolism.

In the present study, it was found that the female control group consumed significantly more total fibre and more crude fibre from bread and bakery products than did the female cholelithiasis group (Tables 7 and 9). The male control group also consumed more total fibre than the male cholelithiasis group but this difference was not significant (Tables 7 and 9). Therefore, the major difference in crude fibre intake between the two female groups would appear to be the consumption of whole wheat flour.

Fisher (1973) reports that 93% of the crude fibre in whole wheat or whole grain flour is obtained from the bran portion of the wheat kernel. Bran is composed of 22% (by weight) cellulose and lignin, 25% hemicellulose and a variety of other constituents (Fisher, 1973). These figures vary according to reference source however (Southgate et al., 1976). It is tempting to speculate that a low fibre intake, particularly from bread and bakery sources (whole wheat flour) may have contributed to gallstone formation in the cholelithiasis group. Also, it is possible that a higher intake of crude fibre by the control group may have promoted a high turnover of bile salts. This may have favored reduced cholesterol secretion into bile, thus protecting these individuals from gallstones.

Studies cited earlier would suggest that it is perhaps the lignin fraction of wheat bran which has the most signif-

icant effect. It should be emphasized that the proportion of crude fibre in relation to total energy intake was the same for both the control group and the cholelithiasis group. The control subjects merely consumed more of all nutrients including crude fibre. If the hypothesis is true it would probably take a long time for gallstones to develop since the difference in crude fibre intake between the two groups was not great although it was significant for the females. Much more research is needed in this area before the role of fibre in the pathogenesis of gallstones can be elucidated.

3. Bile Composition and Lithogenicity

The bile analysis indicated that, although the mean lithogenic index of the cholelithiasis subgroups was higher than that of the control subgroup, this difference was significant only for the males (Table 11). The reason for this discrepancy is unknown, but it may have been due to the small size of the subgroups. There was a difference between the female control group and cholelithiasis group however, and the lithogenic index for the cholelithiasis group was very close to 1.0 (0.95 ± .26). Metzger et al. (1972) have described lithogenic indices greater than or equal to one as representative of lithogenic bile.

In the case of bile samples from the male cholelithiasis subgroup, the mean lithogenic index was actually greater than one (1.17 ± .36), whereas the mean lithogenic index for bile from the male control group was less than one (.74 ± .26).

It should be noted that there was some overlap between cholelithiasis and control subjects with respect to the lithogenic index (see Appendix 9 and 10). This overlap was also noted when the relative percentages of bile salt, phospholipid and cholesterol were plotted as single points on a triangular phase diagram (Figures 1 and 2). It is interesting to note that proportionately more male than female cholelithiasis subjects had lithogenic bile at the time of sampling. This was somewhat surprising considering the higher prevalence of gallstones in females. An overlap between cholelithiasis and control subjects with respect to bile lithogenicity has been shown in several studies.

Smallwood et al. (1972) observed that only 55% of cholelithiasis patients examined in their study had lithogenic bile. Similarly, Metzger et al. (1973) noted that in 30 to 40% of patients with gallstones, bile was not found to be lithogenic upon a single sampling. In fact, as has been discussed previously, lithogenic bile appears to be secreted intermittently; the length of time of lithogenic bile secretion being dependent upon fasting and feeding. The observation by others (Bell et al., 1975) that a clear separation exists between cholelithiasis and control subjects with respect to bile lithogenicity may have been due to the fact that Indian women were used as the experimental subjects. The mechanism of gallstone formation in these individuals may vary somewhat from that of the general population due to observed differences in bile salt and cholesterol metabolism

(Grundy et al., 1972).

It is doubtful that the bile sampling techniques employed in the present study influenced the composition of the bile samples collected. Although, samples from the cholelithiasis group were collected from the gallbladder, whereas samples from the control group were collected from the duodenum, they differed only in their concentration. The relative percentages of each bile constituent would not differ according to sample source as long as the constituents are separated immediately after collection. This has been confirmed by Dam et al. (1967). There is also little difference between the contraction response of the gallbladder to cholecystokinin - pancreozymin or amino acid perfusion (Ertan et al., 1971).

4. Bile Composition and Lithogenicity as Related to Dietary Intake

In the present study, for the female subgroup, a significant negative correlation was noted between weight and the percentage of bile salt in bile, while a significant positive correlation between weight and both the percentage of cholesterol in bile and lithogenic index (Table 14). This latter observation is in agreement with those of others (Grundy et al., 1974), who noted a significant correlation between body weight and cholesterol output in bile. In light of the results of the present study, it would be interesting to establish whether this correlation was a result of the dietary

habits of obese individuals or a result of their body composition. Presently it appears that obesity itself favors the secretion of excess cholesterol into bile (Mabee, 1976), but considerably more research is required in this area.

It was disappointing that few significant correlations were observed between the dietary intake of the subgroup and the four bile parameters examined. This was probably a result of the small group size. One interesting finding, however, was the significant positive correlation between protein intake and the percentage of the salt in bile for the female subgroup (Table 15). This observation perhaps can be explained by the influence that amino acids have on the release of gastrointestinal hormones (eg. CCK-PZ) as discussed earlier. It may be that higher protein intakes favor the release of gastrointestinal hormones in concentrations above basal levels for a prolonged period after each meal. The influence of gastrointestinal hormones on bile salt secretion and bile lithogenicity has been discussed previously.

It was not particularly surprising that significant correlations were not noted between nutrient intakes and lithogenic index. The results of the present study indicated possible relationships between low energy, fat, protein or crude fibre intake and cholelithiasis. However, it should be recognized that the two-day recall of food intake which formed the basis of the mean daily intake results did not immediately precede the bile collection period, therefore, one would not

necessarily expect a relationship between these variables. Some relationship may have been evident had a larger group been investigated. Thus far, there are no reports in the literature to suggest that a study of this nature has been attempted.

SUMMARY

1. The results of the dietary survey showed that the daily intake of protein was significantly higher for the male control group (13 subjects) than for the male cholelithiasis group (15 subjects), although the two groups were similar in age, weight and height. This difference in protein intake was not significant when male subjects with modified intakes (eg. Calorie restricted) were excluded from the sample.
2. The daily intakes of energy, protein, fat, carbohydrate and crude fibre were significantly higher for the female control group (73 subjects) than for the female cholelithiasis group (76 subjects), although the cholelithiasis group was significantly heavier and shorter than the control group.
3. Nutrient intakes/1000 calories were similar for the male cholelithiasis and control groups and for the female cholelithiasis and control groups, indicating that the diets consumed by cholelithiasis and control subjects were of similar composition.
4. Total weekly crude fibre intakes and the food sources of crude fibre were similar for the male cholelithiasis and control groups. However, the total weekly intake of crude fibre and the intake of crude fibre specifically

from bread and bakery products was significantly higher for the female control group than for the female cholelithiasis group.

5. The percentage of cholesterol in bile was significantly higher for the male cholelithiasis subgroup (5 subjects) than for the male control subgroup (8 subjects), although the two subgroups were similar in age, weight, and height. No significant difference in bile composition was detected between the female cholelithiasis subgroup (10 subjects) and the female control subgroup (5 subjects).

6. The lithogenic index of bile from the male cholelithiasis subgroup exceeded 1.0 (1.17 ± 0.36) which indicated lithogenicity. This was significantly higher than the lithogenic index of the male control subgroup. The lithogenic index of bile was similar for female cholelithiasis and control subgroups.

7. Cholelithiasis and control subjects (especially females) were not clearly differentiated by Admirand and Small's cholesterol solubility line.

8. For the female subgroups, a significant negative correlation was noted between weight and the percentage of bile salt in bile, while a significant positive correlation was noted between weight and both the percentage of cholesterol in bile and lithogenic index.

9. A significant positive correlation was noted between protein intake and the percentage of bile salt in bile for the female subgroup when the analysis was performed controlling for age, weight, height and oral contraceptive use.

CONCLUSIONS

The results of these studies indicate a possible relationship between a low intake of energy, protein, fat or crude fibre and cholelithiasis, but more than one of these nutrients could be involved. The significant positive correlation between protein intake and the percentage of bile salt in bile suggests that protein intake particularly may influence the solubility of cholesterol in bile. Other relationships may exist, however, and these might have been evident had the subgroups been large

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APPENDIX

Appendix 1: Forty-eight hour dietary recall form (one day only)

Time of Day Enter Time from 00:01 to 24:00 in hours and minutes	Food Code	Food or Drink	Work Area	Amount Consumed Whole | Decimal	Food Unit or Model No.

Are you following a special diet?

No: ___ 1

Yes, Low Calorie: ___ 2

Diabetic: ___ 3

Sodium Restricted: ___ 4

Modified Fat: ___ 5

Other: ___ 6

Is what you ate yesterday typical of your meal pattern?

Yes: ___ 1

No: ___ 2

Have you experienced any significant change in food habits during your life?

Yes: ___

No: ___

Give details:

Date of interview: Day ___ Month ___ Year 19__

Interviewer:

Appendix 2: Instructions to dietary interviewers

CONDUCTING THE DIETARY INTERVIEW

General Information

The techniques of dietary interview employed will determine greatly the accuracy of the results obtained. It is essential that all interviewers utilize similar techniques to minimize bias. This is not to say that every interview should be exactly the same. Interviews may be modified according to the individual who is being interviewed. The interviewer should be able to judge the intelligence of the respondent and also how apprehensive he is about the interview itself.

The interview should be approached in a calm manner. Don't be in a hurry to pick up information because chances are some important food items will be missed.

1: Greet the respondent warmly at the door. Identify yourself.

2. Establish rapport with the respondent before beginning the interview. Explain why you are there, how long you will be there, and the type of information you are seeking. Don't give too much information about the exact nature of the study however.

3. Arrange yourself in a seat beside the respondent and place the food model kit on the table beside you. It may be appropriate to have the suitcase on a small chair and food models can be extracted and placed on the table as required.

4. Be sincere and straightforward about the interview. Don't be machine-like. Ask questions as if you expect them to be answered.
5. Do not show surprise or disapproval of the respondent's replies, either by facial expression or tone of voice.
6. Listen carefully to the respondent's replies. You may get the answers to several questions at one time.
7. Repeat back what the respondent has told you to make sure you understand the information which has been reported.
8. Maintain a friendly manner but do not engage in small talk throughout the interview.
9. If an unexpected visitor interrupts your interview - come back another time. Don't try to finish the interview when you have lost the respondent's attention.
10. If the respondent appears to be ill - come back another time.

Specific Points on Conducting the Dietary Interview

1. When doing the two day recall of food intake, have the respondent recall the day he remembers the best (usually yesterday).
2. Do not refer to any meals in the day - such as breakfast, lunch and supper. Some people do not follow such a pattern. Simply ask for an account of all the food items consumed throughout the day.

   eg. Ask, "What was the first thing you did when you got up yesterday morning?" If the respondent

says, "I had breakfast", then ask her what she had for breakfast.

3. Try having the respondent recall the activities of the previous days as these are often associated with food intakes.

4. Don't give negative or closed questions like: "Didn't you have anything else to eat last night?"
Use open-ended questions such as: "Can you think of anything else you had to eat last night?"

5. After you have acquired a list of foods in the order of consumption, go back and enquire about the amounts. Place all appropriate food models of the same type on the table equidistant from the respondent (eg. all the glass models).

6. Ask the respondent if any of the models resemble the amount she had to eat. eg. In the case of a beverage, ask:

   i) from what type of container did you drink?

   ii) If she says, "a glass", then display all the glasses and ask which glass resembles the one she drank from.

   iii) Then ask how full that particular glass was.

   iv) Did she drink its entire contents?

7. Always recheck a days intake but do not suggest foods unless absolutely necessary as this will introduce a bias into the results.

8. If the respondent cannot remember her intake for a particular day, then have her look in the cupboards

or the refrigerator. This may help to jar her memory.

9. Although the subject should be interviewed alone where possible, a male respondent may require the help of his wife, especially if she had prepared the meals for him.

10. Remember to ask the respondent if he or she ate everything on his or her plate. Also enquire about second helpings.

Appendix 3: Nutrient composition of additional foods

Food	Nutrient/100g				
	Energy	Protein	Fat	Carbohydrate	Crude Fibre
	(Cal)	(g)	(g)	(g)	(g)
Harvest Crunch (Quaker)[1]	500	11.27	24.67	58.36	1.6
Harvest Crunch (Quaker)[1] with Raisins and Dates	479	10.24	21.41	61.40	1.6
100% Whole Wheat Bread[1]	243	10.5	3.0	47.7	2.3
Ayds (diet candy)[1]	368	1.9	4.8	82.8	.1
Date-nut loaf[2]	281	6.0	7.9	48.7	0.7
Poppyseed cake[2]	364	4.6	16.0	54.0	.5

[1]Product manufacturer supplied nutrient information

[2]Calculated from standard recipes

Appendix 4: Weekly crude fibre intake questionnaire

Instructions to Interviewers

For each of the following food items, ask the subject if he has consumed it in the past seven days (not including the day of interview). Determine serving sizes by using the food models if necessary. Calculate the total amount consumed for the week.

Guidelines for Recording

The form does not have all foods containing crude fibre listed. The following guidelines will help determine which extra food items should be recorded.

Cereals

The foods listed provide at least 1 gm crude fibre/100 gm. Please list all other cereals consumed in the past week.

Fruit

Add other fruit which was not included on the list. Do not include fruit juices unless a pulpy nectar was consumed.

Fruit Pie

Record other fruit pie not included on the list.

Bread and Bakery Products

Record the consumption of bread containing whole grain flours only. Do not record white bread, or plain cakes and cookies. Whole wheat crackers include Stoned Wheat Thins, Triscuits and Canadian Harvest. Other fruit or nut cookies, e.g. fruit peel, raisin, peanut butter. Other bakery products: Record the consumption of all products containing whole wheat flour or other whole grain flour, cereals, fruit (dried or

fresh), nuts, and seeds.

Soup

Record the consumption of all soups.

Nuts

Record the type consumed. Also record seeds such as sunflower seeds.

Vegetables

Record the consumption of all vegetables - raw or cooked.

Cereal: cooked and dry

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
All-bran			
Bran flakes			
Cracked wheat (cooked)			
Crunchy Granola			
Cheerios or Frosty O's			
Grapenuts Flakes			
Muffets			
Pep (Kelloggs)			
Product 19 or Special K			
Raisin bran			
Romanmeal (cooked)			
Red River Cereal			
Shredded Wheat			
Shreddies, etc.			
Sunnyboy			
Oatmeal (cooked)			
Team flakes			
Wheaties			
Wheatabix			
Puffed wheat (Quaker)			
Zoom			
Other: specify			

Fruit

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
Raw apple			
Apple sauce			
Apricots			
Banana			
Canned or fresh blackberries			
Canteloupe			
Cherries			
Dates			
Currants			
Figs			
Canned fruit cocktail			
Grapes			
Grapefruit			
Mango			
Orange			
Canned peaches			
Fresh peach			
Canned or fresh pineapple			
Canned or fresh plums			
Prunes			
Canned pumpkin			
Raisins			
Canned or fresh pears			

Fruit continued

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
Raspberries			
Rhubarb			
Strawberries			
Tangerine			
Watermelon			
Other: specify			

Fruit Pie (use Food Model)

Apple			
Cherry			
Blueberry			
Blackberry			
Peach			
Pineapple			
Mince			
Rhubarb			
Pumpkin			
Raisin			
Other fruit pie: specify			

Bread and Bakery Products

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
100% whole wheat bread			
50-60% whole wheat bread			
Pumpernickel bread			
Rye bread			
Raisin bread			
Cracked wheat bread			
"Brown" bread			
Other bread: specify			
whole wheat crackers, specify:			
Bran muffin			
Graham cracker			
Ry[illegible]afers			
Da[illegible] square			
Hermits			
Other fruit or nut cookies, specify:			
Buckwheat pancakes			
Other bakery products, specify:			

Soup

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
Grean pea			
Bean with bacon			
Homemade bean			
Cream of asparagus			
Cream of celery			
Black bean			
Chili beef			
Minestrone			
Tomato			
Vegetable			
Vegetable bean			
Vegetable beef			
Cream of vegetable			
Vegetarian vegetable			
Beef			
Beef noodle			
Cream of chicken			
Chicken gumbo			
Chicken noodle			
Chicken Vegetable			
Cream of mushroom			
Chicken rice			
Pepper pot			
Clam chowder			

Soup continued

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
Scotch broth			
Tomato vegetable			
Turtle			
Turkey noodle			
Other: specify			

Nuts

Specify			

Vegetables: Cooked unless specified

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
Artichoke			
Asparagus			
Bamboo shoots			
Beans (kidney, lima, etc.			
Green beans			
Wax beans			
an sprouts			
ets			
Beet greens			
Broccoli			
Brussels sprouts			
Cabbage			
Raw cabbage			
Red cabbage (raw)			
Carrots			
Raw carrots			
Cauliflower			
Celery (raw)			
Corn on cob			
Kernel corn			
Raw green pepper			
Cucumber			
Eggplant			
Lettuce			

Vegetables continued

Type	Frequency	Average Serving Size	Total Amount Consumed (past week)
Mushrooms			
Spinach			
Raw Onion			
Onion			
Parsnips			
Peas			
Dill Pickle			
Potatoes Baked with skin			
Mashed			
Boiled (peeled)			
Other			
Radishes			
Rutabaga or turnip			
Sauerkraut			
Squash			
Sweet potato (yam)			
Tomatoes			
Turnip green			
Zucchini			
Other: specify			

Appendix 5: General questionnaire

1. Age
2. Weight
3. Height
4. Have you ever used oral contraceptives?

   For how long (months)?
5. Gravida (number of pregnancies)
6. Parity (number of children)
7. Recent illness or operation which has affected your food habits?

Appendix 6: Intakes of energy, protein, fat, carbohydrate and crude fibre on Day 1 and Day 2 for the cholelithiasis and control groups

Group	No. of Subjects	Day	Amount Consumed/Day				
			Energy (Cal)	Protein (g)	Fat (g)	Carbohydrate (g)	Crude Fibre (g)
Male							
Cholelithiasis	15	1	2290±593	88.0±26.3	101,8±39.1	250.7±89.4	4.9±1.7
		2	2448±492	90.3±22.8	116.5±37.4	257.0±80.7	4.8±2.0
		$\bar{X}^1$	2369±541	89.2±24.2	109.1±38.3	253.8±83.7	4.8±1.8
Control	13	1	2777±976	120.8±50.4	112.9±42.7	306.8±131.2	5.8±2.9
		2	3085±1174	128.1±51.5	142.0±66.6	315.1±133.8	7.0±3.4
		$\bar{X}^1$	2931±1069	124.5±50.1	127.5±56.8	311.0±130.0	6.4±3.2
Female							
Cholelithiasis	76	1	1527±608	61.9±25.0	66.5±36.2	169.0±78.5	3.3±2.4
		2	1488±543	57.3±20.3	64.3±31.7	169.0±68.4	3.2±2.0
		$\bar{X}^1$	1508±575	59.6±22.8	65.4±33.9	169.0±73.4	3.3±2.2
Control	73	1	1800±727	69.4±23.5	83.5±54.6	194.5±74.5	4.3±2.2
		2	1813±695	71.6±25.4	82.6±50.4	197.0±75.2	3.7±2.0
		$\bar{X}^1$	1806±709	70.5±24.4	83.1±52.3	195.7±74.6	4.0±2.1

[1]Mean of day 1 and day 2

Appendix 7: Typical days, weekend days and classification of dietary intake for the cholelithiasis and control subgroups

Subgroup	No. of Subjects	No. of Typical Days[1]	No. of Weekend Days[2]	Classification of Dietary Intake: No. and (Percent) of Subjects			
				U[3]	CR[4]	SR[5]	MF[6]
Male							
Cholelithiasis	3	1.3[7,a]	1.0[a]	3(100)	0	0	0
Control	6	2.0[a]	.2[a]	6(100)	0	0	0
Female							
Cholelithiasis	9	1.2[a]	.2[a]	7(77.8)	2(22.1)	0	0
Control	4	1.3[a]	.5[a]	3(75.5)	1(25.0)	0	0

[1]Mean number of days out of the two days recalled that were typical days

[2]Mean number of days out of the two days recalled that were weekend days

[3]Unmodified

[4]Calorie-restricted

[5]Sodium-restricted

[6]Modified fat

[7]Values for males or females for a given parameter without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was Chi-square.

Appendix 8: Daily intakes of energy, protein, fat, carbohydrate and crude fibre for the cholelithiasis and control subgroups

Subgroup	No. of Subjects	Amount Consumed/Day[1]				
		Energy (Cal)	Protein (g)	Fat (g)	Carbohydrate (g)	Crude Fibre (g)
Male						
Cholelithiasis	3	2248±192[2,a]	89.6±8.3[a]	100.9±31.0[a]	228.2±76.9[a]	5.0±1.4[a]
Control	6	3340±1119[b]	155.0±40.6[b]	142.8±53.4[a]	344.0±146[a]	7.5±3.0[a]
Female						
Cholelithiasis	9	1654±646[a]	63.6±22.1[a]	70.4±27.9[a]	186.9±73.2[a]	3.2±2.1[a]
Control	4	1978±897[a]	69.0±16.0[a]	88.1±41.6[a]	224.3±113.8[a]	5.0±2.2[b]

[1]Each value is the mean of two days.

[2]Values are means ± s.d. Values for males or females for a given parameter, without a common letter in their superscript are significantly different ($P<0.05$). Statistical test used was analysis of covariance adjusting mean values for age and weight.

Appendix 9: Characteristics, Bile Composition, lithogenic indices and percent cholesterol in gallstones for the cholelithiasis subgroup

Sex	Age	Weight	Height	Bile Constituent						Lithogenic Index[3]	Cholesterol in Gallstone
				Bile Salt		Phospholipid		Cholesterol			
	(yr)	(lbs)	(in)	mM/l[1]	%[2]	mM/l[1]	%[2]	mM/l[1]	%[2]		(%)[4]
F	22	115	61.4	136	73	33.7	18	18.2	9	.9	52
F	19	234	65.0	173	63	68.5	25	33.9	12	1.17	76
F	28	130	66.25	109	65	41.2	25	18.4	10	.97	60
F	49	132	65.0	90	75	20.9	17	9.3	8	.80	71
F	29	137	65.4	188	74	46.8	19	19.4	7	.69	91
F	27	140	65.8	91	67	31.6	23	14.1	10	1.0	70
F	39	125	-	174	78	35.8	16	13.2	6	.59	85
F	28	130	61.75	190	65	80.1	27	22.6	8	.78	87
F	56	148	66.0	95	60	32.8	23	16.8	11	1.09	85
F	17	220	62.0	116	62	44.1	23	28.6	15	1.49	100
M	32	176	-	104	62	37.8	23	26.1	15	1.43	84
M	41	162	66.3	128	56	66.0	29	33.6	15	1.46	96
M	31	162	70.0	20	68	5.3	18	4.0	14	1.39	58
M	50	194	-	46	70	14	21	6.0	9	.88	-
M	62	132	70.9	94	71	28.2	22	9.7	7	.69	46

[1] Millimoles per liter of bile

[2] Percent millimoles of bile constituent per total millimoles of bile salt, phospholipid and cholesterol

[3] Lithogenic index = $\frac{\text{actual amount of cholesterol in bile sample}}{\text{maximum amount of cholesterol which could be dissolved by the amounts of bile salt and phospholipid in bile sample}}$

[4] Mean % cholesterol = 75.8

Appendix 10: Characteristics, bile composition and lithogenic indices for the control subgroup

Sex	Age	Weight	Height	Bile Constituent						Lithogenic Index[3]
				Bile Salt		Phospholipid		Cholesterol		
	(yrs)	(lbs)	(in)	mM/l[1]	%[2]	mM/l[1]	%[2]	mM/l[1]	%[2]	
F	45	140	63.5	38	69	6.6	12	10.8	19	1.19
F	45	130	67.0	36	71	3.4	7	11.6	22	.69
F	23	112	64.0	47	78	3.1	5	10.5	17	.49
F	16	130	66.0	57	82	2.7	4	9.5	14	.39
F	23	108	65.0	19	80	1.1	5	2.7	15	.50
M	49	175	70.0	41	69	5.2	9	13.3	22	.89
M	29	162	70.0	26	66	3.2	9	9.0	25	.83
M	42	180	70.0	6.5	61	0.9	9	3.1	30	.87
M	22	150	68.0	5.5	63	0.8	9	2.3	28	.86
M	25	170	72.0	26	76	2.2	6	6.0	18	.59
M	15	175	68.0	16	70	1.2	5	5.7	25	.49
M	17	150	68.0	16	65	2.7	11	6.0	24	1.09
M	18	165	70.0	111	85	3.5	3	16.1	12	.30

[1-3]See footnotes 1, 2 and 3 under Appendix 9