DIETARY HABITS AND LIFE STYLE IN THE ETIOLOGY OF CHOLESTEROL GALLSTONE DISEASE
A MATCHED CASE CONTROL STUDY

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LIST OF ABBREVIATIONS

BA:	Bile Acid
BMI:	Body Mass Index
BS:	Bile Salt
CCS:	Case Control Study
CI:	Confidence Interval
DCA:	Deoxycholic acid
DM:	Diabetes Mellitus
FA:	Fatty acid
FFQ:	Food Frequency Questionnaire
GB:	Gallbladder
GI:	Gastrointestinal
GS:	Gallstones
HD:	Heart Disease
HDL:	High Density Lipoproteins
HRT:	Hormone Replacement Therapy
LDL:	Low Density Lipoproteins
MUFA:	Monounsaturated Fatty Acid
OR:	Odds Ratio
PC:	Phosphatidylcholine
PE:	Phosphatidylethanolamine
PL:	Phospholipid
PS:	Phosphatidylserine

LIST OF ABBREVIATIONS – cont -

PUFA:	Polyunsaturated Fatty Acid
RDA:	Recommended Dietary Allowances
RMR:	Resting Metabolic Rate
RR:	Relative Risk
TG:	Triglyceride
US:	Ultrasonography
UTI:	Urinary Tract Infection
VLDL:	Very Low Density Lipoproteins
WHO:	World Health Organization
WW:	World War

1. INTRODUCTION

A number of studies from Romania, Germany, The Netherlands, France, Japan have suggested that the prevalence of cholesterol gallstones (GS), especially in affluent countries, has increased substantially during the twentieth century[1] [2] [3] [4], whereas it is rare in Asia and Africa[5] [6]. These findings indicate that cholelithiasis might be a disease affected by genetic factors, but environmental factors and lifestyle habits might be just as or more important.

Cholelithiasis is today, together with Heart disease (HD) and Diabetes Mellitus (DM), one of the most frequent diseases in industrialized countries and affects about 15% of Western populations[7]. 10 % of the male population and 20% of the female population suffer from cholelithiasis[8]. In Germany, it was suggested that around 7-9 million people suffer from GSs. In Israel, the prevalence of GS amounts to almost 30% of the population above age 70[9]. Most GS patients suffer from asymptomatic, 'silent', GS and therefore remain unknown. Only about 20% of GS carriers report symptoms, which in most cases lead to operative removal of the gallbladder (GB) [cholecystectomy], which up until about 20 years ago was the only therapy for GS[10].

[1] Acalovschi *et al*, 1987
[2] Balzer *et al*, 1986
[3] Sarles *et al*, 1978
[4] Nakayama *et al*, 1970
[5] Swang WS, 1970
[6] Biss *et al*, 1971
[7] Diehl AK, 1991
[8] Thijs *et al*, 1990
[9] Gilat *et al*, 1985
[10] Barbara *et al*, 1987

Despite the large number of studies in recent decades the etiology and pathogenesis of GS have not been elucidated. Family and twin studies[1] [2] [3] [4] [5] [6] [7] as well as high prevalence in certain populations, such as Pima-, Chippewa-, MicMac-, Sioux- and Navajo Indians[8] confirm the effect of genetic factors. The major increases in GS prevalence within defined populations in a matter of decades indicate the effects of environmental factors. Genetic and environmental factors may well exist, the changing environment affecting mostly those who are genetically predisposed. Diet has long been suspected to be one of these putative environmental factors. Differences in GS prevalence between populations consuming a low calorie subsistence diet and populations in affluent countries having a surfeit of food[9] [10]. The rapid formation of GS in obesity[11] [12] [13] [14] [15] [16] [17], rapid weight loss[18] [19] [20], parenteral feeding[21], as well as in people consuming special diets[22] all point in that direction.

[1] Sarin *et al*, 1995
[2] Nurnberg *et al*, 1989
[3] Danziger *et al*, 1972
[4] Gilat *et al*, 1983
[5] Antero Kesaniemi *et al*, 1989
[6] Doig RK, 1957
[7] Brown *et al*, 1968
[8] Sampliner *et al*, 1970
[9] Sarles *et al*, 1969 & 1978
[10] Gilat *et al*, 1985
[11] Friedman *et al*, 1966
[12] Kern F Jr, 1983
[13] Scragg *et al*, 1984
[14] Diehl *et al*, 1987
[15] Barbara *et al*, 1987
[16] GREPCO, 1988
[17] Maclure *et al*, 1989
[18] Liddle *et al*, 1989
[19] Sichieri *et al*, 1991
[20] Yang *et al*, 1992
[21] Akierman *et al*, 1984
[22] Sturdevant *et al*, 1973

Danziger *et al* [1] showed that in some cholesterol GS patients who were fed high doses of chenodeoxycholic acid (and later its epimer ursodeoxycholic acid), the GS dissolved. Over the years, it became clear that this kind of treatment is only suitable for around 20% or less of GS carriers[2]. Therefore cholecystectomy is presently one of the most common operations and accounts for a high percentage of hospital expenses and health insurance companies.

1.1 Aims and Hypothesis

There have been quite a large number of studies analyzing diet and GS, however no definitive conclusions have emerged. On reviewing these studies several flaws in the research protocols have been identified:

- inclusion of symptomatic GS carriers (might influence usual eating habits)
- no ascertainment of GB status of all cases and controls
- bad choice of study subjects (volunteers, nurses, hospital-based controls), who do not necessarily represent the entire population for whom the results are supposedly applicable
- multiple interviewers (might introduce inter-observer bias)
- limited questionnaires unable to give a true picture of habitual dietary intake, and other problems discussed later.

[1] Danziger *et al*, 1972
[2] Fromm *et al*, 1986

The present study evaluated the potential association between GS and diet trying to avoid some of the shortcomings of previous study protocols. Lifestyle habits were also analyzed. All study subjects (cases and controls) had US examination of their GB to ascertain the presence of GS. All GS carriers were asymptomatic, allowing analysis of diet and lifestyle prior to onset of symptoms.

The matching was complete and included not only gender and age but also community group (which may heavily influence dietary habits). Two hundred and six subjects were studied, 103 GS carriers and 103 controls, without GS.

2. SCIENTIFIC BACKGROUND

Below you will find a literature review. The first part is a short review about bile and GS formation.

The major part though, reviews literature on factors associated with GS in the past. It was difficult to decide on a structure in which to best present them. This was mainly due to the fact that most of the studies examined more than one factor in their study. Finally, I divided the chapter into the main factors and cited every publication that found any association, be it positive or negative, in the relevant sections. Furthermore I described most of the studies in more detail. This was done randomly, in different sections of this chapter, so as to, for example not overload the section on energy intake, which is mentioned in numerous studies.

2.1 Hepatocyte

The hepatocyte is a highly polarized epithelial cell with a sinusoidal receptor-rich domain in contact with the plasma, a lateral domain attached to adjacent cells and the canalicular domain, or apical pole, delimited by tight junctions[1] in contact with bile. Lipids are secreted through the sinusoidal membrane in the form of lipoproteins and through the canalicular membrane as monomers (bile acids) and vesicles (phospholipids [PL], esp. phosphatidylcholine [PC], and cholesterol)[2] [3].

[1] Rigotti *et al*, 1994
[2] Ulloa *et al*, 1987
[3] Cohen *et al*, 1989

2.2 Bile

2.2.1 Flow and composition

Bile is secreted from the liver into the biliary tree, through which it flows to the duodenum. In the interdigestive period bile is stored in the GB. Following cholecystokinin stimulation during digestion bile is delivered in a concentrated form into the gut[1]. It is composed of over 90% water and about 10% solutes, mainly biliary lipids – bile acids (BAs), PL (in bile, mainly as lecithin) and free cholesterol. Other components of bile include various proteins (albumin, glycoproteins, mucins etc.), pigment (bilirubin), electrolytes and xenobiotics[1 2 3]. As mentioned above, cholesterol in bile is present as free cholesterol. BAs are composed of primary BA (cholic and chenodeoxycholic acid) and their intestinal de-hydroxylation products (deoxycholic and lithocholic acid, respectively). All BAs in bile are conjugated with glycine or taurine[1]. Biliary PL are composed mainly of PC (~95%) and small amounts of phosphatidylethanolamine (PE) and phosphatidylserine (PS)[4]. These PL are a mixture of molecular species having primarily palmitic or stearic acid in the sn-1 position and less saturated fatty acyl chains in the sn-2 position[5].

[1] Carey *et al*, 1988
[2] Carey MC, 1989
[3] Harvey *et al*, 1985
[4] Angelico *et al*, 1992
[5] Hay *et al*, 1990

2.2.2 Lipid secretion

An important part of biliary PLs and cholesterol is derived from high density lipoprotein (HDL) lipids. About 3-20% of biliary PLs and cholesterol are newly synthesized in the endoplasmic reticulum prior to transport into bile[1] [2]. Patton and colleagues[3] suggest that acyl remodeling of pre-existing PCs as well as hepatic triglyceride (TG) provides most of the microsomal substrates required for synthesis of bile targeted PCs.

Because of the hydrophobic properties of PLs, these molecules do not move by spontaneous intracellular aqueous diffusion and must be translocated within the cell, either associated to lipid binding proteins or in a vesicular form. PL-transfer proteins have been described and purified[4] [5] [6]. The major fraction of cholesterol and PLs transfer to the canalicular domain of the hepatocyte is believed to be transported by vesicular movement[7] as well as lateral diffusion in the membrane.

The intra-hepatocytic transport of PL-cholesterol vesicles to the canalicular membrane for secretion into bile has not been elucidated with certainty. Indirect evidence suggests that a fraction of biliary lipids (de-novo synthesized cholesterol and PLs) originates in a specific region of the endoplasmic reticulum, where bile-destined lipids are sorted and packed into intracellular vesicles to be transported vectorially through the Golgi apparatus and secreted finally into bile[8] [9].

[1] Turley *et al*, 1981
[2] Robinson *et al*, 1988
[3] Patton *et al*, 1994
[4] Reinhart, 1990
[5] Wirtz KWA, 1991
[6] Cohen *et al*, 1994
[7] Crawford *et al*, 1995
[8] Marzolo *et al*, 1990
[9] Coleman *et al*, 1992

Monensin, a carboxylic ionophore that was found to interfere with the storage and transport of proteins from the Golgi apparatus[1], decreased both the sinusoidal secretion of very low density lipoprotein (VLDL)[2] and canalicular secretion of cholesterol and PL[3]. Another fraction of biliary cholesterol and PC (preformed lipids) could be presumably transported to the canalicular region from the endosomal compartment after processing of lipoproteins internalized by receptor-mediated uptake. Multivesicular bodies, which are derived from lipoprotein internalization, may be an intracellular precursor of biliary lipid secretion[4]. However Cohen and colleagues have shown that transcytosolic movement of vesicles from the endoplasmic reticulum to the bile canalicular domain cannot account quantitatively for the flow of membrane PC into bile, thereby suggesting that both vesicular transport and coupling to lipid-binding proteins are responsible for the movement of cholesterol and PL to the canalicular membrane[5]. After insertion in the canalicular membrane, PC is thought to reside in micro-domains that are particularly rich in PC. This assumption is based on the fact that there is a striking difference between the composition of biliary PL and that of the canalicular membrane.

Biliary PL contains mainly PC (>95%), whereas the canalicular membrane also contains sphingomyelin (±20%), PE (±20%), and PC (±20%)[6]. Furthermore, PC in bile is more hydrophobic than membrane PC[1 7].

[1] Griffith *et al*, 1986
[2] Rustan *et al*, 1985
[3] Casu *et al*, 1990
[4] Hornick *et al*, 1985
[5] Cohen *et al*, 1994
[6] Yusef *et at*, 1987
[7] Coleman *et al*, 1992

Two theories have been proposed to explain the mechanism of biliary lipid secretion at the canalicular membrane: 1) 'fusion-budding' or 'shedding'[1]

2) exocytosis[2 3].

In view of these mechanisms, PL and cholesterol are coupled tightly to BA secretion under physiological conditions. The effectiveness of the various BA species in driving the co-secretory mechanism of biliary PL and cholesterol outputs varies in relation to their hydrophobicity[4 5 6]. The quantitative significance of BAs was found to be relatively minor[5]. The hydrophobicity of bile acid molecules may have a critical role in the process of recruiting and sorting cholesterol-PL vesicles.

2.2.3 Cholesterol solubilization

Bile is the only significant pathway for the excretion of cholesterol from the body, and about half of the biliary cholesterol is lost in feces. The hydrophobic cholesterol is solubilized in bile at a concentration of 5-15 mM, which exceeds over 100,000 fold its water solubility.

This solubilization of cholesterol molecules is made possible by the two amphiphilic lipid molecules – bile salts (BSs) and PL[7]. Together, the three biliary lipids form micellar and lamellar-vesicular structures that serve as cholesterol 'carriers'[8 9 10]. These structures incorporate the insoluble cholesterol molecules during bile secretion, flow, and bile storage in the GB.

[1] Lowe *et al*, 1984
[2] Evans W, 1981
[3] Marzolo *et al*, 1990
[4] Roda *et al*, 1988
[5] Bilharz *et al*, 1989
[6] Hofmann A, 1990
[7] Cabral *et al*, 1989
[8] Carey *et al*, 1978
[9] Admirand *et al*, 1968
[10] Somjen *et al*, 1983 & 1985

The sequence of physical-chemical events during lipid secretion into bile is still being investigated. It is not clear whether PLs and cholesterol are secreted independently or jointly. Crawford and colleagues[1] showed that biliary PL molecules are secreted by hepatocytes into the bile canalicular lumina as 63 – 67 nm (diameter) unilamellar vesicles which contains cholesterol. This process is rapid and is facilitated by the detergent action of BSs at the exoplasmatic part of the canalicular membrane. Cholesterol is solubilized by vesicles and transported into hepatic bile, which normally has a cholesterol:PL ratio of about 1:3. In lithogenic bile, which is supersaturated with cholesterol this ratio is higher especially in the vesicles, where it can reach about 2:1[2]. During flow in the biliary tree, vesicular cholesterol is gradually taken up into BS rich micelles. Since mixed micelle formation requires the solubilization of more PLs than cholesterol, the excess cholesterol remains solubilized in vesicles, which then become supersaturated and thermodynamically metastable.

2.2.4 Phospholipids (PLs) in bile

Recent evidence suggests that PLs are of major importance in biliary pathophysiology. Biliary cholesterol is probably secreted as PL vesicles[3] [4] and PLs are probably the main cholesterol carriers in the bile[5] [6]. A reduction of biliary PLs by intake of dietary legumes was associated with an increased cholesterol saturation of the bile[7].

[1] Crawford *et al*, 1995
[2] Donovan *et al*, 1990
[3] Cohen *et al* 1989
[4] Pattison NR, 1985
[5] Somjen *et al*, 1990
[6] Gilat *et al*, 1990
[7] Nervi *et al*, 1989

During the last decade, some intermediate lamellar structures have also been described in model and native biles, but their nature and possible role in biliary cholesterol solubilization and transport is controversial[1] [2]. When BS concentration is sufficient, all cholesterol will be solubilized in micelles. However, more commonly human bile is supersaturated and only part of the cholesterol is solubilized in micelles, with the rest remaining metastable vesicles. When the cholesterol carrying capacity of the lipid aggregates is exceeded, cholesterol may precipitate and form cholesterol monohydrate crystals[3].

Most available data suggest that biliary cholesterol crystallizes from cholesterol rich vesicles, though at least one study showed that supersaturated mixed micelles may also be the source of cholesterol crystal precipitation[4]. Ultimately, within the GB cholesterol crystals are agglomerated with an organic matrix of mucin glycoproteins to form cholesterol GS[5].

2.2.5 Cholesterol crystallization (calculi formation)

Cholesterol monohydrate crystals are microscopic building blocks of cholesterol GSs[6]. Hence it is obvious that the process of cholesterol crystallization is a crucial and obligatory step in cholesterol GS formation.

[1] Somjen *et al*, 1990
[2] Cohen *et al*, 1993
[3] Small DM, 1980
[4] Ahrendt *et al*, 1994
[5] Lee *et al*, 1979
[6] Womack *et al*, 1963

This process can be subdivided into 3 successive steps: nucleation, formation and precipitation of solid crystals (crystallization), and crystal growth[1]. Despite their importance, these steps are difficult to observe and investigate separately.

Most research on cholesterol crystallization to date has been based on the nucleation time assay which measures the crystal detection time in crystal-free bile samples incubated *ex-vivo*[2]. In 1992, Konikoff and his colleagues[3] have shown that the process of cholesterol crystallization is more complex than hitherto believed. Biliary cholesterol was found to initially crystallize as thin filamentous crystals that transform through intermediate microcrystalline structures before becoming thermodynamically stable classical monohydrate crystals. The nucleation time of bile from GS patients has been shown to be significantly shorter than that of GS free subjects[4]. This is commonly interpreted as evidence for the presence of pro-nucleating agents (mainly proteins) in human lithogenic bile[5]. On the other hand, normal human biles have longer nucleation times than identically matched model lipid solutions[6], suggesting the presence of anti-nucleators in normal non-lithogenic biles. These pro- and anti-nucleating factors have not all been convincingly documented and there is a great deal of confusion as to their actual role in GS pathogenesis[7].

A clear understanding of the microstructural events occurring during the earliest phases of cholesterol crystallization in bile is crucial for the identification of factors possibly delaying or preventing precipitation of cholesterol crystals and therefore, GS formation in bile.

[1] Wang *et al*, 1996
[2] Holan *et al*, 1979
[3] Konikoff *et al*, 1992
[4] Burnstein *et al*, 1983
[5] Portincasa *et al*, 1997
[6] Holzbach *et al*, 1984
[7] Harvey *et al*, 1993

Carey[1] suggests that GSs occur as a result of a change in the proportions of bile lipids in the bile. Cholesterol saturation or supersaturation in the bile occurs as a result of any of the two following situations:

1. Hyposecretion of BAs (for example due to increased gastrointestinal loss)
2. Hypersecretion of cholesterol.

The latter can occur as a result of:

- increased cholesterol synthesis in the liver
- increased consumption of dietary cholesterol
- increased influx of plasma lipo-proteins into the liver.

Supersaturation of cholesterol in the bile leads to precipitation of cholesterol crystals which favor the development of GSs. Dietary factors might be responsible for cholesterol supersaturation in the bile, but most probably do not play a role in the crystallization and/or crystal growth.

2.2.6 Cholelithiasis – formation or presence of calculi (Gallstones, GS) in the GB

This section has been taken from the Merck Manual[2].

'In GS disease we differentiate between cholesterol GS, which are made up of more than 70% cholesterol and pigment stones, made up mainly of calcium salts of bilirubin and fatty acids (FAs) as well as mucins, and proteins.

GS are formed and located mostly in the GB, but also in the extra-hepatic or intra-hepatic bile ducts. The site of the GSs can give us information on the make up of the stones. Cholesterol GSs are mostly found in the GB.

[1] Carey MC, 1989
[2] Beers *et al*, (pp 400) 1999

Of the pigment stones, the black stones are usually found in the GB, whereas the brown stones are bile duct concrements. GSs originally located in the GB can move into the bile duct.

2.2.6.1 Symptoms and signs

The clinical consequences of GS formation in the GB are exceedingly variable. Most patients remain asymptomatic for long periods, frequently for life. GS may traverse the cystic duct with or without symptoms of obstruction. Transient cystic duct obstruction results in colicky pain, whereas persistent obstruction usually produces inflammation and acute cholecystitis. In contrast to other types of colic, biliary colic typically is constant, with pain progressively rising to a plateau and falling gradually, lasting up to several hours. Nausea and vomiting are often associated. Fever and chills are absent in uncomplicated GB colic. Pain most often occurs in the epigastrium or right upper quadrant, radiating to the right lower scapula.

Symptoms of dyspepsia and fatty food intolerance are often inaccurately ascribed to GB disease. Belching, bloating, fullness, and nausea are equally associated with cholelithiasis, peptic ulcer disease, or functional disorders. Such symptoms may disappear after cholecystectomy but should not be the only indication for operation. Postprandial fatty food intolerance maybe be caused by cholelithiasis if symptoms include right upper quadrant pain; however, the prevalence of postprandial functional distress is so high in the general population that these symptoms alone are insufficient for diagnosis of GB disease without supportive clinical signs and diagnostic studies.

2.2.6.2 Diagnosis

Few calculi escape detection, but the relative accuracy, ease, safety, and cost of diagnostic methods are subject to change, to debate, and to local availability and skills. US examination is the method of choice for diagnosing possible GB calculi. Sensitivity (probability of a positive test when disease is present) is 98%; specificity (probability of a negative test when the disease is absent) is 95%. Static B mode US and oral cholecystography are also sensitive and specific.

2.2.6.3 Treatment

Asymptomatic GS: Because asymptomatic GS are often discovered during evaluation of other problems, the question arises whether to recommend observation or elective cholecystectomy. Neither choice applies to all circumstances. Although the natural history is unpredictable, there is a cumulative chance (about 2% per year) that symptoms will develop. Most patients with clinically silent stones decide that the discomfort, expense, and risk of elective surgery are not worth removing an organ that may never cause clinical illness, although the potential complications represent serious disease. If symptoms appear, prompt therapy is advisable.

Symptomatic GS: Biliary colic recurs with irregular, pain-free intervals of days or months. Symptoms often do not progress in severity or frequency, but neither do they cease. Symptomatic patients are at increased risk of developing complications, and cholecystectomy is indicated. Symptoms attributable to the GB can be expected to disappear after cholecystectomy; nonspecific symptoms of postprandial dyspepsia usually also remit in patients who have had colic. Recurring colic, even years later, should prompt an evaluation for possible common duct stones (choledocholithiasis).

Cholecystectomy does not result in nutritional problems, and no dietary limitations are required postoperatively.

The standard operation for GB removal through a right subcostal or midline incision is open cholecystectomy. When performed electively during a period free of complications, the procedure is relatively safe, with a mortality rate of 0.1 to 0.5%. However, since its introduction in 1988, laparoscopic cholecystectomy has been the treatment of choice for symptomatic GS. This technique was popularized largely because of a shorter convalescence, decreased postoperative discomfort, and improved cosmetic results. The procedure entails the insertion of specialized surgical instruments and a video camera into the peritoneal cavity through multiple small incisions in the abdominal wall. After insufflation of the peritoneal cavity, the GB is removed under video monitoring. Laparoscopic cholecystectomy is converted to an open procedure in approximately 5% of cases, usually because of an inability to identify the anatomy of the GB or to manage a complication.

For patients declining surgical treatment or for whom surgical treatment is inappropriate, GB calculi may sometimes be dissolved in vivo by giving BAs orally for many months. Stones must not be calcified, and demonstration of normal GB function on oral cholecystography is essential. Ursodeoxycholic acid 10 mg/kg/day reduces biliary secretion of cholesterol and decreases the cholesterol saturation of bile, resulting in gradual dissolution of cholesterol-containing stones in 30 to 40% of patients. Recurrence of stones is common after cessation of the drug. Alternative methods of stone dissolution (methyl-*tert*-butyl ether) or stone fragmentation (extracorporeal shock wave lithotripsy) are now largely unavailable owing to greater patient acceptance of laparoscopic cholecystectomy.'

2.3 Non Diet-Related Factors associated with GS

Cholesterol GSs are found in 5-30% of the population of Central and Northern Europe, the USA, Canada, Australia and New-Zealand and in 70-90% of people suffering from GSs[1]. Geographical and ethnic factors, together with gender and age differences, seem to be major risk factors in the etiology of cholelithiasis[2].

2.3.1 Geographic and ethnic influences

Genetic and ethnic influences can be observed, especially in North America, where 70% of Pima Indians in Arizona suffer from GS[3]. Regarding both sexes, significantly more GB disease was found in the Indians than in the population of Framingham, Massachusetts. No association was apparent between either body weight or serum cholesterol and GB disease in the Pima group[2]. This is different from findings in other populations, discussed later. Though genetics have been suggested as the cause in this case, no genetic marker could be identified so far.

Comparison of the number of GB operations carried out in 1961 and 1971 in three similar towns in Canada, England, and France suggests that the incidence of GB disease was six times higher in North America than in Western Europe and nine times higher for patients below the age of 35. The number of cholecystectomies had doubled in all three countries over ten years[4]. The operation rate, however, does not reliably reflect prevalence rates.

[1] Sama *et al*, 1990
[2] Bennion *et al*, 1978
[3] Comess *et al*, 1967
[4] Plant *et al*, 1973

Chileans also have a higher risk of GS disease[1]. Further in Chilean Indians and Hispanics cholesterol lithogenic genes are widely spread and this might explain the high prevalence of GB diseases among some South American populations[2].

A study on Mexican Americans[3] was based on the San Antonio Heart Study, a population-based survey whose phase one was performed between 1979 and 1982, which assessed cardiovascular risk factors and health status in a bi-ethnic sample of Mexican Americans and non-Hispanic whites, aged 25-64 and non-pregnant women. The participants were randomly selected from three neighborhoods. The participants were questioned about any history of GB disease and interviewed to determine their dietary intake using the 24-hour recall method. This was followed by a medical examination in a mobile clinic, including skinfold measurements. Even after adjusting for differences in dietary intake between the ethnic groups Mexican American women had a higher risk of GS disease.

[1] Covarrubias *et al*, 1984
[2] Miquel *et al*, 1998
[3] Haffner *et al*, 1989

2.3.2 Demographic differences (age and gender)

The prevalence of GSs is approximately two times higher in women than in man. This is true for ages between puberty and menopause, after which the difference in prevalence between females and males narrows.

In a study conducted on Caucasian women from a rural Canadian community, the authors documented a relatively high prevalence of GSs (11.3%) in young Caucasian women (20-50 years of age), with a peak prevalence in the fourth decade of life (33%)[1]. In this population the risk factors identified were obesity, a narrow range of daily energy intake, a low daily calcium intake and a low level of activity.

The GS prevalence was studied in 11, 840 consecutive autopsies from 1940-1975 in the University hospitals of Essen, Germany. The total prevalence was 20.7%, 13.1% for men and 33.7% women. The crude prevalence for three 12-year periods showed a significant increase from 8.2% to 15% in men, and from 25.7% to 36.3% in women. A detailed analysis showed that this increase occurred only in the age groups over 60 and was the consequence of the fact that a greater proportion of women over 60 came to autopsy. When age and gender-specific morbidity ratio was calculated to standardize the data, the authors found considerable fluctuations in 3-year periods since 1940, and therefore concluded that there was no real increase in the prevalence of GSs in the past 30 years[2].

[1] Williams *et al*, 1980
[2] Balzer *et al*, 1986

The prevalence of GS disease (cholelithiasis and previous cholecystectomies) in the population of the town of Sirmione, Italy, was examined by US examinations, was 6.7% in men, and 14.6% in women, ranging from 18 to 65 years af age (overall prevalence = 11%)[1]. The prevalence of cholelithiasis in the same age span was 6.9% (4.5% in men and 8.9% in women). Prevalence of cholelithiasis increased with age in both sexes. Prevalence of GS disease was found to be higher in obese and hypertriglyceridemic subjects and to increase with the number of pregnancies[1].

The prevalence of GS disease in a large Romanian town was determined on 6275 necropsies performed during a 10-year period (1973-1982). The crude prevalence of GS disease in women was 17.1% and 6.9% in men. Age-standardized prevalence was 8.4% in women and 5% in men. This rate is lower than the prevalence of GSs in Northern or Central European countries, but it is higher than that established in some Southern countries of Europe. A comparison of the crude prevalence of GS disease was compared with that calculated for a similar 10-year period 100 years ago (1873-1882), on 1538 necropsies performed in the same town. Prevalence of GSs rose significantly in a century from a mean of 1.2% to 11.3%, a finding consistent with the concept that GS disease is a "disease of civilization"[2].

Overall prevalence in a population of male civil servants in Rome was 8.2% and increased with age from 2.3% in the 20- to 25-year-old age group to 14.4% in the 60– to 69-year-old age group, based on both presence of GSs and history of cholecystectomy[3].

[1] Barbara *et al*, 1987
[2] Acalovschi *et al*, 1987
[3] GREPCO 1988

One hundred elderly black women in Soweto, aged 55 – 85, who had no clinical evidence of gastrointestinal (GI) disease underwent US. 10 patients were positive for GS (10%). The body mass index (BMI) was significantly higher in the GS carriers, although no differences were found in nutritional habits (using the 24-hour recall method)[1].

The prevalence of GS disease in Maastricht was studied by US screening of a hospital population of 424 men and 631 women, admitted for elective surgery unrelated to GS disease. The prevalence increased with age. In the oldest age category (70-79 years of age) 16% of the men and 40% of the women had GS disease. These prevalence data are on the lower side within the range of prevalences found in other West European countries[2].

The prevalence of GS disease in a stratified random sample of 1896 British adults was established using real time US (performed for the purpose of the study just after having been enrolled). The prevalence rose with age, except in women of 40-49 years, so that at 60-69 years, 22.4% of women and 11.5% of men had GSs or had undergone cholecystectomy[3].

A total of 29 739 Italians were screened in the MICOL study. 6.5% of the males, and 10.5% of the women had GS. Prevalence of GS disease (GSs and cholecystectomy) increased linearly with age in both sexes[4].

[1] Walker *et al*, 1989
[2] Thijs *et al*, 1990
[3] Heaton *et al*, 1991
[4] Attili *et al*, 1995

2.3.3 Parity and hormonal intake

The number of births, as well as the intake of estrogens in the form of birth control pills and/or estrogen preparations often taken after menopause, are said to increase the cholesterol saturation in the bile and thereby increase the risk of cholesterol GS formation[1]. Gilat and Konikoff[2] believe that the controversies regarding the true relationship of pregnancies and GS disease, in the past, were mainly due to disregarding of potential confounders. They argue that comparisons should have only been made within a defined population having similar genetic and environmental backgrounds, with the number of pregnancies being regarded as the major variable. In 2000 they analyzed some more recent publications that had much more uniform results. These included The Rome Group for the Epidemiology and Prevention of Cholelithiasis (GREPCO) study[3], which investigated employees of government ministries in Rome, the Sirmione study[4], which evaluated inhabitants of a village in northern Italy, and a study performed in Denmark[5], investigating a random sample in Copenhagen county. All three studies described a two to three times higher crude prevalence of GSs in women with two to three or more pregnancies compared with nulliparous women. Other studies have also provided some evidence that multiple pregnancies are actually a risk factor for the development of GS, nevertheless their adjustments regarding potential confounding factors were not uniform[4 6 7 8 9].

[1] Scragg *et al*, 1984
[2] Gilat *et al*, 2000
[3] GREPCO, 1984
[4] Barbara *et al*, 1987
[5] Jørgensen T, 1988
[6] Friedman *et al*, 1966
[7] Comess *et al*, 1967
[8] Attili *et al*, 1997
[9] Singh *et al*, 2001

In Benha City[1], neither age of menarche, duration of menstrual life, age at first pregnancy, multiparity nor duration of contraceptive pill use showed any differences between the female cases and controls studied. Jørgensen[2] described significant associations with young age at menarche, abortions and multiple childbirth.

Pixley and colleagues[3] could neither prove an association between parity nor exogenous hormones with GS disease in vegetarian women. Wheeler's group[4] could observe a higher risk with pregnancy and GS disease. They suggested that pregnancy could have had an influence on dietary intake, and thereby on the subcutaneous fat, but this will remain unknown as the participants were not asked about change in dietary habits during pregnancy.

In a CCS in Australia[5] it was observed that contraceptive use increased the risk of GS development in young women, whereas decreased it in the older females. It was also observed that risk increased with increasing parity especially among the young women. The risk fell with progressing age at first pregnancy, independent of parity. The authors suggested that there were subpopulations of women susceptible to early formation of GSs after exposure to either oral contraceptives or pregnancy. An Italian study in Castellana[6] observed an increased risk with pregnancy.

[1] Abdel-Rahman *et al*, 1993
[2] Jørgensen T, 1988
[3] Pixley *et al*, 1985
[4] Wheeler *et al*, 1970
[5] Scragg *et al*, 1984
[6] Misciagna *et al*, 1996

2.3.4 Metabolic diseases

People suffering from DM were shown to have a higher risk for cholelithiasis, though the mechanism remains unknown. Although there was no association between DM and GB disease in Pima females, an association between these diseases was actually present in older Pima males[1]. Between 1986 and 1990 screening US of the GB was performed in 2,756 male self-defense officials in Japan who received a retirement health examination[2]. Risk factors were investigated in 61 men with GSs and 38 with previous GB removal (overall prevalence of 3.6%). DM could not be associated with GS disease in this setting.

101 newly diagnosed GS carriers identified from a small town in Italy were matched to 303 controls[3]. It was found that even in subjects with no clinical diagnosis of DM, insulin was associated with GSs.

2.3.5 Gastrointestinal (GI) diseases

Several GI diseases seem to predispose cholelithiasis. This was shown for diseases of colon and ileum[4 5], as well as subjects suffering from cystic fibrosis[6 7 8 9] and people suffering from bile malabsorption[7]. In gastrectomized patients the GB volume was significantly increased[10].

Heaton[11] suggested in a review article that the role of colonic transit may play an important role in the etiology of GS formation.

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[1] Comess *et al*, 1967
[2] Kono *et al*, 1992
[3] Misciagna *et al*, 2000
[4] Nightingale *et al*, 1993
[5] Andersson *et al*, 1987
[6] Jebbink *et al*, 1992
[7] Henschke *et al*, 1983
[8] Bennion *et al*, 1987
[9] L'heureux *et al*, 1977
[10] Hahm *et al*, 2000
[11] Heaton KW, 2000

He based his suggestion on a number of studies which proved high prevalence in people with high usage of laxatives (indication for constipation) and in people with high biliary deoxycholic acid (DCA) (colonic BA)[1], and on studies which proved slow transit in non-obese GS carriers[2]. Enrichment of bile with DCA leads to enrichment of the bile with cholesterol. Biliary DCA can be raised and lowered by slowing down and speeding up colonic transit, respectively.

Dowling and his group[3] showed in patients receiving octreotide that slow large bowel transit increases the production (and absorption) of DCA and are lithogenic. Furthermore in acromegalic patients, octreotide increased the proportions of arachidonic acid-rich phospholipids, with associated rises in (a) the cholesterol saturation index and percentage of vesicular cholesterol, and (b) the percentage of DCA in GB bile[4].

2.3.6 Physical activity

As part of the *Nurses Health Study*, a sub-study investigated recreational physical activity and sedentary behavior in relation to the risk cholecystectomy in females[5]. Out of 60,290 women who were 40-65 years of age in 1986, 3257 cases of cholecystectomy were identified. Analysis of the questionnaires on physical activity that was mailed every 2 years showed that recreational physical activity (such as jogging, running, cycling) was associated with a decreased risk of cholecystectomy (Relative Risk [RR]: 0.69).

[1] Petroni ML, 1996
[2] Spathis *et al*, 1997
[3] Veysey *et al*, 1999
[4] Pereira *et al*, 2001
[5] Leitzmann *et al*, 1999

Women who spent between 41-60 hours sitting per week as compared to those who sat less than 6 hours per week while at work or driving, showed an increased risk of 1.42. The risk-association was strengthened in women who sat more than 60 hours per week (RR: 2.32). The association persisted when controlled for body weight or weight changes.

With regard to men, 828 male symptomatic GS carriers were identified after a 8 year prospective, follow-up study of 45,813 health professionals, aged 40-75 in 1986[1]. Physical activity was assessed by analyzing five questionnaires that were mailed every two years. Increased physical activity was inversely related to symptomatic GS disease, where the association was stronger in those younger than 65, than in the older group (RR: 0.58 and 0.75, respectively). In contrast, sedentary behavior (more than 40 hours watching TV per week) was associated with an increased risk. The RR in the older group was 3.32, in the younger ones the association was somewhat less prominent with an increased risk of 1.58.

2.3.7 Smoking habits

A matched CCS performed on 96 cases and 118 age- and gender matched controls[2] actually found smoking as well as jobs demanding hard labor to be protective. In Australia[3] it was observed that current-smokers versus never-smokers had an increased risk of 1.3 in females and 1.6 in males. It was further shown that current-smoker females, less than 35 of age, had an increased risk of 3.5, and women who had been smoking for 1-8 years increased their risk by 2.8.

[1] Leitzmann *et al*, 1998
[2] Linos *et al*, 1989
[3] McMichael *et al*, 1992

It was concluded that smoking-related risk was greatest soon after first exposure, which might be an indication that exposure factors could be different between early-occurring and late-occurring cases. In Italy[1] smoking was found to be associated with risk of GSs.

2.3.8 Serum lipids

Barbara and colleagues[2] found that hypertriglyceridemic but not hypercholesteremic patients were at higher risk. Nevertheless, a Japanese study[3] could not confirm any relation, neither positive nor negative, between serum TGs and total serum cholesterol and GS disease. Tandon[4] demonstrated in a study in Northern India that female and male GS carriers had significantly higher TG values, whereas only the male cases had significantly higher serum cholesterol levels than their controls. In an Italian study[5] it was observed that young women with GSs were slightly hypercholesteremic as compared to non-carriers.

[1] Misciagna *et al*, 1996
[2] Barbara *et al*, 1987
[3] Kono *et al*, 1988
[4] Tandon *et al*, 1996
[5] Cavallini *et al*, 1987

2.4. Diet-Related Risk Factors associated with GS

The following section is partly based on the article published by Trautwein[1], in which the author reviewed a substantial number of studies that examined dietary factors in the development of GS disease. It has been updated and augmented by additional, old and new studies.

Epidemiological studies which have examined the relationship between lifestyle and/or dietary habits and cholesterol GS, including CCSs which compared dietary habits of patients suffering from GSs with those of healthy "stone-free" individuals, as well as examinations aimed particularly at the impact of nutrients on cholesterol saturation of the bile, have provided valuable information about that relationship. The most important putative dietary risk factors are increased caloric intake, as well as elevated intake of cholesterol and fat (especially of animal origin). Simple carbohydrates and saccharine also seem to play a part as well as obesity and dieting. However, vegetable protein, dietary fiber and alcohol seem to have an inverse relationship to the development of GSs.

The importance of CCSs lies in the fact, that dietary habits of study subjects can be compared to those of disease-free individuals, which provide a picture on the dietary impact on the disease being studied. Many or most of the published CCSs dealing with the impact of dietary factors on cholelithiasis are subject to methodological deficiencies.

[1] Trautwein EA, 1994

They contained either a small number of study subjects, or GS status was not ascertained properly, or an unsuitable control group (hospital-based controls suffering from other diseases) was selected. Often the cases examined were symptomatic. In this context it is also important to remember that patients often change their eating habits after their knowledge of their GSs. Therefore when conducting CCSs some patients might have already changed their dietary habits prior to being questioned about their usual intakes, and will find it hard to reconstruct their diet prior to knowledge of the disease. Additionally many of the studies published had questionnaires containing only a narrow variety of foods. Others used 24-hour or 48-hour diet recall methods, which do not provide a true picture of usual dietary intake habits.

There have been a great number of intervention studies in humans and also in animals, in which the effect of specific dietary components on the bile; its lipids, as well as on cholesterol saturation was evaluated. In particular, the influence of total energy intake, the amount of fat intake as well the FA composition (especially of polyunsaturated fatty acids [PUFAs]), the cholesterol intake and the dietary fiber intake were examined. In those kinds of examinations care needs to be taken regarding the interactions among the nutrients, which make it difficult to differentiate which single nutrient exerted the effect. For example, the intake of one nutrient often goes together with the intake or reduction of another one:

- ✓ a high intake of simple sugars comes often with a decreased intake of complex carbohydrates and dietary fiber
- ✓ the intake of animal fat goes along with intake of animal protein
- ✓ the consumption of animal fat also means consumption of cholesterol etc.

2.4.1 Increased energy intake and obesity

Sarles and his colleagues were a group of scientists who pioneered the research on the etiology of GS disease. Their first matched case- control study[1] included 101 female patients, aged 20-55, with GSs, and 101 age-matched controls, of the same race, with a similar profession (or husband's occupation). Presence of GSs was proven by operation and/ or radiology. Of the 101 patients, 56 were operated on and they were ascertained to have cholesterol GSs. Controls did not undergo cholangiography, and therefore some of them might have had 'silent' stones. The questionnaire consisted of 141 questions on the way of life. Questions on dietary habits for one week before the onset of the disease, or, if silent, before the diagnosis were asked during the first interview. A second interview took place either after the first attack, the diagnosis of disease, or the surgical operation, and all modifications of the usual dietary habits were noted. Taking their previous two studies (1959 and 1965) into consideration, the authors finally concluded, that, as in each study the methodology slightly differed, different dietitians interviewed the participants, and the studies took place at different points in time, an increased caloric intake irrespective of the composition of diet and the weight of the patients, was a cause of GS formation. In two later studies[2] the progressive decrease of differences found in the three previous studies of Sarles *et al* was confirmed. They therefore concluded that the over-consumption of total energy, mainly foods of animal origin, in France in the aftermath of World War (WW) II was a risk factor, whereas starvation during WW II was protective against cholesterol GS disease.

[1] Sarles *et al*, 1969
[2] Sarles *et al*, 1978

In the *Nurses' Health Study*[1] a prospective cohort study 88,837 US nurses, aged 34-59 years, completed a semiquantitative (61 items) food frequency questionnaire (FFQ) in 1980 and were without prior diagnosis of cholelithiasis or prior symptoms later attributed to cholecystitis. Women were asked to report diagnoses of GSs or cholecystectomy on follow-up questionnaires sent in 1982 and 1984. Many articles have been published from the results of this study, with regard to a large number of different diseases and factors.

The authors referred to those subjects included as symptom-associated GS carriers, though recognizing that they might have included a presumably large number of asymptomatic, 'silent' GS carriers having GI symptoms due to other causes. Their results have to be taken with caution due to potential bias in their study design. Potential bias due to only having examined nurses is very probable, as the nurses are not a representative sample of the population at large. They are highly motivated women, with health education and awareness of a healthy life style. Secondly, the study included women only. Most importantly the study excluded the great majority of asymptomatic GS carriers. Nevertheless the authors argued that in their opinion, there could not be bias of food intake as the data was collected before onset of disease. It was observed that high energy intake in itself appeared to be a significant risk factor for symptomatic GS disease. This association was also observed in Northern India[2] and in Mexican Americans[3].

[1] Maclure *et al*, 1989 & 1990
[2] Tandon *et al*, 1996
[3] Tseng *et al*, 2000

A great number of studies have managed to provide us with data that strengthen the hypothesis that obesity increases the risk of GS development [1] [2] [3] [4] [5] [6] [7]. The relation between obesity and GSs was shown to be more noticeable in women, than in men[6] [8], and specifically in women under the age of fifty[8]. Haffner and his colleagues[9] went even further and examined the distribution of fat in the body. They found that the risk was higher in women who were classified as having a central fat distribution, as opposed to overall adiposity. Obese people were shown to actually suffer three-to-four times more often from cholesterol GS disease than normal weight individuals.

Nevertheless, there are studies that could not find an association between GB disease and increased weight[10].

As weight is very relative, it is more accurate to take into consideration the height and age of the subjects. Therefore the studies which have proven a direct correlation between BMI and GS disease, might be a little more conclusive regarding the influence of obesity[5] [11] [12] [13] [14]. Since obesity itself is an important risk factor for GS formation, it is rather difficult to differentiate between the influences of obesity and those of hypercaloric dietary habits. Nevertheless there were studies that found a correlation with obesity, but no difference in nutrient intake between cases and 'stone-free' subjects[10].

[1] Williams *et al*, 1980
[2] Barbara *et al*, 1987
[3] GREPCO 1988
[4] Maclure *et al*, 1989
[5] Maclure *et al*, 1990
[6] Sichieri *et al*, 1991
[7] Stampfer MJ, 1992
[8] Scragg *et al*, 1984
[9] Haffner *et al*, 1989
[10] Comess *et al*, 1967
[11] Walker *et al*, 1989
[12] Kono *et al*, 1992
[13] Kodama *et al*, 1999
[14] Misciagna *et al*, 1999

2.4.2 Dieting

Dieting however, which in general is the healthiest cure to obesity, does not necessarily protect from the development of GSs. In studies that examined obese people who tried to lose weight, a marked increased incidence in cholesterol GS disease could be observed[1] [2] [3].

A weight reduction with an average loss of 16.5 kg led to GS formation in 25% of the obese participants (women and men) who ate an energy-reduced diet (500 kcal per day) for a period of eight weeks[1]. In most of the cases the asymptomatic GSs disappeared and the cholesterol saturation of the bile normalized after reaching 'normal weight'. Nevertheless in 20% of the cases GS disease was symptomatic[1]. Another study of this kind showed that not only the period of dieting but also the degree of obesity and loss of weight as well as the plasma TG concentration, increase the risk of GS development[3]. On the other hand, a Dutch CCS found a stronger co-relation between BMI and the risk of GSs rather than between dieting and GSs[4]. In that study the influence of dieting on GS disease was strongly dependent on the pre-dieting BMI, where the influence decreased with increased obesity. An increased GS risk was observed in thin people with a BMI between 14 and 21, whereas with obese people there was no increase in risk. Therefore the authors of this study concluded that diets for the obese do not increase the risk of GSs in the long-term.

In the 'Nurses Health Study' [5] it was also observed that dieting had more of a moderate effect on the risk of symptomatic GSs. The influence of adipositas as a risk factor was markedly higher than the influence of dieting.

[1] Liddle *et al*, 1989
[2] Sichieri *et al*, 1991
[3] Yang *et al*, 1992
[4] Thijs *et al*, 1992
[5] Stampfer *et al*, 1992

2.4.3 Influence of fats (refer to table 2.1)

The direct influence of fat intake on bile lipids and GS incidence in humans is not entirely clear. Studies concerned with the question of how dietary fat has an influence on the development of GSs, examined on the one hand the effect of the amount of total dietary fat intake and on the other hand the influence of specific FAs, especially the proportion of PUFAs. In almost all of the studies, which examined the influence of total fat on bile lipids, fat was substituted with carbohydrates. Since the proportion of fat and carbohydrates have been changed at the same time, it is difficult to decide which of the two nutrients has brought about the change in the bile lipid composition. In this context it is rather interesting that such a diet leads to elevated serum TG concentrations and lowers HDL-cholesterol concentrations, which have also been mentioned as risk factors for the development of cholesterol GSs.

Studies which examined the influence of fat quality, especially the effect of PUFAs on the bile lipids showed inconsistent results. In a dietary study for the prevention of HD conducted in Los Angeles, it was observed that men ingesting a diet high in PUFAs, had double the GS prevalence than men who continued with their typical American diet. In other studies it was observed that in men, but not in women, a high PUFA intake elevated the secretion of cholesterol in the bile. In epidemiological studies no influence of dietary fat or FA composition on bile lipids was observed. In the 'Nurses Health Study' it was observed though, that women ingesting a diet high in PUFAs had a decreased risk of developing symptomatic cholelithiasis.

Summarizing the effect of PUFAs, there seems to be a gender difference in terms of the influence, with a higher risk for men than women.

Table 2.1: Influence of fat & cholesterol intake on bile lipids &/or risk of GS disease

TYPE OF FAT	EFFECT	LITERATURE
Total fat	Risk elevated with increased fat intake Risk elevated with increased fat intake (males) No effect found	[1] [2] [3] [4] [5]
Animal fat	Risk elevated with increased animal fat intake	[6]
Vegetable fat	Protective effect of vegetable fat	[7] [8]
Saturated fat	Risk elevated with increased fat intake	[9]
PUFA	Risk elevated with high intake of PUFA (especially in men) Risk decreased in women with high intake of PUFA No effect of PUFA (especially in women)	[4] [10] [11] [7] [12] [13] [14]
MUFA	Protective effect (also from olive oil)	[6, 9]
Dietary cholesterol	Increase of biliary cholesterol saturation Protective effect through dietary cholesterol No effect through dietary cholesterol	[15] [16] [9] [8] [10] [17] [18]

Adapted from Trautwein, 1994 and modified

[1] Diehl *et al*, 1987
[2] Tandon *et al*, 1996
[3] Miettinen *et al*, 1976
[4] Bennion *et al*, 1978
[5] Sichieri *et al*, 1991
[6] Linos *et al*, 1989
[7] Maclure *et al*, 1990
[8] Pixley *et al*, 1985
[9] Misciagna *et al*, 1999
[10] Diehl *et al*, 1989
[11] Sturdevant *et al*, 1973
[12] Dam *et al*, 1966
[13] Kohlmeier *et al*, 1985
[14] Kohlmeier *et al*, 1988
[15] Den Besten *et al*, 1973
[16] Lee *et al*, 1985
[17] Comess *et al*, 1967
[18] Scragg *et al*, 1984

2.4.4 Dietary cholesterol intake

At present the role of dietary cholesterol in the etiology of GSs is not entirely clear. The results of studies done so far are inconsistent. Examinations in humans, who had consumed great amounts of cholesterol, did not show a correlation between dietary cholesterol and cholesterol saturation in the bile[1] [2]. Contrary to this two other studies found an elevated cholesterol saturation associated with an increased consumption of dietary cholesterol[3] [4].

CCSs and studies based on population differences do not give a clear indication for a correlation between cholesterol GS and the intake of dietary cholesterol[5] [6]. In the 'Adelaide study' the authors even found in young women a negative correlation between cholesterol intake and development of GS[6].

A large cross-sectional study including 1081 female civil servants in Rome[7] aimed to investigate relations between type of dietary fat habitually consumed and risk of GS development by comparing the erythrocyte FA composition of women with and without GSs. The GB status was determined by US and/or oral cholecystography. Determination of red blood cell FA pattern has been demonstrated to be an objective index of FA composition of habitual diet over a long period. The results did not demonstrate significant differences between the study groups. The authors suggested that dietary fats might not play a major role in the etiology of cholelithiasis.

[1] Andersen *et al*, 1979
[2] Dam *et al*, 1971
[3] Den Besten *et al*, 1973
[4] Lee *et al*, 1985
[5] Diehl *et al*, 1989
[6] Scragg *et al*, 1984
[7] Arca *et al*, 1987

Misciagna and colleagues[1] conducted a CCS on 100 newly diagnosed GS carriers to whom they matched 290 population-based controls. The presence of GSs was ascertained by US examination. The usual diet and physical activity was recorded by self-completion of a questionnaire. They observed a positive association between GSs and dietary cholesterol intake.

2.4.5 Protein intake and quality

Concerning protein intake as well as protein quality, there is not much known about an association with GSs. The MICOL study showed a slight positive association with total protein intake only in their male subjects[2]. Nevertheless it was observed that vegetarians have only half the incidence of GSs than non-vegetarians[3]. The *Nurses Health Study*[4] also concluded that protein of plant origin might have a protective effect on the risk of symptomatic GSs. Here care must be taken, as the authors could not clearly differentiate between an effect of vegetable protein and an effect of vegetable fat or a higher consumption of dietary fiber.

A study on Chilean men[5] showed that intake of legumes elevated the cholesterol saturation significantly in the bile, but the exact mechanism is not understood.

[1] Misciagna *et al*, 1999
[2] Attili *et al*, 1998
[3] Pixley *et al*, 1985
[4] Maclure *et al*, 1990
[5] Nervi *et al*, 1989

2.4.6 Carbohydrate intake

Epidemiological examinations suggest that an increased consumption of simple carbohydrates increase the risk of GSs[1] [2] [3]. In the *Adelaide Study*[3] it was found that the consumption of foods containing sugars, such as soft drinks and sweets was higher in GS patients than in the control groups. A study in Northern India[4] found that intake of refined carbohydrates was significantly higher among the female cases as compared to their controls, whereas a significantly higher total carbohydrate intake in cases compared to their disease-free controls was true for both sexes. The *Nurses Health Study*[5] and a study in vegetarians[6] on the other hand, could not find any relation between total carbohydrate intake and GSs. The MICOL study found a slight positive association with total carbohydrates in males and females[7].

[1] Misciagna *et al*, 1999
[2] Diehl *et al,* 1989
[3] Scragg *et al*, 1984
[4] Tandon *et al*, 1996
[5] Maclure *et al,* 1990
[6] Pixley *et al*, 1988
[7] Attili *et al,* 1998

2.4.7 Dietary fiber

In a CCS in Adelaide[1], 267 hospitalized patients with cholelithiasis, proven by US or cholecystography were matched to 241 controls from the community (no US examination) and 359 hospital controls who had also undergone US. Subjects were below 70 years of age. They underwent a first interview for medical history and demographics (double-blind), then filled out a self-administered quantitative FFQ containing 105 items, which was followed by a double-blind check-up interview. They found an inverse association between GS and dietary fiber intake that presumably would have been stronger had they adjusted for energy intake. Attili and his colleagues[2] found a negative association with the intake of dietary fiber in the female subjects. In another Italian CCS[3] an inverse association was observed between dietary fiber from cellulose and the presence of GSs. In yet another study, no effect was observed[4].

2.4.8 Drinks (incl. alcohol)

Alcohol, which is known to elevate the concentration of HDL-cholesterol, has most probably an effect on cholesterol concentration in the bile[5]. In epidemiological studies it was observed that the risk of GSs decreases with the consumption of alcohol[1] [6]. In the *Nurses Health Study* the consumption 5g and more alcohol per day was related to a 40 % decrease in GS risk in obese women[7].

[1] Scragg *et al*, 1984
[2] Attili *et al*, 1998
[3] Misciagna *et al*, 1999
[4] Nervi *et al*, 1989
[5] Thornton *et al*, 1983
[6] Kono *et al*, 1992
[7] Maclure *et al*, 1990

As part of a health professional follow-up in the USA, 46 008 men, aged 40-75 in 1986 and with no history of GSs, self reported food and drink intake by completing a FFQ for follow-up until 1996[1]. The authors went further than just assessing alcohol consumption in relation to symptomatic GS disease, it also aimed to assess alcohol consumption patterns and specific types of alcohol. Consumption patterns that reflected any amount of intake 5-7 days per week was associated with a decreased risk of symptomatic GSs as compared to non-drinkers. In contrast, infrequent consumption (up to 2 days per week) showed no association with risk. All types of alcohol were inversely related to the risk of symptomatic GS disease.

In the same prospective study, the caffeine intake was assessed and the results published elsewhere[2]. After controlling for known or suspected risk factors, the RR for men in the highest category of caffeine intake (>800 mg/d) compared with men in the lowest category (≤25 mg/d) was 0.55. In contrast decaffeinated coffee was not associated with a decreased risk of symptomatic GSs.

Misciagna *et al*[3] found that wine and coffee were protective in GS disease.

[1] Leitzmann *et al*, 1999
[2] Leitzmann *et al*, 1999
[3] Misciagna *et al*, 1996

2.4.9 Vegetarianism

In a CCS, 109 non-vegetarian GS carriers and only 12 vegetarian GS carriers were matched to GS-free subjects according to age[1]. Most cases were recently identified, though 31 were post-cholecystectomy. Participants had to complete a four-day fixed format dietary diary. Within a month of completing the diary the subjects were interviewed by one of the two specially trained interviewers to clarify any discrepancies in the diary and to collect further clinical information, such as weight and height. An appreciably reduced frequency of GSs was found in vegetarians as compared with non-vegetarian women (11.5% and 24.6%, respectively), eating a more typical Western diet, though the nutrient intake did not differ between cases and controls. When considering nutrient intakes for non-vegetarians and vegetarians, however striking differences emerged. Non-vegetarians had a significantly higher consumption of total calories, total protein, total fat, cholesterol, and alcohol. On the other hand they had a significantly lower intake of non-animal (vegetable) protein, total carbohydrates, refined sugars, dietary fiber. Cases were significantly more obese as measured by the BMI. Considering the response rate of 69%, non-responder bias could be considered. Also an appreciable under-estimation of intake by the obese might be possible.

[1] Pixley *et al*, 1988

3. METHODS

3.1 Case Control Studies (CCSs)

Cohort and case-control methodologies are the main tools for analytical epidemiological research. Other important types of epidemiological studies mainly for generating hypotheses include cross-sectional and ecological, or correlation studies. The conclusions that can be drawn from findings of these types of studies are, however, much weaker compared to those of cohort and CCSs. This is not to say that findings from cohort and CCSs always reflect true associations which can be universally generalized. Epidemiological research is, to a large extent, of an observational character as opposed to experimental research.

The study discussed in this thesis is known as a CCS. The cases' history of exposure or other characteristics, or both, prior to onset of the disease, is recorded by interview and sometimes referring to records and other sources. A comparison group consisting of individuals without the disease under study but similar in other pre-specified criteria (controls) are selected, and their past history is recorded in the same fashion as for the cases. The purpose of the control group is to provide an estimate of the frequency and amount of exposure in subjects in the population without the disease being studied. This means that whereas cohort studies are concerned with frequency of disease in exposed and non-exposed individuals, CCSs are concerned with the frequency and amount of exposure in subjects with a specific disease (cases) and people without the disease (controls).

3.1.1 Measure of association

In CCSs, data are not available to calculate the incidence rate of the disease being studied, and the actual RR cannot be determined.

The measure of association between exposure and occurrence of disease in CCSs is the so-called odds ratio (OR): the ratio of odds of exposure in diseased subjects to the odds of exposure in the non-diseased. The following table exemplifies the basic method of calculating the OR in a CCS.

Table 3.1: Basis of calculating ORs in CCSs

Exposure	**Disease**	
	Yes (cases)	No (controls)
Yes	a	b
No	c	d
Odds of exposure	a/c	b/d

The OR is thus given by $\frac{[a/c]}{[b/d]}$ (or ad/bc). The OR is generally a good estimate of the relative risk. The terms OR and RR are in fact interchangeable when used in CCSs.

3.1.2 Confounding and bias

As mentioned earlier, CCSs are observational studies and are potentially subject to the effect of extraneous factors which may distort the findings of these studies. The term confounding (or confounding factor) used in this context refers to an extraneous variable that satisfies both of two conditions: it is a risk factor for the disease being studied, and it is associated with the exposure being studied but is not a consequence of exposure[1].

[1] Schlesselman JJ, 1992

Adjusting for the effects of confounding factors is evidently important in observational epidemiological studies, and can be dealt with in the study design by matching or stratifying sampling of study subjects, or in the data analysis by stratification or multivariate analyses[1] [2] [3].

Another potential complicating factor of not only observational but practically all types of research, is bias. Bias has been defined as any systematic error in the design, conduct, or analysis of a study that results in a mistaken estimate of an exposure's effect on the risk of disease[1]. Sackett[4] has provided an extensive discussion of various types of bias. One type of bias frequently referred to in epidemiological research is "recall bias", namely the propensity of diseased subjects (cases) when interviewed, to scrutinize their memory and report more accurately on past exposure and possible causes of their disease than non-diseased subjects (controls) would do. Such recall bias has been documented[5] [6] [7].

[1] Schlesselman JJ, 1992
[2] Kleinbaum *et al*, 1982
[3] Rothman KJ, 1986
[4] Sackett DL, 1979
[5] Hogue CJ, 1975
[6] Klemetti *et al*, 1967
[7] Lindefors-Harris *et al*, 1991

3.1.3 Advantages and disadvantages of CCSs

When faced with a research question concerning the association between a possible etiologic factor and disease, the epidemiologist has to choose an appropriate strategy to resolve the matter. A number of circumstances have to be considered before a certain type of research is chosen, such as the incidence rate of disease, time elapsing between exposure and clinical manifestation of the disease, whether the exposure is associated with only one or more diseases, the urgency of the research question, ethical issues, and funding available for the research, etc. Below are listed some of the advantages and disadvantages of CCSs.

Table 3.2: Advantages and Disadvantages of CCSs

Advantages	**Disadvantages**
☺ Permit the study of rare diseases	☹ Information on exposure and past history is primarily based on interview and may be subject to recall bias
☺ Permit the study of diseases with long latency between exposure and manifestation	☹ Validation of information on exposure is difficult, or incomplete, or even impossible
☺ Can be launched and conducted over relatively short time periods	☹ By definition, concerned with one disease only
☺ Relatively inexpensive as compared to cohort studies	☹ Generally incomplete control of extraneous variables
☺ Can study multiple potential causes of disease	☹ Choice of appropriate control group may be difficult
	☹ Correct interpretation of results may be difficult

3.1.4 Assessment of causality

One of the more difficult tasks in epidemiological research is to assess whether associations between exposure and disease derived from observational epidemiological studies are of a causal nature or not. It has been underlined above that observational epidemiological studies are subject to the influence of factors over which the investigators most often do not have full control, and that findings from these studies are less reliable than those of studies with an experimental research design. It is therefore imperative that findings from analytical epidemiological studies are critically scrutinized before any judgment of causality is made. Furthermore, findings from one single epidemiological study only exceptionally provide conclusive evidence of a causal relationship between exposure and disease. Discussions and reasoning concerned with which criteria to apply for the assessment of causality have been given by several authors[1] [2] [3].

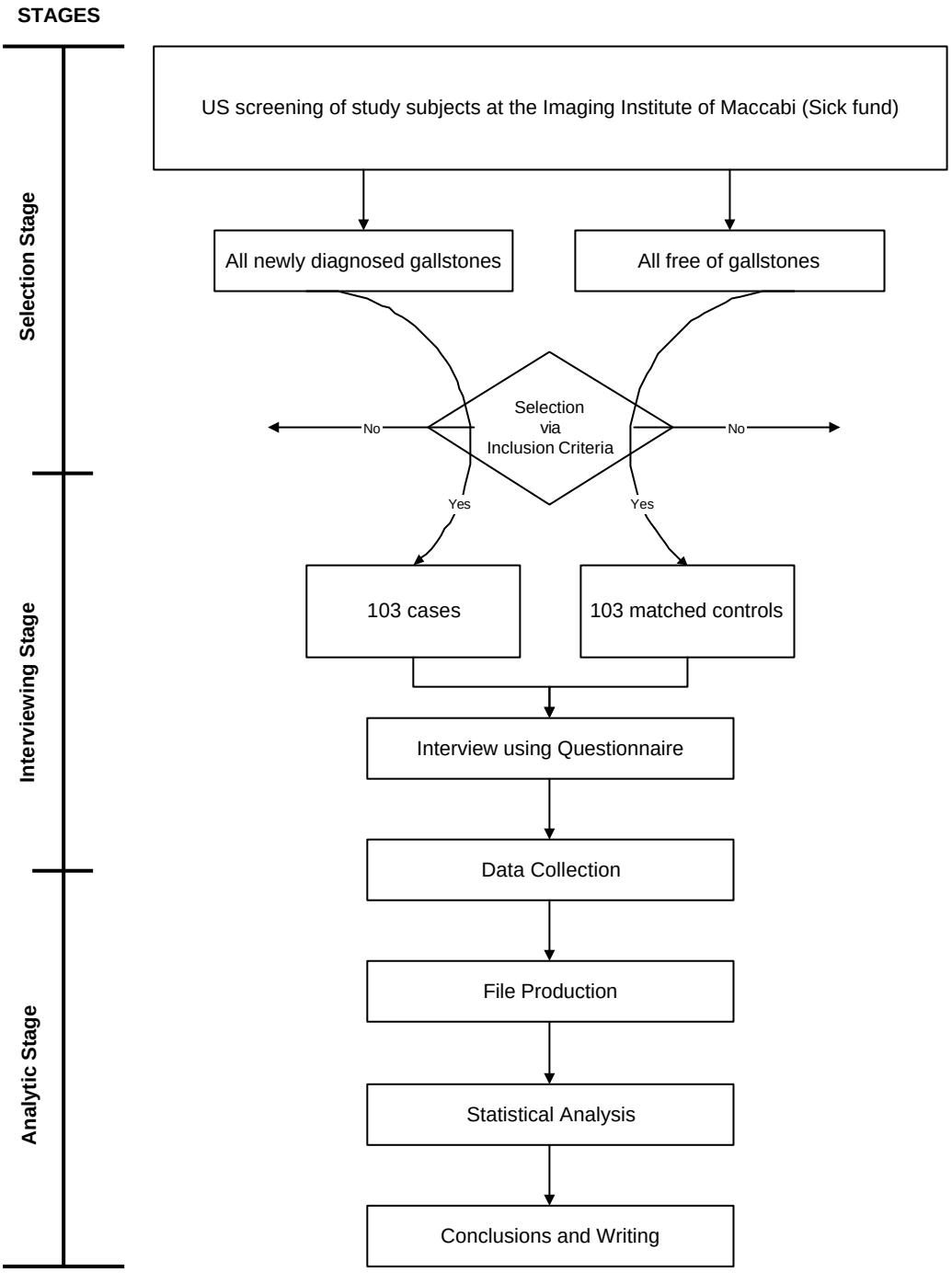
Bradford Hill[2] has listed nine aspects concerned with the association between exposure and disease which need to be considered. The first of these is the *strength of association*. A strongly elevated RR is more likely to reflect a causal association than is a slightly or moderately increased risk. *Consistency* of findings across studies conducted with different methodologies and in different settings, is another aspect. A third characteristic is *specificity,* that the exposure causes a particular disease. An important condition is the *sequence of events*: the potentially causative factor must precede the effect (disease). The *dose-response relationship, or biological gradient*, is another aspect.

[1] Evans AS, 1978
[2] Hill AB, 1965
[3] Lilienfeld *et al*, 1980

Biological plausibility is an aspect which is important, but depends on the biological knowledge of the day. The association should be consistent with what is generally known about the *occurrence of the disease,* its natural history and pathophysiology, and should not conflict with its knowledge. The causal interpretation of an association is furthered if there is *experimental evidence* in support of it, for example if elimination of exposure reduces the incidence of the disease. The ninths aspect is *analogy*. For example, if a virus is shown to be oncogenic in animal studies, we are more prone to accept that the human papilloma virus may be the cause of cervical cancer in humans. In this essay on association and causation, Bradford Hill notes that none of his nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a *sine qua non* (equivalent).

3.2 Work Plan

Figure 3.1: Stages of research protocol



During the **first stage**, subjects were selected from 'Ramat Marpe', an Imaging Institute, which serves a large number of private patients and those of one of the largest sick funds in Israel, after having undergone US examination (after a fast of at least eight hours). 61 cases and 51 controls came to the Imaging Institute for abdominal pain, and 19 cases and 20 controls came for a general checkup. Other reasons for US examination included urinary tract infection (UTI), chronic liver or kidney problems, or general ill-feeling. There were no significant differences in the reasons for undergoing US between the subjects in both groups. All potential cases, as well as controls had to fit clearly specified inclusion and exclusion criteria.

Stage two of the study comprised an interview where all the participants were questioned primarily about their usual food intake and eating habits using a FFQ. This was designed to obtain qualitative, descriptive data on usual intakes of foods or classes of foods over a long time period. The FFQ also contained general questions on other factors which might play a role in the etiology of cholesterol GS disease (cholelithiasis), such as weight and height, physical fitness, concomitant diseases and use of prescribed drugs in the past 15-20 years, education, occupation and workplaces in the last 20 years, family history, and serum cholesterol and TG levels.

The **third stage** consisted of data collection, analysis of existing data using a validated computer software, statistical calculations and drawing of conclusions.

3.3 Study Subjects

The study was conducted in Tel Aviv and its vicinity. 278 subjects were approached, after they had undergone US examination in the imaging institute 'Ramat Marpe'. 31 subjects could not be enrolled as they did not fit one or more of the inclusion or exclusion criteria. 24 of the approached subjects (11 cases and 13 controls) refused to participate, due to various reasons, such as no time, too personal questions, no interest at all. At the end 11 controls remained that did not have a case to which to compare. We hope in the future to be able to approach them again for additional research. The final number of participants was 206 subjects, of which 103 were recently diagnosed, asymptomatic GS patients and 103 were controls, matched to the cases by gender, age and region of birth. Altogether 75 female pairs and 28 male pairs were included into the final analysis.

3.3.1 Inclusion and Exclusion Criteria (determined prior to study start)

I. Cases, i.e. recently diagnosed, asymptomatic GS patients

Inclusion Criteria:

- Existence of GSs proven by US examination
- 18-75 years of age at time of interview
- Females and Males
- Existence of asymptomatic('silent') GSs, i.e. no symptoms whatsoever
- Existence of GSs is known for a period less than three months prior to interview

Exclusion Criteria:

Anybody suffering from the following diseases:

- DM
- Inflammatory Bowel Disease (Crohn's disease and Ulcerative Colitis)
- Renal failure
- HD
- Cancer
- Any other chronic, debilitating disease

II. Controls, i.e. matched to the cases by gender, age and region of birth. The latter was divided into the following groups:

A. North Africa and Western Asia (Iraq, Iran, Syria, Yemen, etc.)

B. Europe and USA

C. Balkans (Bulgaria, Greece, Turkey, etc.)

D. Israel

Inclusion Criteria:

- Absence of GSs proven by US examination
- 18-75 years of age at time of interview
- Females and males

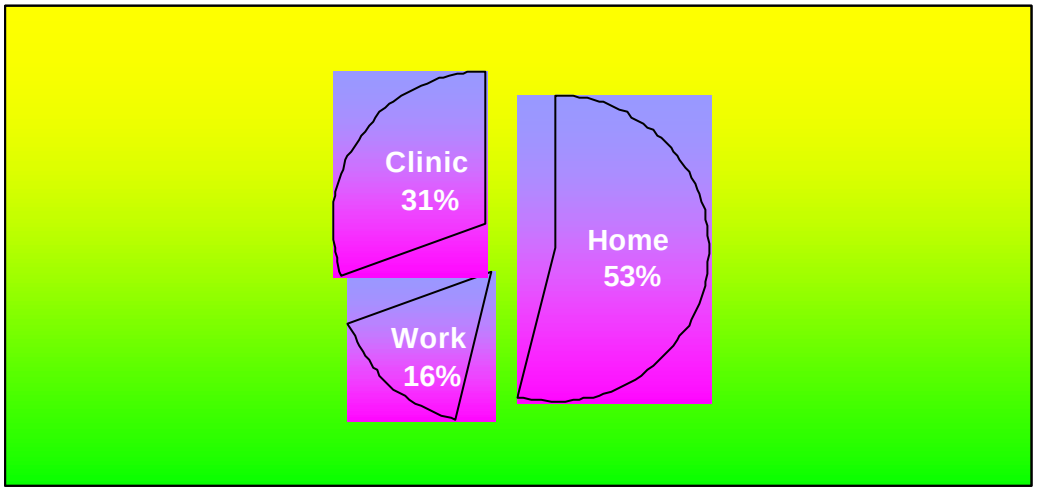
Exclusion Criteria:

Same as for cases

3.4. Questionnaire (see appendix 10.1)

All the participants of the study underwent a standardized interview lasting approximately 1½ hours using a pre-coded questionnaire on diet and lifestyle habits. They were interviewed by the PhD candidate herself (EK), who is a certified nutritionist and has undergone special training to learn the interview technique. The interviews took place primarily at the participants' home (figure 2), its advantage being that the dimensions of certain dishes, utensils and foods could be measured and portion size could therefore be estimated more accurately. The rest were interviewed either at the imaging institute and some at their workplaces. Those interviewed in the clinic were interviewed immediately after their US examination. All other subjects were interviewed within four weeks of their US examination.

Figure 3.2: Places subjects were interviewed



The questionnaire was made up of the following parts:

- Questions on the medical history of the participant, of family members, medication intake, and other therapies.
- Questions on demographics, such as gender, age, country of birth, education, profession, marital status.
- Questions on anthropometric data, such as weight, height, calculation of BMI, and on physical activity, smoking habits.
- Questions for women only, such as hormone intake, age at first pregnancy, age at first menstruation and / or menopause, number of pregnancies, number of births.
- Questions on food intake and dietary habits, which made up the main part of the interview.

The questionnaire on dietary intake:

The questionnaire used was a FFQ. The questionnaire was made up of approximately 175 food items (foods not mentioned on the list, but consumed by the participant, were added), including typical ethnic dishes which are consumed by groups of different origins of the Israeli population, soft drinks and alcoholic beverages. The questionnaire was designed so as to evaluate current and long-term dietary habits.

Concerning the food items, each participant was asked to describe:

A. The frequency of consumption according to daily, weekly or monthly intake.
B. The number of portions consumed on every occasion.
C. The portion size which was measured by standard sizes of packaging, or according to specific sizes which were shown on colored pictures of foods in their actual size.

The interviewee was additionally questioned as to each single item, whether there were changes in the dietary habits prior to the knowledge of the disease (controls: prior to the interview). Furthermore the interviewee was asked to reconstruct as far back as possible, at least ten years, whether there have been any changes in the dietary habits. If there were changes, the previous dietary habits were documented in the same manner as described above. As a result we received information on the eating habits in two different time frames:

- Eating habits of the past
- Current (very recent) eating habits

The gathering of the information on eating habits for both time frames gave me the opportunity to determine whether past as well as current dietary habits may play a role in the etiology of cholesterol GS disease. GSs may take years to develop and the factors in the diet may take many years to produce their effect. This type of questionnaire and its effect on study results was discussed previously[1].

3.4.1 Portion sizes

Natural units, standard units of packaging, and individual portion sizes as determined by life-size color photographs, were used to quantify portion size. In cases where the interview took place at home, participants were asked to show typical dishes, and/or the item itself for accurate recording.

[1] Lubin *et al*, 1998

3.4.2 Frequency of consumption

The adult population is known to change nutritional habits for health and other reasons. Therefore, for each item, the interviewer (EK) asked whether any changes had occurred in consumption habits during the last 10 years. If yes, first the more recent and then the pre-change habits ('past' consumption) were recorded in an identical set of columns including the same questions as for the more recent period (frequency, number of portions and portion size) as well as the number of years since the change occurred. For items in which no change was reported, present habits were equivalent to past habits and used for the analysis. This method allowed evaluation of pre-change habits (past consumption), which represent long-term life habits. Because a longer period of exposure to a nutritional factor is expected to be of greater biological importance, the data in the present study are given for past consumption (≥2 years), providing information more relevant to cholesterol GS etiology.

The reliability of the dietary history questionnaire with respect to reporting past habits has been evaluated in previous epidemiological studies on nutrition and cancer[1] [2] [3] [4]. In the present study EK re-interviewed a random sample of 10 cases and controls after one year (± 1 month) at the same setting. The reproducibility of the habitual (long-term consumption) frequency was 83%, for the number of portions 89%, and for the portion sizes 90%.

[1] Lubin *et al*, 1998
[2] Lubin *et al*, 1997
[3] Lubin *et al*, 1986
[4] Modan *et al*, 1974 and 1975

3.4.3 Food item-and-frequency coding

In order to make the analysis easier the food items were encoded in such a manner that food items of the same family, such as milk products would always start with the same number. The frequency of consumption was encoded too, so that for example, monthly consumption was labeled with an 'A', bi-monthly consumption was labeled 'B', etc. Regarding weekly consumption, the number of days were directly copied (see appendix 10.1). Certain food items are only available during certain time periods and this seasonality was accounted for (see p.62).

3.5 Food Supplement-and Medication Intake

Subjects were asked to recall whether they are taking, or have taken any food supplements, such as multivitamins, minerals in the past, and for how long. Any information on regular medication intake, its indication and period of intake was collected and recorded.

3.6 Parity and Hormonal Intake (females only)

From the literature it is known that women in general, suffer more from cholelithiasis than men[1] [2] [3], therefore all female participants were asked several questions concerning menarche, menopause, number of pregnancies, number of births, use of hormones, so as to give us a clue to potential mechanisms.

[1] Balzer *et al*, 1986
[2] Barbara *et al*, 1987
[3] Attili *et al*, 1995

3.7 Physical Activity

The questionnaire was designed so as to estimate the average level of physical activity of the study participants. It included information about weekly to monthly hours spent on different activities at work, at home, and at leisure. The description of activity categories in the questionnaire were similar to those described in the recommended dietary allowances (RDA) of the National Research Council (NRC)[1] for hours spent resting and sitting, and hours spent performing very light, light, moderate and heavy physical activity. The category of very heavy (exceptional) activity was not applicable to our more sedentary population.

Physical activity was categorized into three levels of intensity (low, medium, high) according to the distribution of an individual 'activity score', consisting of the number of weekly hours spent in activities of moderate to heavy intensity plus one-half the hours of light-intensity activity. The OR was determined comparing the upper to the lower tertile. This method was found previously to be effective in other population studies[2] [3].

[1] NRC, 1989
[2] Modan *et al*, 1986
[3] Lubin *et al*, 1997

3.8 Education, Profession and Work Places

In order to determine the socio-economic background of the participants, years of education, as well as the profession of each participant was recorded. The participants were also asked to record their current occupation, as well as all workplaces in the past, with special attention to chemical exposure at work, and or at home (eg. cleaning material).

3.9 Smoking Habits

Lifetime tobacco smoking habits were ascertained by asking the participants whether they were smoking or not, smokers were asked about their age when they started and/or stoped smoking, number of cigarettes smoked per day at present and/or in the past.

To assess tobacco use, a score based on the mean number of cigarettes smoked per day times 365, times the number of years of smoking, was used for present and past smokers. Two levels of smoking were determined according to the median score, and ORs were derived using ‘never smoked’ as a reference category.

3.10 Weight, Height and Body Mass Index (BMI)

For the weight history, current weight was measured, without shoes and jackets, using the same scale for all participants, and all subjects had to report their weight during most of their adult life and their weight at 18 years of age. Those who could not remember, were asked to at least determine whether weight was less or more than at the time of interview.

Height, without shoes, was measured with the same instrument for all participants. The BMI was calculated, using the formula weight/(height)^2, kg/m^2. Weight change from age 18 years to adulthood was calculated from the difference between both values.

3.11 Family Disease

All study subjects were questioned about disease in the family, specifically for first and second degree relatives. Disease histories were recorded for parents, sisters and brothers, aunts and uncles, and grandparents. Those who were not sure completed the information over the phone.

3.12 Serum Lipids

Cases and controls were asked whether they had any blood tests taken not longer than three months prior to the interview. Those who had (n=178), agreed to forward their TG and cholesterol levels to us. 28 of the subjects had no recent blood tests taken, but agreed to have them following the interview. Therefore we had complete laboratory data on serum lipids for all subjects.

At this point it is worth mentioning that all subjects were recruited from the same Imaging Institute, and were all insured at the same sick fund, which uses the same laboratory for the Tel Aviv area.

3.13 Data Analysis and Statistical Calculations

The statistical analyses were made using SAS (version 6.12) software on a UNIX platform.

3.13.1 Study population size

The sample size calculation was performed for one risk factor. Since the study was of a matched case-control type, only discordant pairs (i.e. case exposed and control not exposed or vice versa) take place in the analysis. We assume one third of the general population to be exposed to the major risk factor (dietary fat) and the standard alpha, a = **0.05** and beta, b = **0.80**. Since the direction of the influence of the factor is obvious, a one sided test was done.

Under these assumptions, the required sample size is calculated by a formula similar to the one used for testing a single binomial proportion[1].

[1] Schlesselman JJ, 1992

Implementing the formula and assuming the 80% power to be achieved at a relative risk (OR) of 2 we finally arrive at: **107 pairs**, i.e. sample size of 214.

OR (controls) = p_0 / q_0; where $q_0 = 1\text{-}p_0$

OR (cases) = p_1 / q_1; where $q_1 = 1\text{-}p_1$

<u>McNemar's test</u>: $m = \frac{[Z_a/2 + Z_b \times \sqrt{p(1-p)}]^2}{[(p-1)/2]^2}$

3.13.2 Energy and nutrient intake

Reported food consumption (monthly and weekly) was converted into average daily amounts consumed. A comprehensive computer program was developed at the Department of Clinical Epidemiology, Chaim Sheba Medical Center. The program enabled quantitative analysis of the dietary profile at any level desired, including foods, food groups, and food components. First, the program converted all frequencies into weekly frequency and all portion sizes to total weekly amounts for both periods, the past and present, and then to mean daily consumption. Length of season was taken into account when calculating average weekly consumption of fruits and vegetables that are consumed in season.

This was done using the formula below:

$$\text{Nº of calories consumed per day of the food item} = \left[\frac{\text{kcal } x \text{ grams } x \text{ nº of portions } x \text{ nº of times per week}}{7 \text{ (days)}}\right]\left[\frac{\text{nº of mths}}{12 \text{ (mths)}}\right]$$

A similar formula was used in order to calculate the exact amounts of the macronutrients consumed daily (fat, proteins, carbohydrates) as well as the micronutrients, such as the intake of vitamins and minerals of each individual.

The food analysis software was based on Israeli food tables[1], where the dietary fibre analysis was taken from tables of 'Mc Cance and Widdowson's Compositions of food'[2]. The PL content of foods was specially analyzed in our labs and compared to those of Souci *et al*[3] and added to the software.

Table 3.3: List of nutrients analyzed

NUTRIENTS	
➔ Carotin	➔ Phosphatidylcholine
➔ Calcium	➔ Phosphatidylethanolamine
➔ Carbohydrates	➔ Phosphatidylinositol
➔ Cholesterol	➔ Phosphatidylserine
➔ Dietary fiber	➔ Phospholipids, total
➔ Energy	➔ Potassium
➔ Fat, total	➔ Proteins, total
➔ Fat, animal	➔ Proteins, animal
➔ Fat, vegetables	➔ Proteins, vegetables
➔ Fat, polyunsaturated	➔ Retinol
➔ Fat, saturated	➔ Sphingomyelin
➔ Fat, monounsaturated	➔ Starch
➔ Magnesium	➔ Sugar
➔ Nitrogen	➔ Vitamin C
➔ Nitrate	➔ Vitamin E
➔ Nitrit	➔ Zinc

Table 3.4: List of foods and food groups analyzed

FOODS / FOOD GROUPS	
➔ Alcoholic beverages	➔ Milk products
➔ Beef	➔ Oils, animal
➔ Bread and Cereals	➔ Oils, vegetables
➔ Chicken	➔ Olives
➔ Coffee & Tea	➔ Pulses
➔ Dried Fruits	➔ Seeds
➔ Eggs	➔ Soft Drinks
➔ Ethnic Dishes	➔ Sweets
➔ Fish	➔ Vegetables
➔ Fruits	➔ Water

[1] Guggenheim *et al*, 1991
[2] Paul *et al*, 1978
[3] Souci *et al*, 1989

Analysis of the incidence tables

The incidence tables of the type 2*x*2 have been calculated for cases matched by two dichotomous variables (e.g. gender and disease). Using those tables we tried to ascertain the potential dependency of those two variables using the method described by Fisher[1]. Concerning the 2*x*C tables (C>2) is to check whether a linear relation exists between an ordinal variable and another. The test is therefore the Mantel Haenszel test[2].

On the basis of the linear distribution of intake by the total population (cases and controls), three levels of intake (low, medium, high) were determined.

ORs associated with these tertiles of mean daily consumption of each food item or food component was calculated by conditional logistic regression analysis[3], adjusting for energy intake. The lowest level was used as a reference category. Corresponding, 95% confidence intervals (CIs) were determined, and the significance for linear trend was calculated[1]. ORs presented in the following chapter, will be comparing the upper to the lower tertile, although figures for medium vs lower levels are available too, which are important in proving linear association.

3.13.3 Life style parameters

Cutpoints for three levels of physical activity, body weight, weight change, BMI, and tobacco use were defined as for the other parameters, according to the linear distribution of values of both cases and controls.

To assess the independent effect of each of the lifestyle parameters studied, adjusting for all others, a multivariate logistic model was also fitted[4].

[1] Sokal *et al*, 1981
[2] Mantel *et al*, 1959
[3] Breslow *et al*, 1980
[4] Schlesselman JJ, 1992

4. RESULTS

4.1 Study Subjects (demographic background)

Table 4.1: N°. of subjects in each of the subgroups, which were analyzed

	Cases	Controls
All	103	103
Females	75	75
Males	28	28
< 45	51	51
> 45	52	52
Israel born	64	64

4.1.1 Age

Initially the age span for inclusion was set from 18-75. Nevertheless, as can be seen in table 4.2, the youngest subject in the trial was 24 years of age at the time of the interview, and the oldest was 73.

Table 4.2: Age (years) distribution among study subjects

	CASES			CONTROLS			ALL
	Females	**Males**	**Total Group**	**Females**	**Males**	**Total Group**	
N	75	28	103	75	28	103	206
MEAN	**45**	**48**	**45.8**	**44.7**	**46.1**	**45.1**	**45.4**
STD	13.7	13.9	13.7	13.1	13.4	13.1	13.4
Min	25	27	25	24	27	24	24
Max	69	73	73	73	70	73	73

Table 4.3: Division of subjects into two age groups

	Cases		Controls	
	N	**%**	**N**	**%**
< 45 years	51	48.5	51	51.5
> 45 years	52	51.5	52	48.5

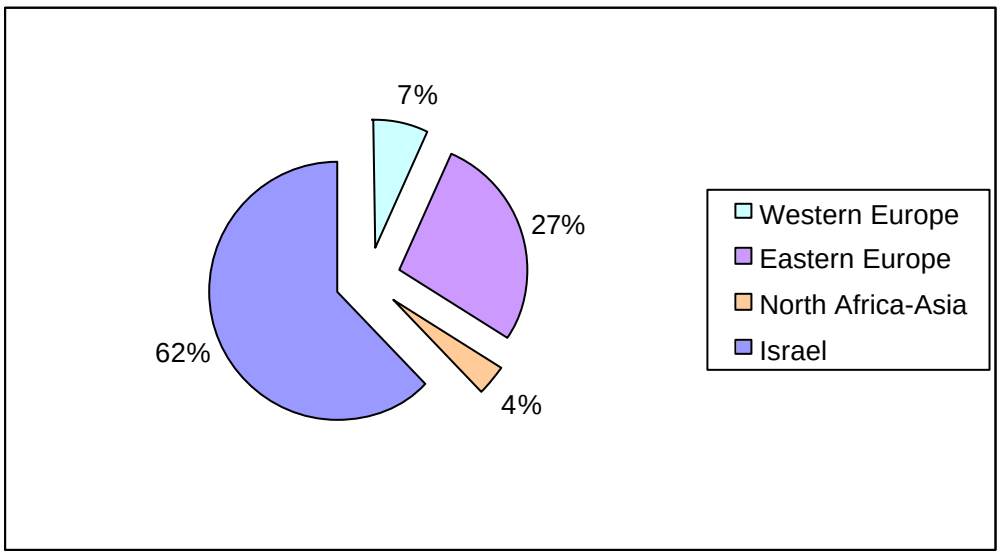
4.1.2 Ethnicity

The study subjects were also matched according to region of birth, so as to ensure appropriate comparison between cases and controls. Israel is known for the fact that the population is diverse, as Jews have immigrated from many parts in the world. All study subjects were subdivided for analysis into 4 groups (see table 4.4). Subjects who were born in Israel were matched according to their mother's region of birth. Analysis in this paper was made only for the latter group, which made up 64 of the 103 pairs (figure 4.1). The three other groups were too small to derive any meaningful conclusions.

Table 4.4: Regions of birth: Division

Area Codes *(Place of birth):*	
Western Europe, North America, South Africa, Australia, New Zealand:	01
Eastern Europe:	02
North Africa, Asia:	03
Israel:	04

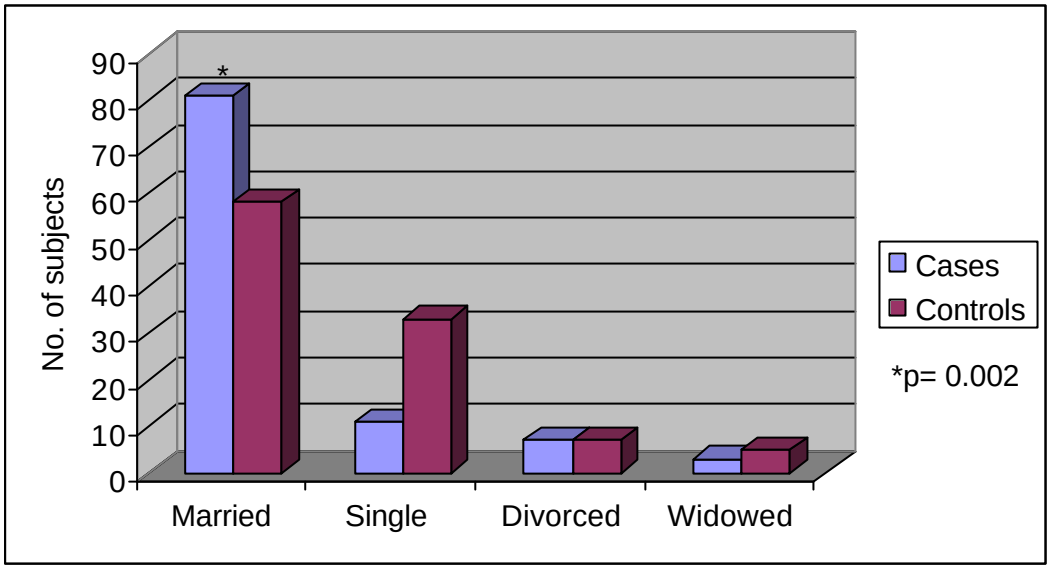
Figure 4.1: Participants' place of birth



4.1.3 Family status

All subjects were questioned about their current family status. We found that significantly more cases were married than their controls in this study (figure 4.2).

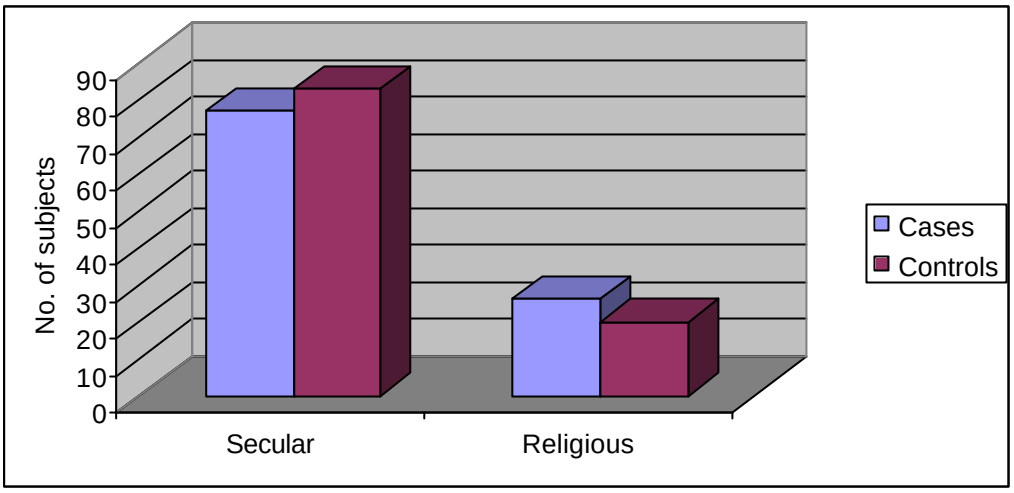
Figure 4.2: Distribution of family status



4.1.4 Religious dietary laws

Religion in the context of this study was used solely to differentiate those subjects that eat kosher food only from those who do not. In terms of dietary habits this means that subjects do not consume pork, certain fish and seafood, while meat and milk products are not consumed at the same time. The distribution among cases and controls was similar and almost homogenous (figure 4.3).

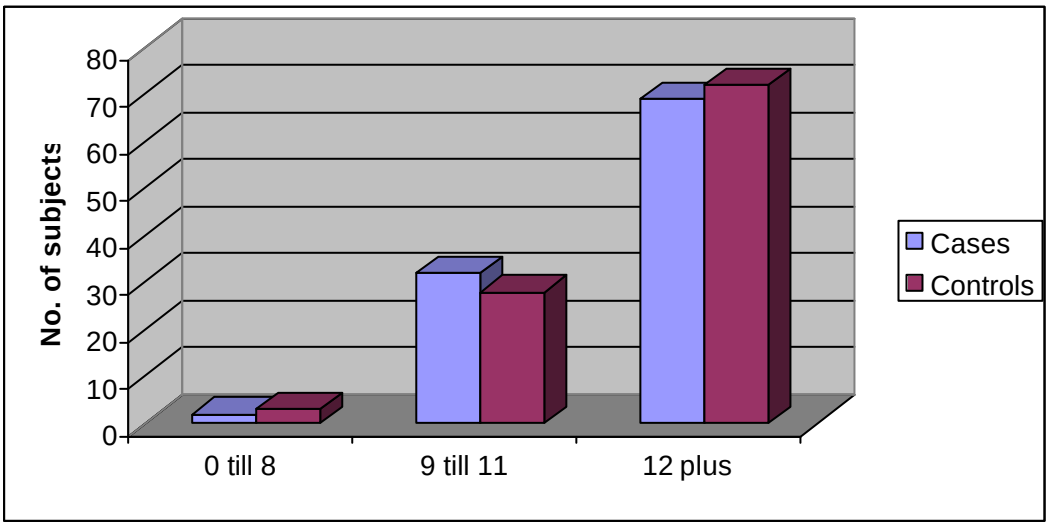
Figure 4.3: Religious habits of study subjects



4.1.5 Education and profession

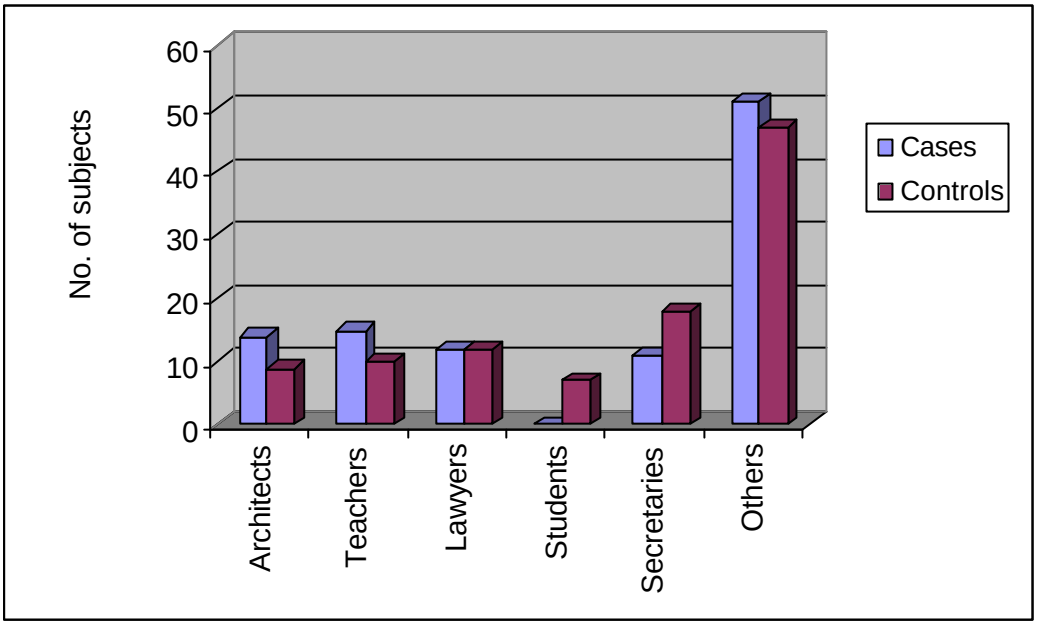
Regarding the study subjects' formal education, the mean number of study years was almost identical for cases and controls, 14.41 & 14.72 respectively. The distribution among cases and controls was very similar (figure 4.4).

Figure 4.4: Formal education



Additionally the participants provided information about their professions and their current and previous occupations. It was observed that the distribution of professions (figure 4.5), as well as places of work were similar in both study groups (figures 4.6 & 4.7).

Figure 4.5: Profession distribution of study subjects



Though the control group included significantly more subjects who were students, or working in a medical institution, and/or working in a bank, the numbers of people were only a fraction of the entire study population, and therefore no relationship to GS development has been suggested.

Figure 4.6: Cases' places of work

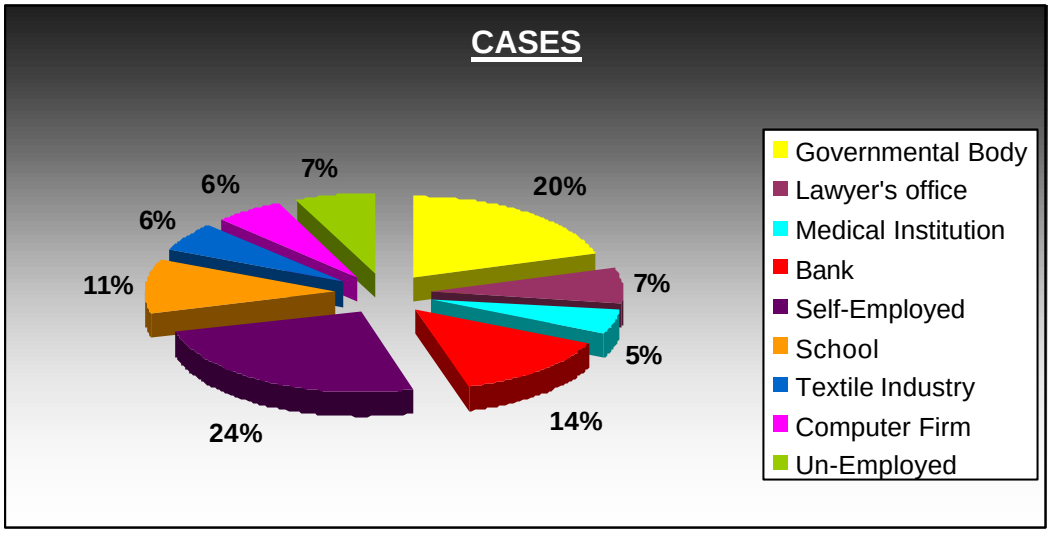
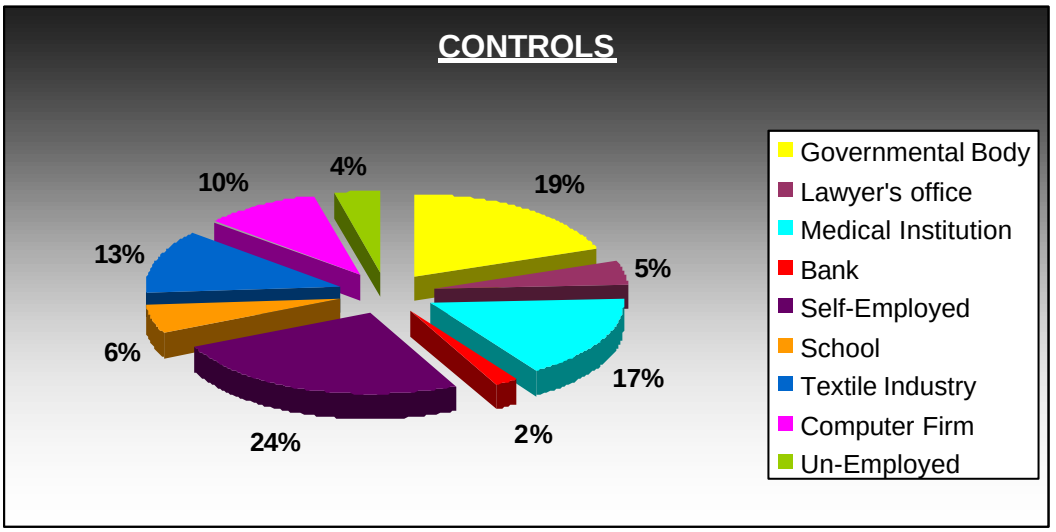


Figure 4.7: Controls' places of work



Controls were significantly more frequently exposed to some sort of chemical, be it during work and/or at leisure time (figure 4.8). Here again the number of those exposed to particular chemicals was too small in order to allow any conclusions. The specification of the chemicals is given below (table 4.5).

Figure 4.8: Exposure to chemicals at work and at leisure

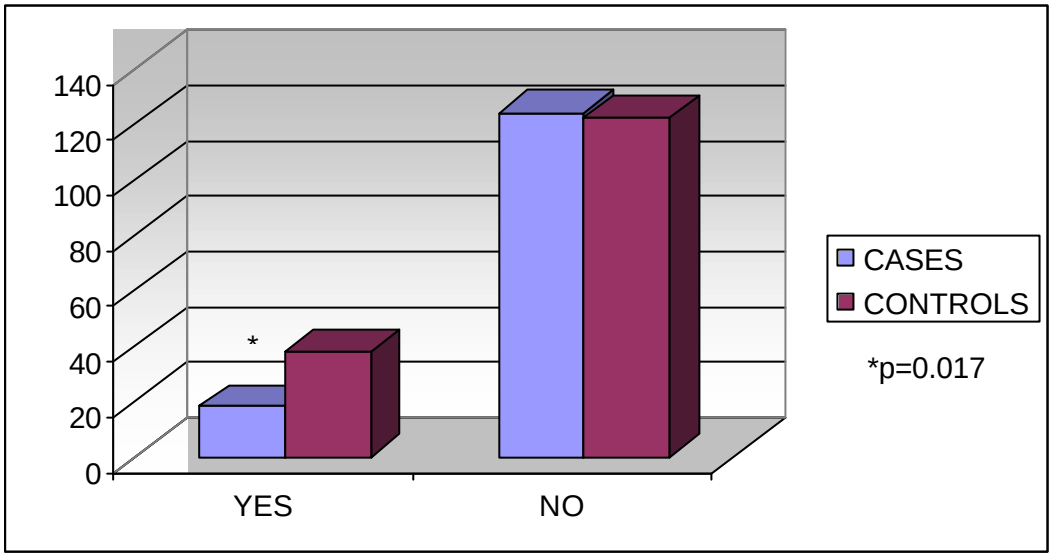


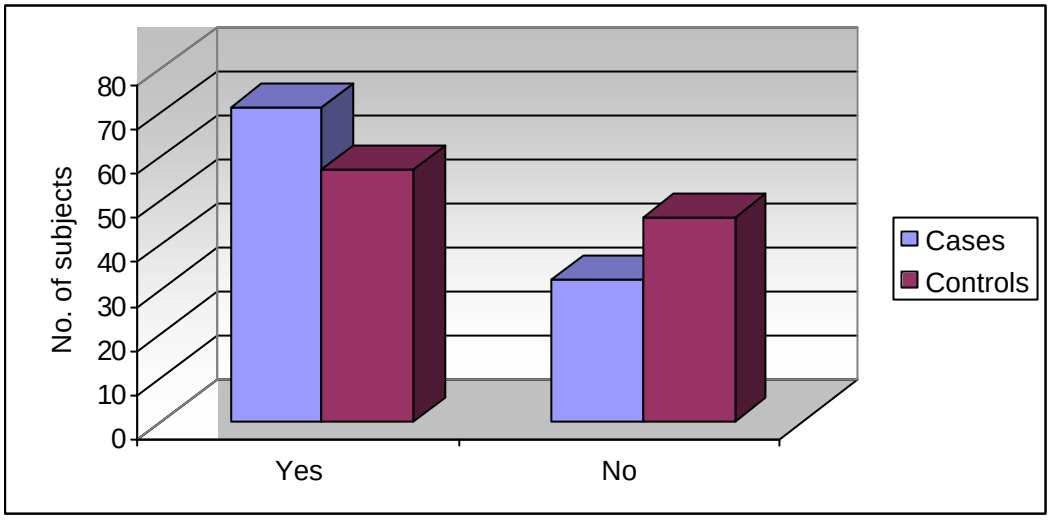
Table 4.5: Exposure to chemicals

Chemical	No. of subjects exposed	
	Cases	Controls
Cleaning material	4	8
Lead	1	1
Pesticides	0	0
Organic solvents	3	5
Radiation	2	10
Colour	0	2
Photo developing material	3	5
Chemicals, unspecified	2	3
Other, unspecified	3	4

4.2 Dietary Habits

More cases than controls in the present study had never changed their dietary habits for various reasons. The difference, though, was not significant (p=0.062).

Figure 4.9: Changes in dietary habits

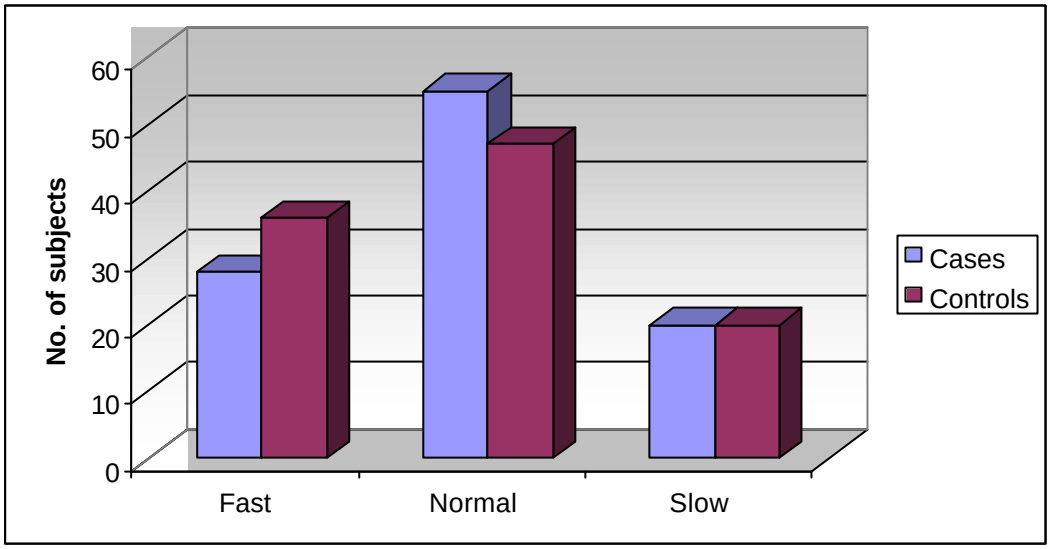


The most important reasons for change in dietary habits are mentioned in the following paragraph. The results show that most subjects changed their eating habits only for short periods in order to loose weight. Others changed their habits completely to try and live a healthier life, some of them with the primary aim of loosing weight. Immigration was the third most important reason for a change in eating habits.

Most subjects changed their habits by reducing their total energy intake. More cases lowered their energy consumption than controls, though the difference was not significant (p=0.068). Nevertheless, more cases reduced the intake of fat than their controls (p=0.051). The third biggest change in habits was the reduction of meat consumption, which was similar between cases and controls.

No differences between cases and controls were found regarding the speed of food consumption (figure 4.10).

Figure 4.10: Speed of food consumption



4.3. Nutrient Intake

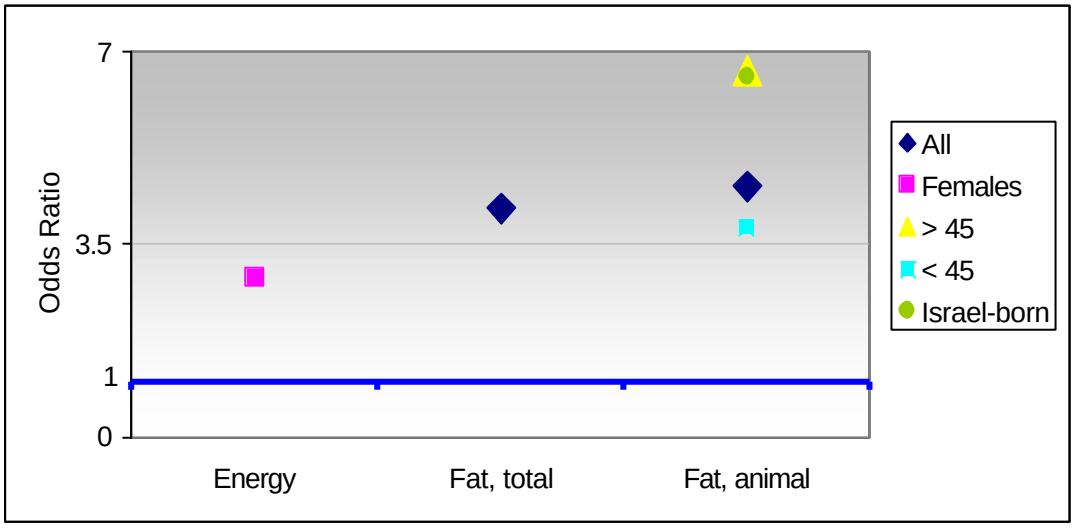
Nutrient and food intake follows on the coming pages and is presented for five groups:

- All subjects
- Females only (females tend to have a higher prevalence it was interesting to determine their risk and protective factors, and the number of male pairs was too small to draw any conclusions)
- Two age groups, above and below age 45 (in the past people above age 40 had a significantly higher prevalence, though these observations seem to diminish, it was interesting to see whether there were marked differences)
- Israeli-born subjects, as they made up the majority of studied subjects.

4.3.1 Total energy-and fat intake

Total energy intake was shown in females to be a risk factor (figure 4.11). With regard to fat we examined total fat intake as well as polyunsaturated, monounsaturated, saturated fat intake, cholesterol intake, fat of animal origin and of vegetable origin. We could observe that total fat, and especially fat originating from animals was a risk factor in all subjects. Animal fat presented itself as a risk factor especially in the older population and those born in Israel with a more than six-fold risk, but the younger subjects also showed a four times higher risk to disease from GSs. The results for sub-groups that emerged (non-significant results) are presented in table 4.7.

Figure 4.11: Risk factors: Energy & fats



We divided food and nutrient intake into three levels. This is shown on table 4.6 and tables that will follow as percentiles, 33% and below (low), 50% (medium), and 66% and above (high), respectively. With regard to energy intake that means that females who ate a diet with an energy content of =1909.48 kcal daily have a 2.9 greater risk of developing GSs than females consuming =1436.82 kcal daily.

Table 4.6: ORs, p-value, mean intake & percentiles of significant results: Energy and fats

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Energy [kcal]	Females	2.895	0.023	1790.51	1436.82	1678.51	1909.48
Total fat [g]	All	4.166	0.038	73.12	55.20	64.63	79.48
Animal fat [g]	All	4.555	0.001	33.14	23.64	28.51	34.58
	> 45 yrs	6.667	0.016	33.34	23.19	26.87	33.36
	< 45 yrs	3.795	0.034	32.94	24.32	28.57	34.89
	Israel-born	6.555	0.004	32.49	23.50	26.39	34.54

Table 4.7: ORs, p-value, mean intake & percentiles of non-significant results: Energy and fats

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Energy [kcal]	All	1.862	0.093	1881.11	1493.29	1779.12	1962.33
	> 45 yrs	1.546	0.388	1811.66	1445.57	1700.15	1908.20
	< 45 yrs	1.937	0.210	1951.92	1538.39	1851.95	2058.18
	Israel-born	2.035	0.113	1955.98	1538.39	1851.68	2039.41
Total fat [g]	Females	0.230	0.239	69.12	52.91	61.66	75.17
	> 45 yrs	5.120	0.109	70.27	53.72	62.15	73.02
	< 45 yrs	1.568	0.629	76.03	56.18	67.69	82.76
	Israel-born	2.417	0.239	76.28	56.18	67.19	83.32
Animal fat [g]	Females	2.591	0.071	31.39	23.19	25.67	31.35

4.3.2 Proteins

The consumption of proteins with regard to risk of GS disease was probably the biggest surprise in our analyses. Never before was such a strong co-relation observed. Total protein intake resulted in an over ten-fold risk elevation (figure 4.12) when consuming =80.20 grams daily as opposed to =62.69 grams per day (table 4.8). This trend was found in the young, old, and Israel-born subjects. Consumption of protein of animal origin was also associated with GS, though the association was less strong, except in the young subjects where the risk was 9.9 times higher. Nevertheless the trend could be observed as a 4-5 times fold elevated risk in all other subgroups analyzed.

Figure 4.12: Risk factor: Proteins

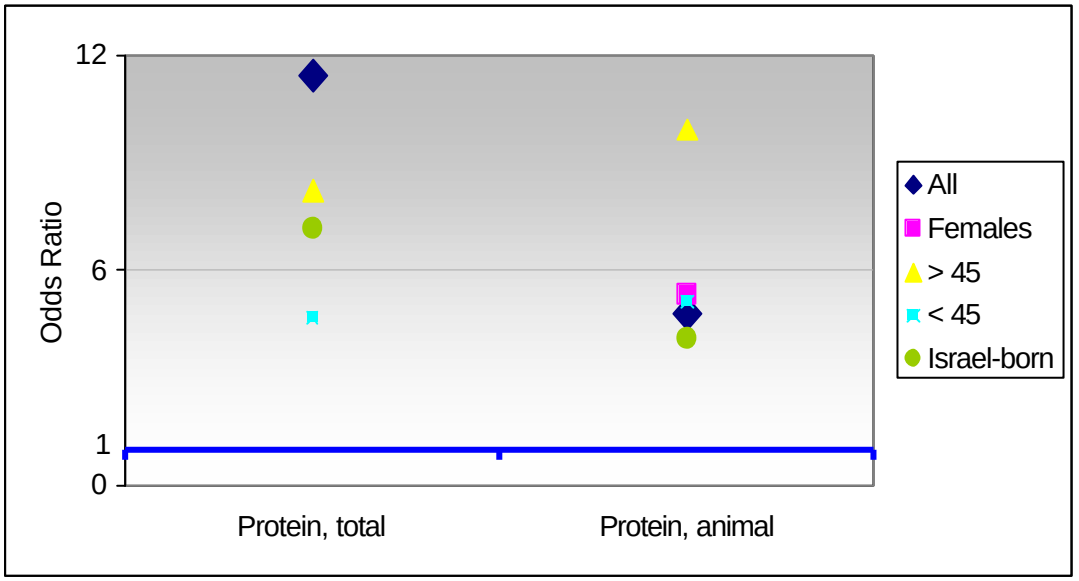


Table 4.8: ORs, p-value, mean intake & percentiles of significant results: Proteins

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Total Protein [g]	All	11.440	0.0001	75.37	62.69	71.59	80.82
	> 45 yrs	8.207	0.025	73.58	62.07	69.60	79.66
	< 45 yrs	4.700	0.040	77.20	62.69	73.64	83.33
	Israel-born	7.187	0.015	77.27	63.92	73.64	83.95
Animal protein [g]	All	4.800	0.0001	41.55	31.67	37.87	46.67
	Females	5.318	0.002	72.21	30.57	36.14	45.00
	> 45 yrs	9.931	0.002	41.98	32.52	38.81	47.71
	< 45 yrs	5.142	0.007	41.11	30.46	36.08	46.31
	Israel-born	4.124	0.006	41.39	31.81	39.87	46.34

Table 4.9: ORs, p-value, mean intake & percentiles of non-significant results: Proteins

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Total Protein [g]	Females	3.664	0.059	72.21	60.85	67.84	77.81
Vegetable Protein [g]	All	0.908	0.822	24.91	19.18	22.61	27.00
	Females	0.789	0.631	24.05	18.55	21.31	25.85
	> 45 yrs	0.406	0.219	23.39	19.15	20.91	24.60
	< 45 yrs	0.975	0.966	26.45	19.18	25.48	29.07
	Israel-born	1.094	0.880	26.03	19.38	24.33	28.48

4.3.3 Carbohydrates

Dietary fiber was shown to be protective in all subjects, and in subjects born in Israel (figure 4.13). In detail, a consumption =20.95 grams per day, compared to =15.21 grams per day reduces the risk of developing GSs by almost four (OR: 0.28) in all subjects. The same association was observed with starch in the older subjects studied, where a consumption =133.96 grams per day, compared to =96.10 grams per day reduces the risk of developing GSs by nearly five (OR: 0.21) (table 4.10).

Figure 4.13: Risk factor: Complex carbohydrates

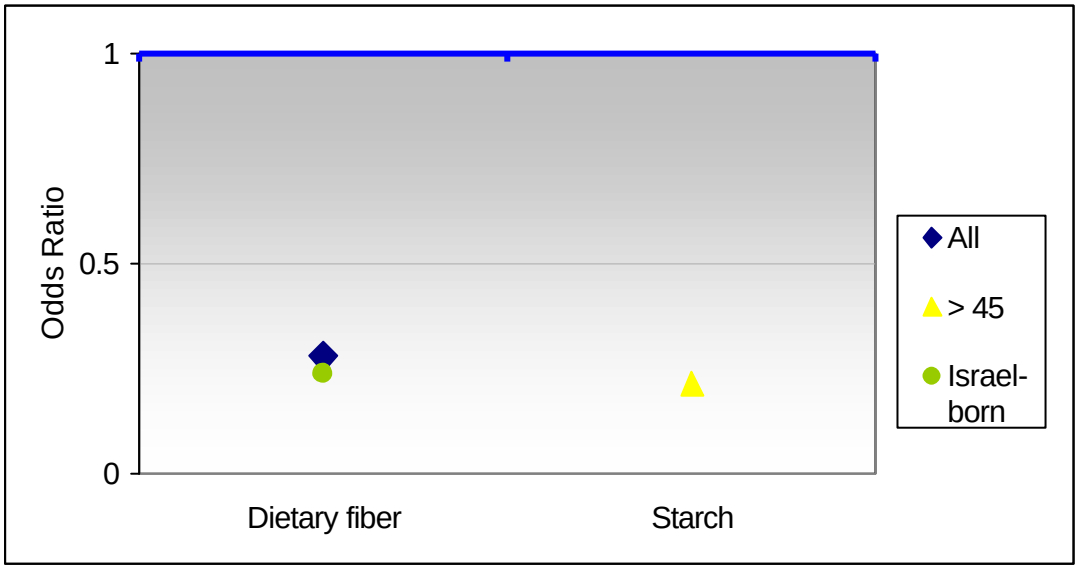


Table 4.10: ORs, p-value, mean intake & percentiles of significant results: Dietary fiber and starch

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Dietary fiber[g]	All	0.279	0.009	19.99	15.21	17.89	20.95
	Israel-born	0.239	0.018	20.90	15.84	18.69	22.26
Starch [g]	> 45 yrs	0.214	0.030	122.94	96.10	114.63	133.96

Table 4.11: ORs, p-value, mean intake & percentiles of non-significant results: Dietary fiber and starch

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Dietary fiber[g]	Females	0.343	0.056	19.99	15.21	17.89	20.95
	> 45 yrs	0.304	0.054	20.90	15.84	18.69	22.26
	< 45 yrs	0.241	0.067	19.39	14.35	16.78	19.72
Starch [g]	All	1.007	0.986	122.94	96.10	114.63	133.96
	Females	1.211	0.702	127.04	97.23	119.24	145.67
	< 45 yrs	1.190	0.806	146.98	114.35	142.23	164.69
	Israel-born	1.112	0.860	142.07	107.29	136.14	159.24

4.3.4 Micronutrients

PS turned out to raise the risk of GS development. Contrary to this, zinc was shown to be protective.

Figure 4.14: Risk factor: Micronutrients

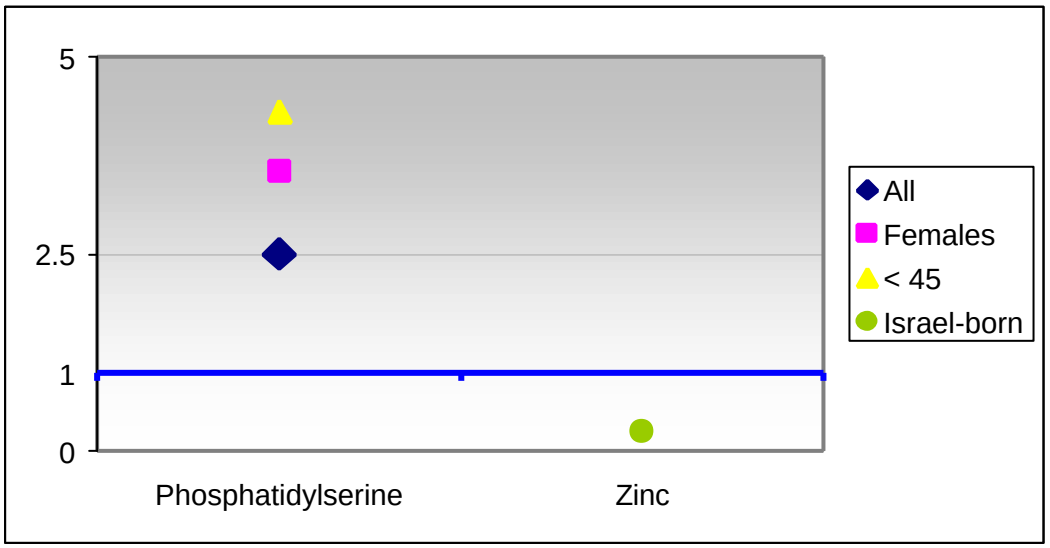


Table 4.12: ORs, p-value, mean intake & percentiles of significant results: PS and zinc

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Phosphatidyl serine [mg]	All	2.506	0.016	34.71	15.49	27.23	37.39
	Females	3.540	0.018	32.80	14.63	24.94	33.96
	< 45 yrs	4.297	0.020	32.33	13.16	19.98	35.47
Zinc [mg]	Israel-born	0.238	0.028	102.10	70.77	84.58	101.70

Table 4.13: ORs, p-value, mean intake & percentiles of non-significant results: PS and zinc

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Phosphatidyl serine [mg]	> 45 yrs	1.794	0.243	37.04	18.17	28.90	37.85
	Israel-born	1.989	0.130	33.03	13.84	22.96	33.11
Zinc [mg]	All	0.391	0.055	93.24	68.09	79.77	95.82
	Females	0.473	0.223	89.73	66.38	77.26	91.05
	< 45 yrs	0.712	0.635	105.30	69.39	85.53	104.43
	> 45 yrs	0.391	0.157	81.40	66.48	77.64	88.14

4.4 Specific Foods and Beverages

Alcohol intake as well as the consumption of soft drinks were inversely associated with the risk of GSs. The same association was observed with the consumption of dried fruits and soft drinks.

In contrast beef, chicken, fish and fruits showed a positive association to GS development. Chicken was associated with an increased risk among all study subjects, whereas the relation to beef was observed only in the younger subjects. Fish was related to an increased risk in the older subjects. Olive intake was associated with an increased risk in Israel-born subjects (figure 4.15).

Figure 4.15: Risk factor: Specific foods

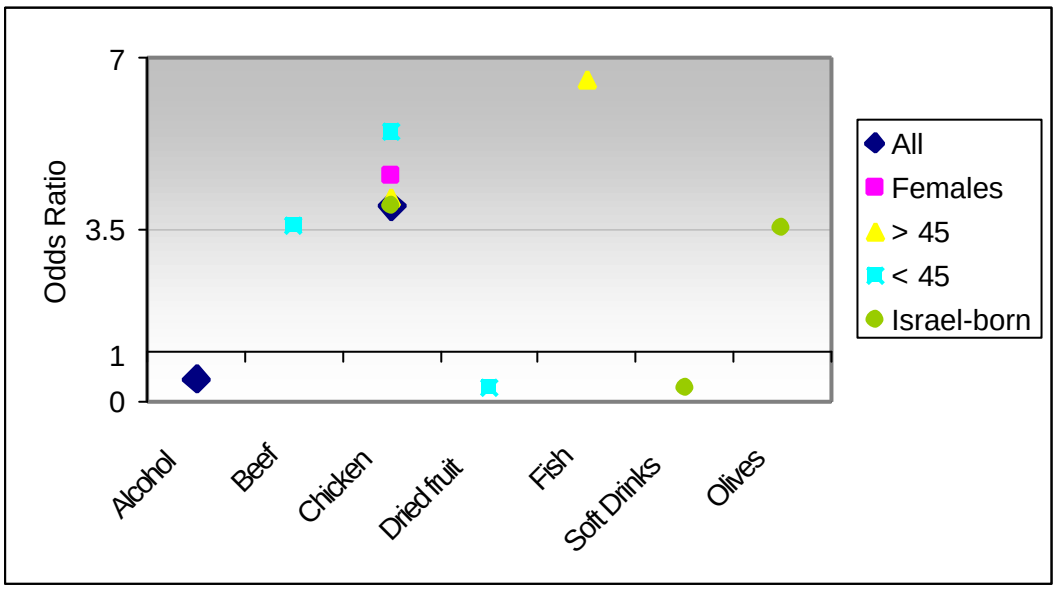


Table 4.14: ORs, p-value, mean intake & percentiles of significant results: Foods

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Alcohol [ml]	All	0.461	0.039	2.35	0.00	0.67	1.71
Beef [g]	< 45 yrs	3.584	0.029	18.55	3.93	10.35	20.00
Chicken [g]	All	3.982	0.0001	68.39	45.43	59.89	81.43
	Females	4.609	0.001	64.31	42.72	55.69	71.43
	< 45 yrs	5.481	0.007	68.48	45.71	59.54	72.27
	> 45 yrs	4.146	0.009	68.30	43.60	60.80	82.06
	Israel-born	3.948	0.006	70.36	45.43	60.80	88.09
Dried fruits [piece]	< 45 yrs	0.294	0.022	8.37	0.00	0.00	2.30
Fish [g]	> 45 yrs	6.573	0.002	24.16	10.82	20.01	28.15
Olives [piece]	Israel-born	3.553	0.031	3.65	0.86	1.43	2.86
Soft Drinks [ml]	Israel-born	0.271	0.016	975.40	640.00	864.76	1154.29

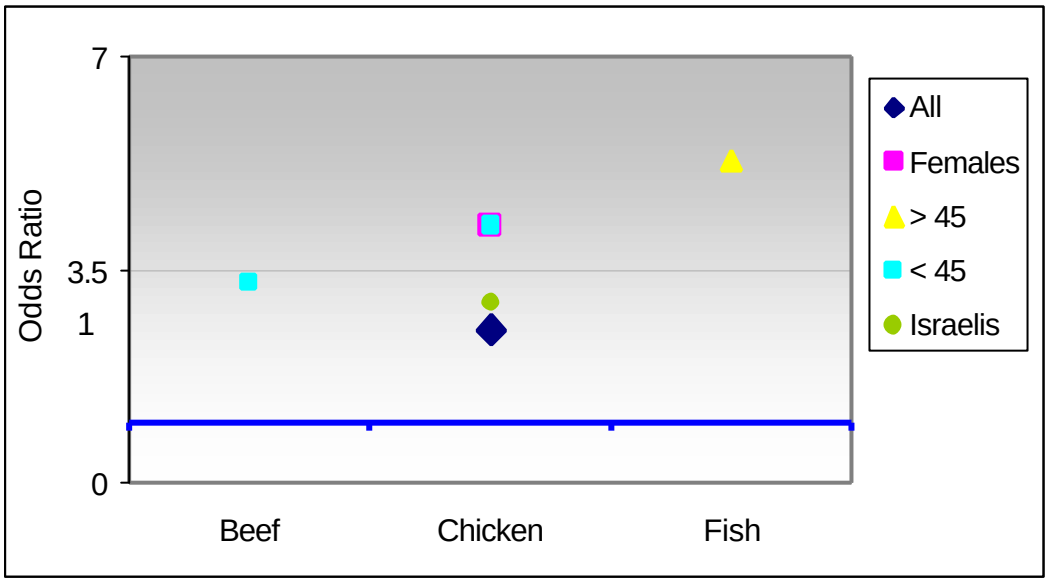
Table 4.15: ORs, p-value, mean intake & percentiles of non-significant results: Foods

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Alcohol [ml]	Females	0.489	0.074	1.48	0.00	0.10	1.18
	< 45 yrs	0.476	0.155	2.17	0.00	0.67	1.71
	> 45 yrs	0.497	0.190	2.53	0.00	0.61	1.71
	Israel-born	0.566	0.187	2.15	0.00	0.54	1.71
Beef [g]	All	1.306	0.470	16.26	2.86	7.91	17.14
	Females	1.163	0.731	12.82	1.97	5.81	12.46
	> 45 yrs	0.783	0.674	14.00	0.82	5.25	14.29
	Israel-born	2.042	0.125	16.24	2.86	7.87	17.47
Dried fruits [piece]	All	0.568	0.112	9.05	0.00	0.00	4.59
	Females	0.551	0.137	9.87	0.00	0.66	4.59
	> 45 yrs	1.186	0.747	9.73	0.00	1.64	4.92
	Israel-born	0.751	0.507	9.50	0.00	0.66	5.71
Fish [g]	All	1.822	0.08	22.16	9.51	16.72	26.07
	Females	1.771	0.170	21.42	10.00	16.89	25.36
	< 45 yrs	0.674	0.469	20.12	5.57	12.59	24.27
	Israel-born	1.935	0.152	19.53	7.70	13.85	23.28
Olives [piece]	All	1.494	0.328	3.36	0.79	1.43	2.86
	Females	0.529	0.454	2.99	0.66	1.43	2.86
	< 45 yrs	2.000	0.236	3.47	0.79	1.43	2.86
	> 45 yrs	1.208	0.751	3.26	0.66	1.57	2.86
Soft drinks [ml]	All	0.484	0.051	1008.54	670.48	906.67	1226.67
	Females	0.512	0.128	980.69	640.00	880.00	1177.14
	< 45 yrs	0.334	0.052	1053.58	708.57	929.52	1241.90
	> 45 yrs	0.641	0.384	964.35	640.00	851.43	1120.00

4.5 Nutrients and Foods after Multivariate Analysis

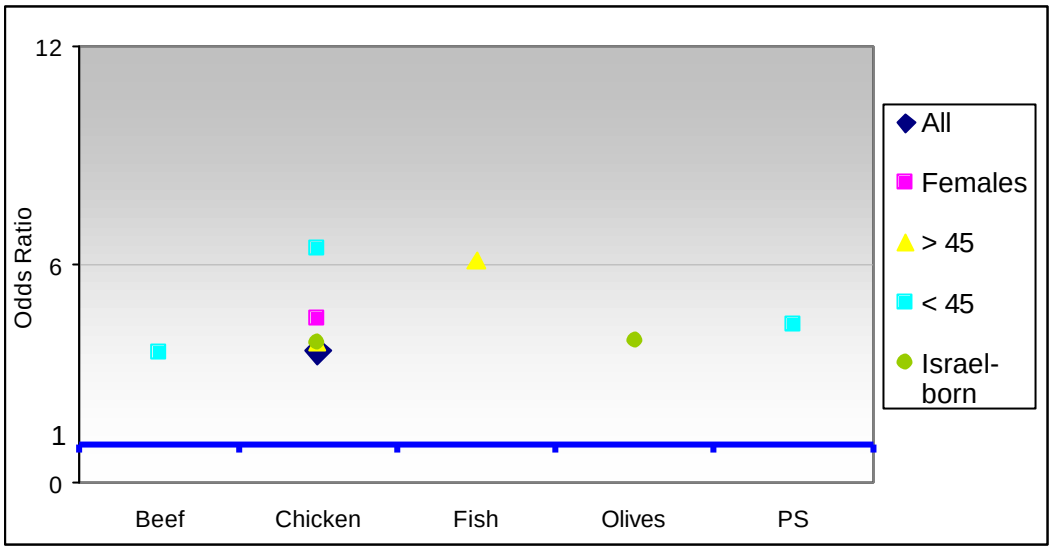
When we realized that **proteins** had such a strong association with the disease under investigation, an additional analysis was performed, when all nutrients were adjusted to energy and proteins. Chicken persisted as a major risk factor. Beef remained a risk factor in the young subjects and so did fish in the older subjects.

Figure 4.16: Risk factors when adjusted to energy and proteins



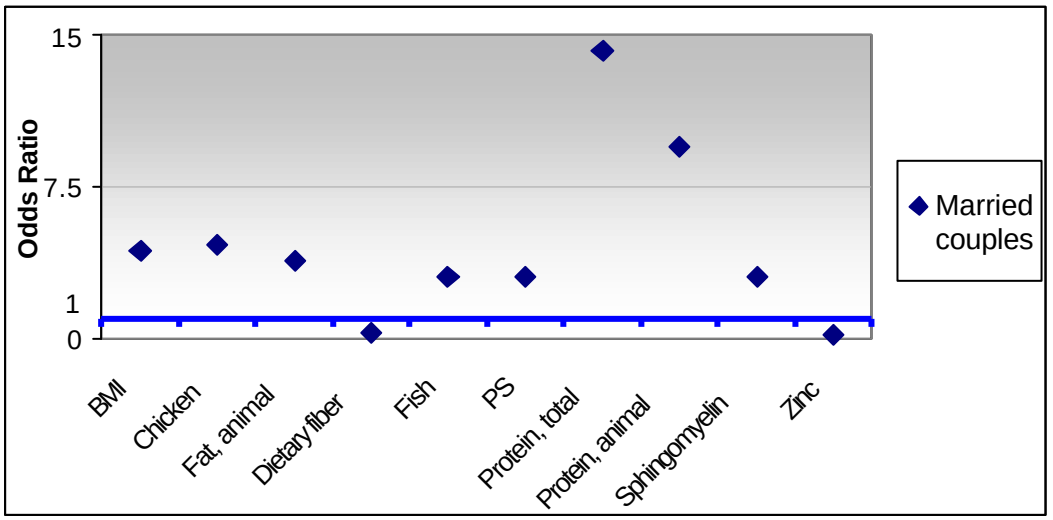
We also did additional analysis, when all nutrients were adjusted to energy and fat. In figure 4.17 can be seen that again that risk factors remained as they were in the initial analysis which was adjusted to total energy only.

Figure 4.17: Risk factors when adjusted to energy and fat



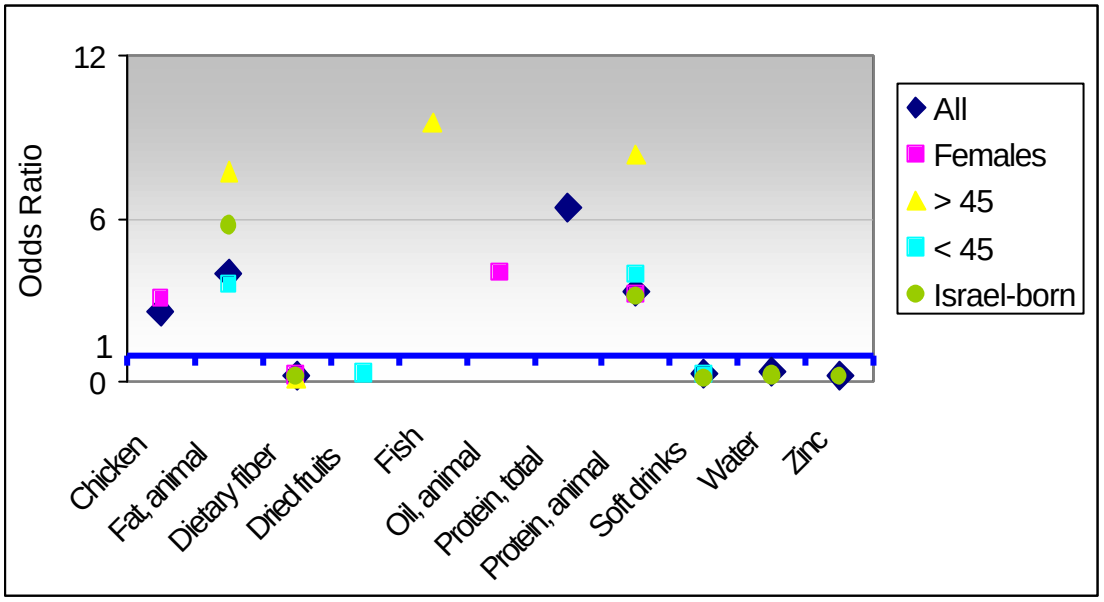
As we found that significantly more cases were married, we wanted to determine which factors might play a role, as the fact of being married could obviously not be the reason for GS development. In figure 4.18 we can see the results for those matched pairs where both, case and control, were married.

Figure 4.18: Risk factors for married individuals only (n=61 pairs)



BMI turned out to be a risk factor (see section 4.6), so we did sub-analysis to control for relative weight. As can be seen on the figure below the risk factors remained almost identical (beef, olives and total fat disappeared as risk factors, and alcohol disappeared as a protective factor) as were observed without controlling for relative weight (being a risk factor in itself).

Figure 4.19: Risk factors when adjusted to energy and BMI



4.6 Anthropometric Influences

Weight was shown to be an important risk factors for the development of GSs. The weight distribution among the study subjects is shown in table 4.16. Nevertheless, I chose to show the results for BMI, as it is well known that weight in relation to height is a more exact way of presenting weight-related risk factors. In all subjects, and in all subgroups analyzed, BMI turned out an important risk factor (figure 4.20). The exact data can be seen in table 4.17.

Table 4.16: Weight [kg] distribution among study subjects

	CASES			CONTROLS			ALL
	Females	**Males**	**Total Group**	**Females**	**Males**	**Total Group**	
N	75	28	103	75	28	103	206
MEAN	**69.2**	**80.3**	**72.2**	**62.2**	**79.0**	**66.7**	**69.5**
STD	15.2	14.3	15.7	9.7	17.4	14.2	15.2
Min	47.0	54.0	47.0	45.0	56.0	45.0	45.0
Max	140.0	120.0	140	86.0	137.0	137.0	140.0

Figure 4.20: Risk factor: Body Mass Index

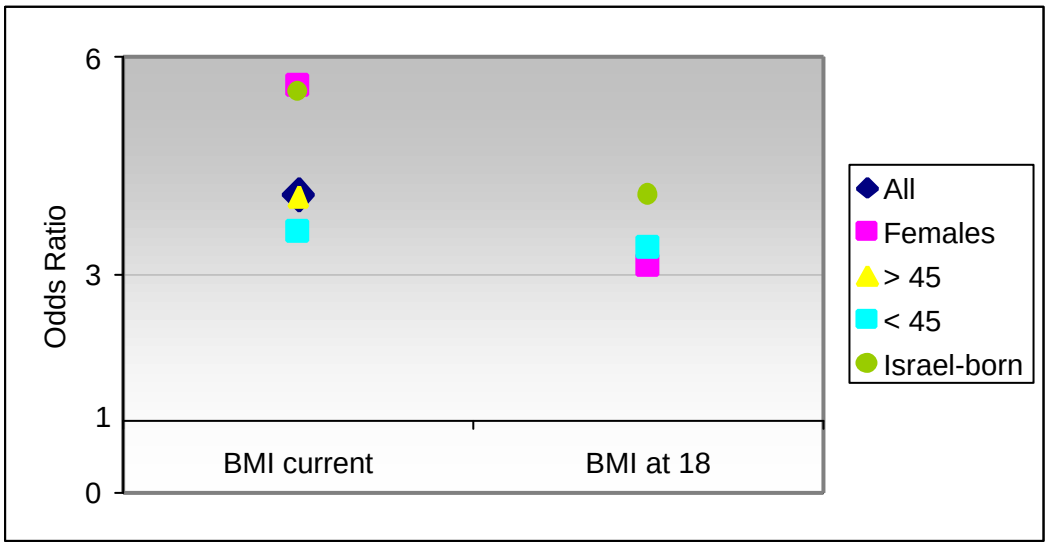


Table 4.17: ORs, p-value, mean intake & percentiles of significant results:

BMI

Item	Group	OR	p-value	Mean	33.3 %	50 %	66.6 %
Current BMI [kg/m^2]	All	4.492	0.0001	24.64	22.23	24.10	25.70
	Females	5.601	0.001	24.46	22.04	23.55	25.33
	> 45 yrs	4.079	0.018	24.99	22.48	24.37	26.31
	< 45 yrs	4.035	0.023	24.26	21.74	23.44	25.42
	Israel-born	5.521	0.004	24.49	22.04	23.32	25.00
BMI at age 18 [kg/m^2]	Females	3.850	0.007	17.70	18.75	19.56	20.93
	< 45 yrs	3.364	0.029	17.16	18.75	20.17	21.36
	Israel-born	4.103	0.006	17.24	18.75	20.02	20.96

4.7 Medication-and Supplement Intake

The disease status as well as medications and the intake of food supplement were ascertained by asking the subject whether medication was ever taken for a period longer than three months. There were no significant differences between cases and controls regarding both, medication and supplements (figures 4.21 & 4.22). This might be due to the fact that only a small number of subjects had taken any medication or food supplements at the time of interview or in the past. Therefore no conclusions could be drawn.

Figure 4.21: Intake of food supplement

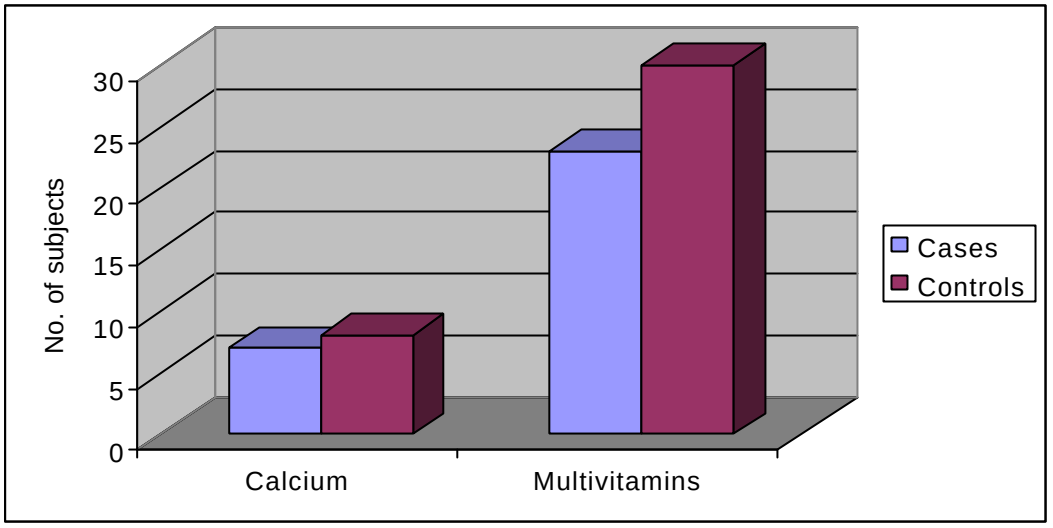
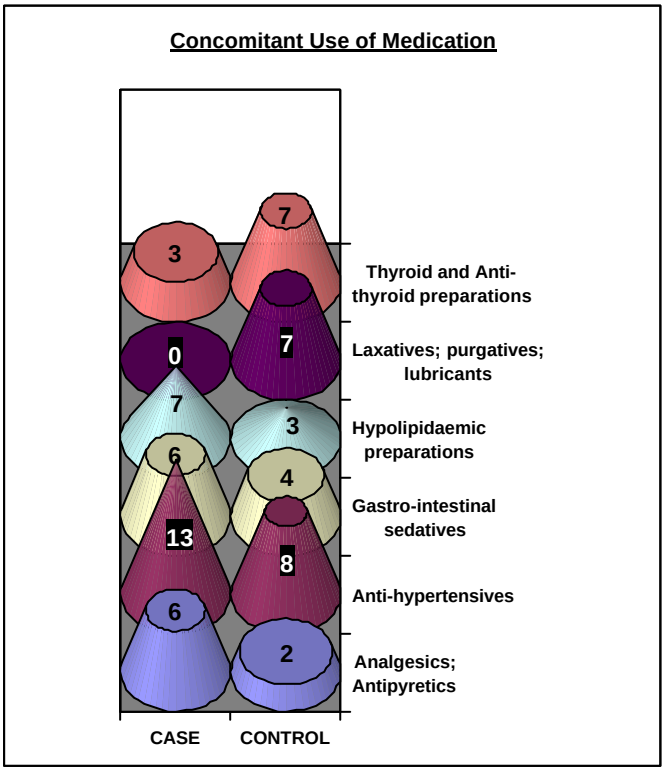


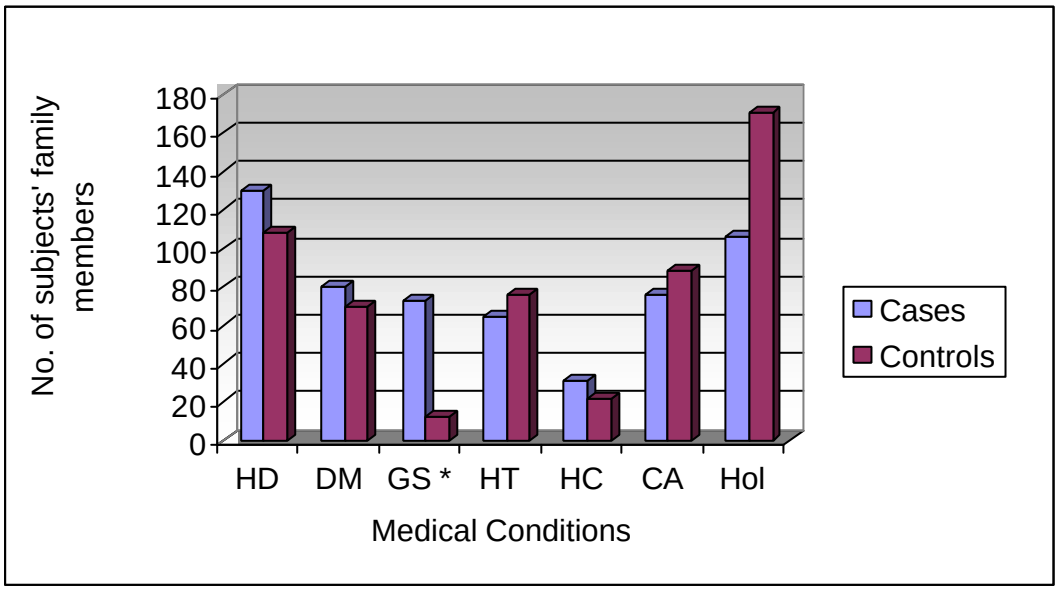
Figure 4.22: Chronic use of medications



4.8 Family History

The most striking difference in family history was the fact that in all subjects and all subgroups analyzed the number of other family members suffering from GSs, was significantly higher in the families of the cases than in those of the controls. This effect is presented in two ways. Figure 4.23 shows results when all family members are counted for all diseases. As sizes of families differ among the subjects, especially in Israel there is often a difference in the number of children per family between families of Ashkenazi (East-European background) and Sephardi heritage (Sephardi families tend to have more children), the results of family history were mainly presented when only one family member was counted per disease (figures 4.24 - 4.29). The way of presentation had no significant effect on the results.

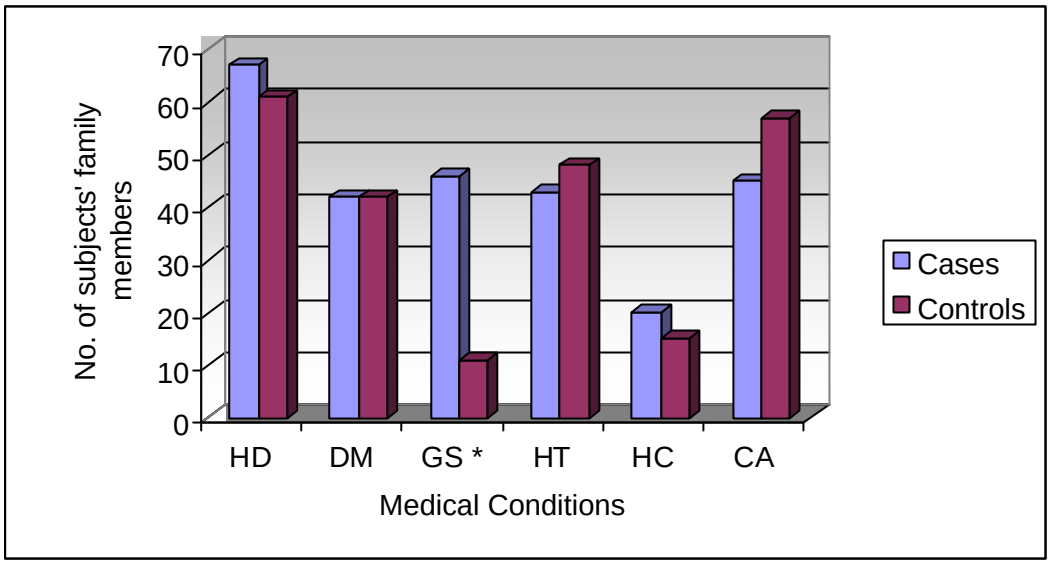
Figure 4.23: Cumulative family disease



* Significant difference ($p<0.001$)

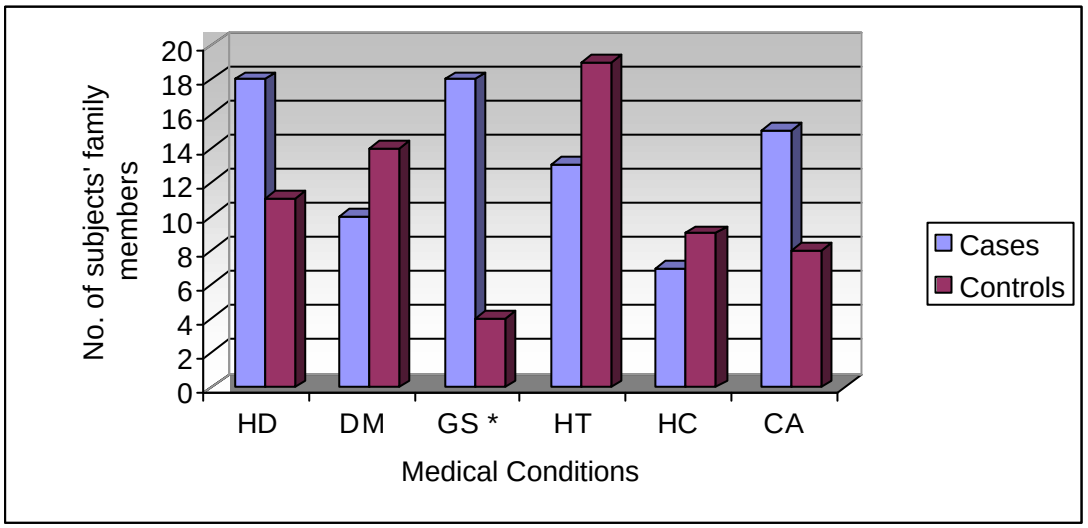
HD – Heart Disease; DM – Diabetes Mellitus; GS – Gallstones; HT – Hypertension; HC – Hypercholesteremia; CA – Cancer; Hol – died during the Holocaust

Figure 4.24: Family diseases (all) when only one person is reported in the family



* Significant difference ($p<0.001$)

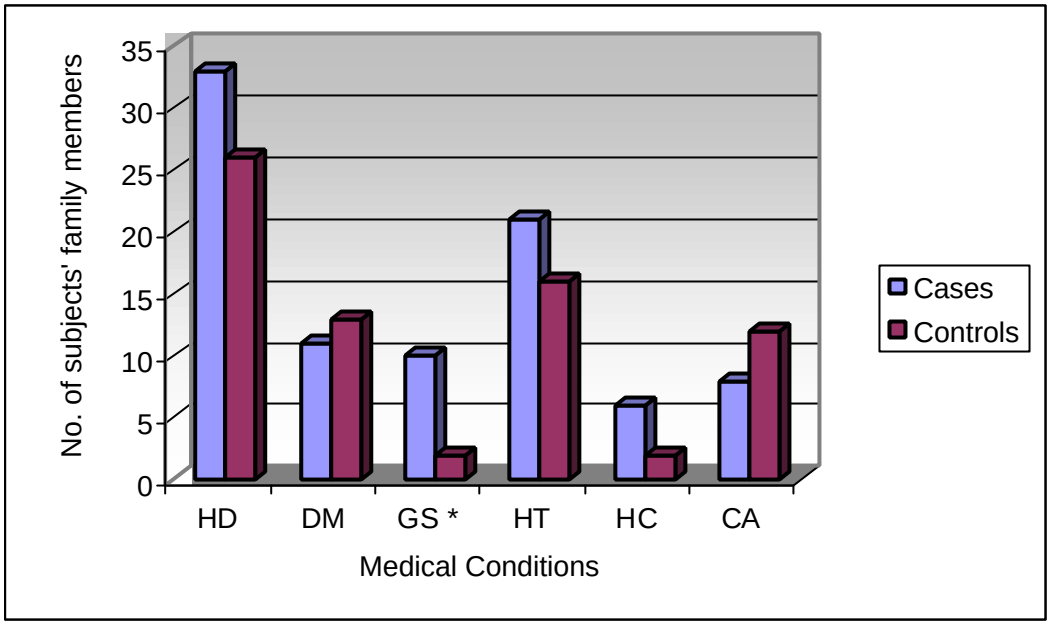
Figure 4.25: Mothers' medical history



* Significant difference ($p<0.001$)

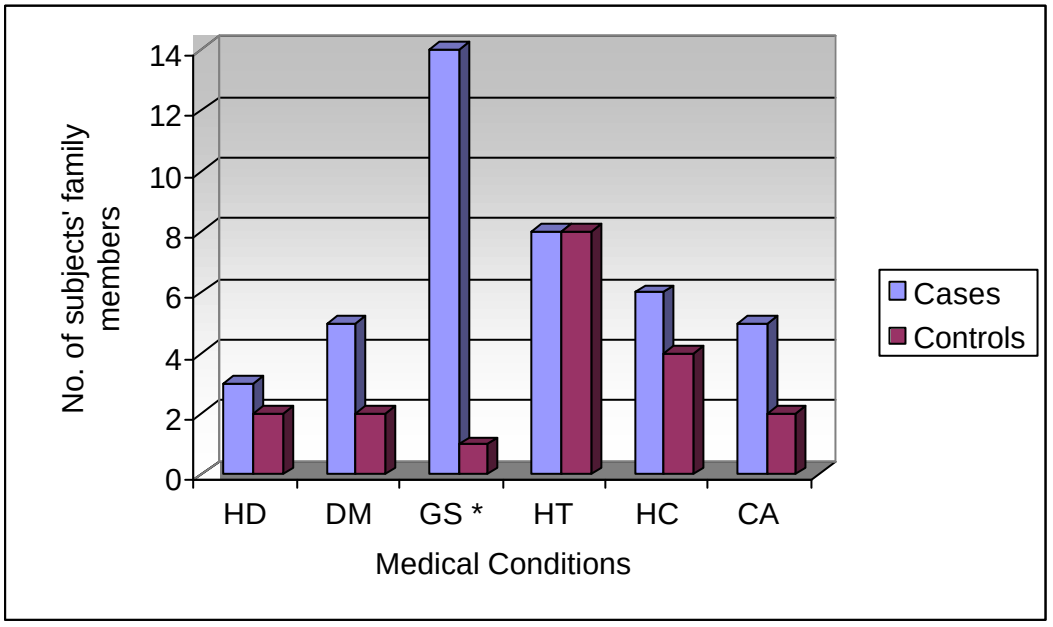
HD – Heart Disease; DM – Diabetes Mellitus; GS – Gallstones; HT – Hypertension; HC – Hypercholesteremia; CA – Cancer; Hol – died during the Holocaust

Figure 4.26: Fathers' medical history



* Significant difference (p=0.033)

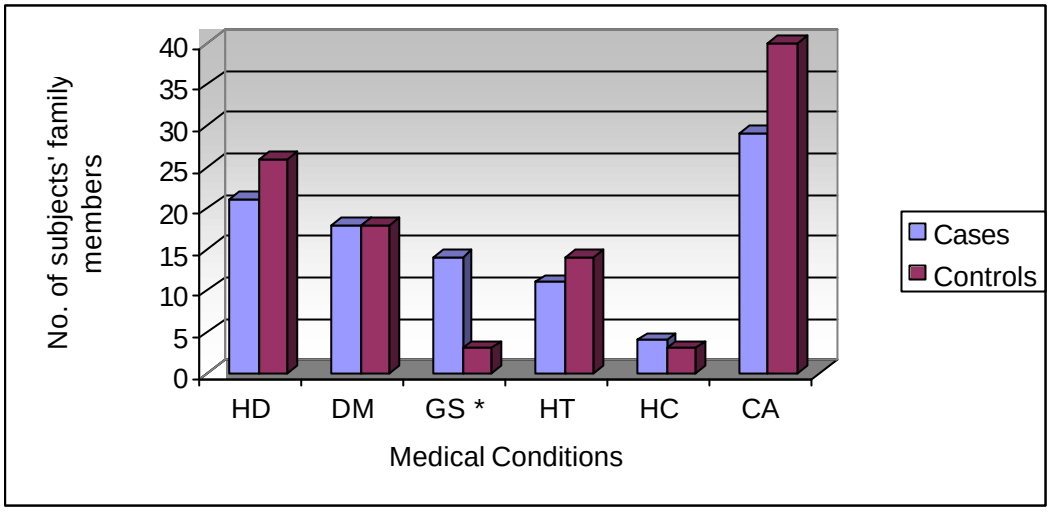
Figure 4.27: Family medical history: Sisters & Brothers



* Significant difference (p<0.001)

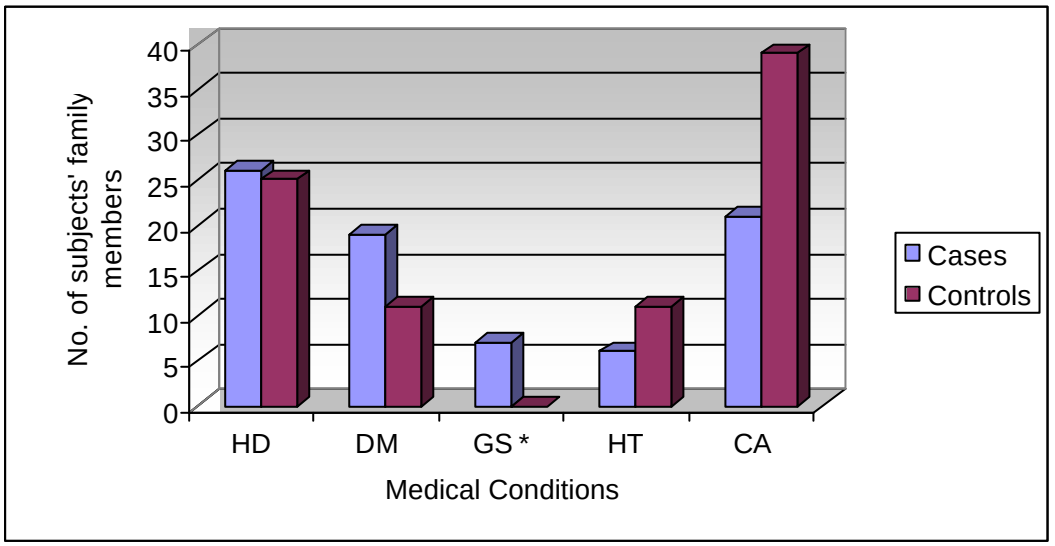
HD – Heart Disease; DM – Diabetes Mellitus; GS – Gallstones; HT – Hypertension; HC – Hypercholesteremia; CA – Cancer; Hol – died during the Holocaust

Figure 4.28: Family medical history: Maternal side of the family (uncles & aunts, grandparents)



* Significant difference ($p<0.001$)

Figure 4.29: Family medical history: Paternal side of the family (uncles & aunts, grandparents)



* Significant difference ($p=0.014$)

HD – Heart Disease; DM – Diabetes Mellitus; GS – Gallstones; HT – Hypertension; HC – Hypercholesteremia; CA – Cancer; Hol – died during the Holocaust

Of the first degree family members we found 44 (86%) members with GSs of cases' families versus 7 of controls' families. Of the 51 GS-carrying members 33 (75%) were females. Of the family members of the maternal side, 18 GS carriers (86%) were of cases' families and 3 of controls'. In the paternal side we found much lower figures, 7 members of cases' families had GSs and none of the controls.

4.9 Physical Activity

No significant difference in physical activity was observed between cases and controls in this study.

4.10 Smoking

At the time of interview only 18 cases and 18 controls reported being smokers. 30 cases and 32 controls, had smoked in the past. None of our analyses could prove a significant difference in smoking habits between cases and controls.

4.11 Parity and Hormonal Intake (females only)

There were no significant differences between female cases and controls in either of the parameters checked, which are shown in figures 4.30 – 4.32. Mean age at menarche, first pregnancy and menopause were almost identical. The same was true for number of pregnancies and deliveries. Use of contraception and hormone replacement therapy (HRT) and breast-feeding was very limited in cases as well as controls. Therefore no significant differences were observed.

Figure 4.30: Mean age at menarche, 1st pregnancy and menopause

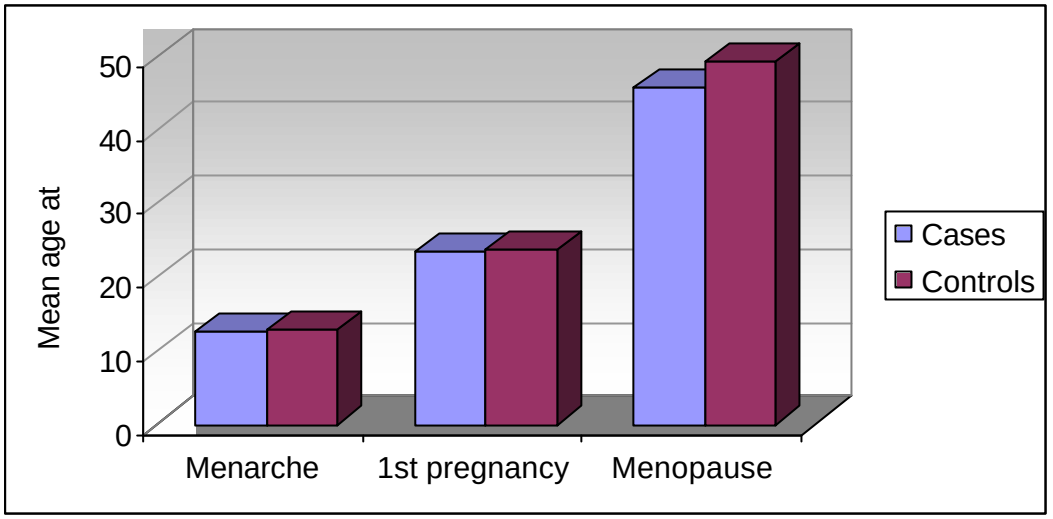


Figure 4.31: Number of pregnancies and deliveries

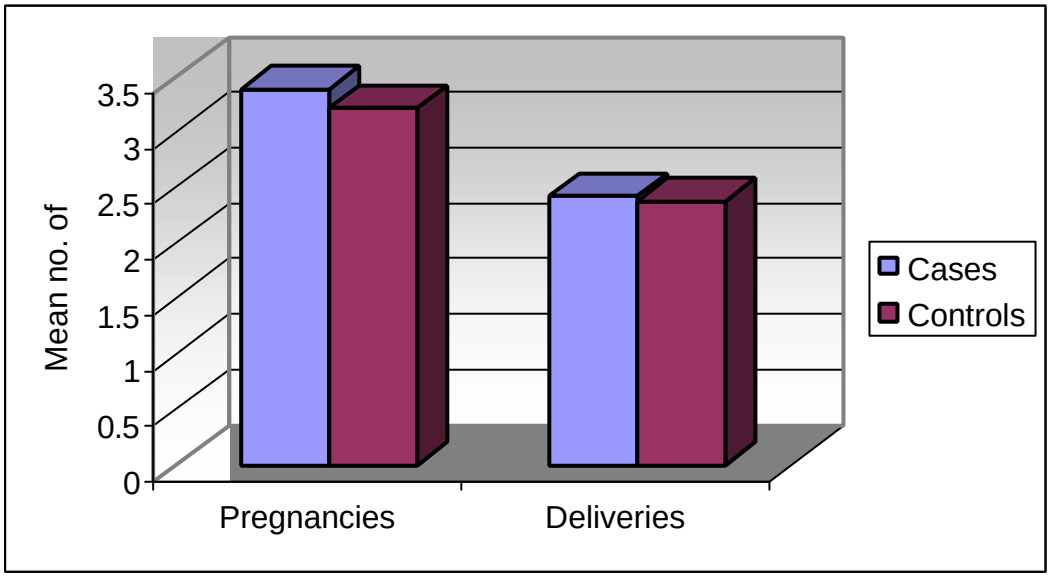
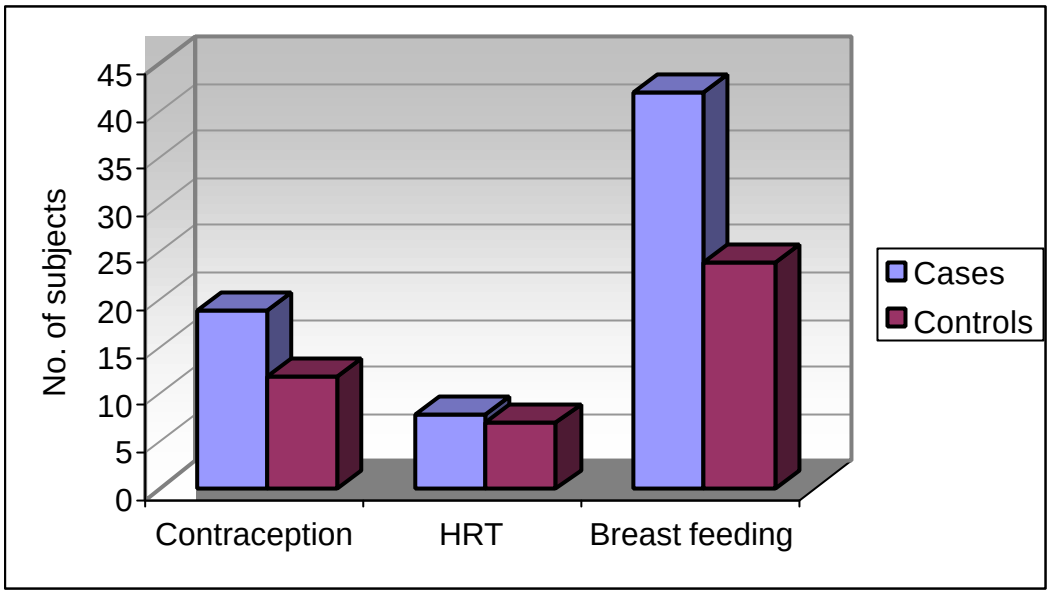


Figure 4.32: Use of contraceptive pills and/or HRT, and breast-feeding



4.12 Serum Lipids

The analysis of all subjects showed that a total serum cholesterol of = 224.00 (table 4.18) elevated the risk of GS development by 2.2 (figure 4.33) as compared to subjects with a figure of = 191.00. The same trend could be observed for those subjects born in Israel. Regarding serum TGs we could also see that subjects with a TG level = 138.00 had a 2.92 fold elevated risk of developing GSs as compared to those with a level = 95.00. The same trend could be observed in females and Israel born subjects.

Figure 4.33: Risk factor: Serum lipids

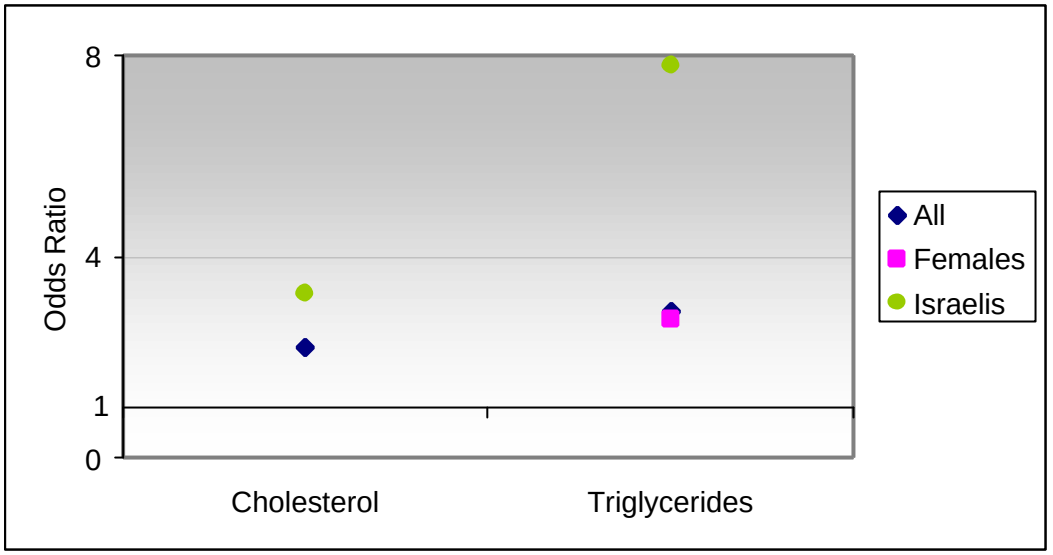


Table 4.18: ORs, p-value, mean intake & percentiles of significant results: Serum lipids

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Total serum cholesterol [mg/dl]	All	2.2	0.042	212.47	191.00	210.00	224.00
	Israel-born	3.274	0.030	209.30	186.00	203.50	221.00
Serum triglycerides [mg/dl]	All	2.923	0.008	124.82	95.00	106.00	138.00
	Females	2.743	0.034	122.33	92.00	106.00	134.00
	Israel-born	7.811	0.001	119.95	92.00	102.00	130.00

Table 4.19: ORs, p-value, mean intake & percentiles of non-significant results: Serum lipids

Item	Group	Odds Ratio	p-value	Mean	33.3 %	50 %	66.6 %
Total serum cholesterol [mg/dl]	Females	2.665	0.054	211.23	189.00	203.50	224.00
	>45 yrs	2.276	0.172	223.86	206.00	221.00	238.00
	<45 yrs	2.808	0.080	200.86	182.00	195.00	210.00
Serum triglycerides [mg/dl]	>45 yrs	1.853	0.262	139.41	107.00	127.50	157.00
	<45 yrs	2.249	0.107	109.94	80.00	93.50	105.00

5. DISCUSSION

Factors associated with GS formation, diet and non-diet related, have been established in the past, and were already presented in depth in chapter 2.4. Following is the discussion of the methodology used in this study and the study results mainly focusing on possible mechanisms and/or biological plausibility.

5.1 Dietary Assessment Method

Measuring energy and nutrient intake in humans is a difficult task. Even under the best possible circumstances, a 10 percent error in precision can be expected[1]. Several different methods have been utilized to assess the energy intakes of specific populations including 24-hour recall, food records, diet history and FFQs. Measured against one another, most of these methods have some degree of validity[2]. The method of choice depends on a number of criteria, including purpose of the survey, the sample size, funds and personnel availability[3]. In this study the FFQ was chosen as the best choice taking into account the criteria mentioned above. A 24-hour recall and food record were not used as the food intake would be limited to a certain day or week, not taking into account seasonality and the months or years prior to GS diagnosis or interview, for cases and controls respectively. Diet histories and FFQs require that study subjects recall their usual dietary intake over a period of time. These methods are employed frequently to estimate the intake of foods by rank or category according to frequency of consumption rather than to provide a quantitative measure of actual intake. The use of these questionnaires is relatively cheap, easy to use and straight forward for analysis.

[1] Stern *et al*, 1984
[2] Block G, 1982
[3] Pekkarinen M, 1970

The FFQ is comprised of a comprehensive list of specific food items to record intakes over a pre-specified period (day, week, month, year). The record is obtained by interview or by self-administered questionnaire.

Pietinen and colleagues[1] examined the reproducibility and validity of a self-administered food use questionnaire with a portion-size picture booklet and a self-administered FFQ, which was developed for a lung cancer intervention trial on 27,000 Finnish men. The reproducibility studies of the questionnaires included 121 men and 107 men, respectively and the validity study included 190 men. The reproducibility was examined by filling out both questionnaires three times at an interval of three months, the validity was compared to 12 day food consumption records, which were recorded twice at an interval of six months. The authors concluded that the food use questionnaire was better suited for the assessment of an individual's habitual intake for a variety of nutrients, whereas the self-administered FFQ was a useful tool for monitoring qualitative changes in the diet, for example after intervention, as its reproducibility was high.

In that context, Wheeler *et al*[2] conducted a study to examine the influence of three different FFQs, and the influence of intervals between their completion. They found no difference between the formats, but observed that the motivation to complete it for the second time was considerably less after 4-6 weeks than after 3 months. Many other validity and reproducibility studies have come to a similar conclusion that FFQs can be used for large populations and gives valid information to estimate nutrient intakes of individuals[3 4].

[1] Pietinen *et al*, 1988
[2] Wheeler *et al*, 1994
[3] Andersen *et al*, 1995
[4] Shimizu *et al*, 1999

The questionnaire can be semi-quantitative (SQFF) when subjects are asked to quantify usual portion sizes of food items, with or without the use of food models and/ or pictures of food portions. There are scholars who have suggested that validity and reproducibility of FFQs need to be assessed for each individual population. Munger *et al*[1] ascertained the intake of women in Iowa using the SQFF which was previously shown by Willett *et al*[2] [3] in Boston women to be valid and reproducible. They found similar levels of reproducibility and agreement relative to the mean of the five 24-hour dietary recalls in Boston women and therefore concluded that the method was accurate enough to classify individuals by nutrient intake in large epidemiological studies.

The major drawbacks of this method include:

- Self-administered FFQ: No control
- FFQ Interview: Inter-and intra-observer bias, less precise quantitation of actual intake

[1] Munger *et al*, 1992
[2] Willett *et al*, 1985
[3] Willett *et al*, 1988

5.2 Diet-Related Factors

5.2.1 BMI and dieting

This study could identify obesity, by means of BMI, as a risk factor, independent of energy intake. This was observed in quite a large number of studies published earlier[1] [2] [3] [4] [5] [6] [7]. The positive association of obesity and GS formation has previously been explained by a hypersecretion of biliary cholesterol, which is more frequently observed in obese people[8] [9]. Salen and collegues[10] observed an elevation of hepatic cholesterol synthesis in patients with GSs, which has been shown in animal studies to be stimulated by insulin[11]. Insulin was observed to be higher in obese subjects and among subjects with GSs [12] [13] [14].

We also found a positive association with high BMI at the age of 18 and GS formation. This was observed earlier by Maclure and his collegues[7]. Though we found BMI at present and BMI at age 18 to be positively associated with GS formation, we did not further examine whether the same subjects that had a high BMI at age 18 were the same as those with an elevated BMI at the time of interview. Therefore no conclusion can be drawn with regard to maintenance of a high BMI or whether some of the subjects had actually lost or gained weight over the course of their life.

[1] Friedman *et al*, 1966
[2] Kern F Jr, 1983
[3] Scragg *et al*, 1984
[4] Diehl *et al*, 1987
[5] Barbara *et al*, 1987
[6] GREPCO, 1988
[7] Maclure *et al*, 1989
[8] Bennion *et al*, 1975
[9] Shaffer *et al*, 1977
[10] Salen *et al*, 1975
[11] Nepokroeff *et al*, 1974
[12] Scragg *et al*, 1984
[13] Stout *et al*, 1978
[14] Norton *et al*, 1968

Rapid loss of weight as a result of fasting in obese subjects has been established as a risk factor in itself[1]. Supersaturation of bile with cholesterol also results from the reduced output of solubilizing BAs that occurs during fasting[2]. Though subjects in this study were questioned about change of dietary habits, including long and/or short term diets, no significant differences among cases and controls were found.

5.2.2 Energy intake

Total energy intake in this study was shown in females to be a risk factor. This is in line with a number of other studies discussed earlier. Other studies found that women with GSs below age 55 ate significantly more[3] [4] [5]. Another study found the opposite in women older than 60[6]. Those observations suggest that other factors might have an effect in GS development, such as hormonal differences among younger and older women. These could be due to menopausal or postmenopausal state and/or use of contraceptives [7] [8] [9]. In this study we did not analyze women separately with regard to age, as the number of women was only 75, and a sub-analysis would have made the results inconclusive due to a small number of subjects in each of the subgroups.

[1] Broomfield *et al*, 1988
[2] Pattinson *et al*, 1986
[3] Sarles *et al*, 1969
[4] Sarles *et al*, 1978
[5] Williams *et al*, 1980
[6] Smith *et al*, 1979
[7] Scragg *et al*, 1984
[8] Royal College of General Practitioners, 1982
[9] Boston Collaborative Drug Surveillance Programme, 1973

5.2.3 Fats and PS

It was observed that total **fat** and especially fat originating from animals was a risk factor in all subjects. This is in agreement with many studies discussed previously. Though animal fat presented itself as a risk factor especially in the older population and those born in Israel with a more than six-fold risk, in the younger subjects also a four times higher risk was shown to disease from GSs. The mechanism of fat has in the past been described as being similar to that of total energy intake and risk of GSs.

The high ORs might be explainable by the fact that foods originating from animal sources have been found to be potential risk factors in several 'Western' diseases and they therefore just might be high risk factors. It might also be due to a generally high consumption of animal foods in Israel, or the subject number was too small after all.

PS turned out to raise the risk of GS development.

Halpern and colleagues[1] on the other hand showed that the addition of egg lecithin to bile prolongs the nucleation time, shifts cholesterol from the vascular phase to the non-vascular phase and reduces the cholesterol/ PLs ratio of the remaining vesicles. These findings suggest a protective effect of PLs.

It was later shown that *in vitro* PS prolongs the nucleation time and diminishes cholesterol crystallization[2].

PS is however a very minor component of biliary PLs in man and in any case ingested PLs are not excreted into bile[3].

If PS indeed promotes GS formation, the mechanism would probably be a metabolic one.

[1] Halpern *et al*, 1993
[2] Ringel *et al*, 1998
[3] Pakula *et al*, 1996

5.2.4 Proteins

The consumption of proteins with regard to risk in GS disease was probably the biggest surprise in our analyses. Never before was protein consumption, especially of animal origin, found to be so strongly associated with the disease under observation. The only explanation we can suggest is that protein consumption raises directly the presence of proteins (pro-nucleating agent) in human bile. This however, was not tested in the present study. The nucleation time of bile from GS patients has been shown to be significantly shorter than that of subjects without GSs[1]. This is commonly interpreted as evidence for the presence of pro-nucleating agents (mainly proteins) in human lithogenic bile[2]. As discussed in Chapter 2.2.4 nucleation is the first step in the formation of GSs. Further studies are required to confirm our findings.

The high ORs might be explainable by a generally high consumption of animal foods in Israel, or the subject number was too small after all.

5.2.5 Dietary fiber

Dietary fiber was shown to be negatively associated with GS development in this study. This has been observed in other studies[3] [4] [5] [6]. The protective properties of complex carbohydrates can also be seen in many other diseases characterized by Western dietary intake. It has been suggested that a low dietary fiber intake increases the risk of GS formation due to decreased colonic motility and the resultant increase in fecal secondary bile acids[7] [8].

[1] Burnstein *et al*, 1983
[2] Portincasa *et al*, 1997
[3] Scragg *et al*, 1984
[4] Jørgensen *et al*, 1989
[5] Sichieri *et al*, 1991
[6] Moerman *et al*, 1994
[7] Heaton *et al*, 1993
[8] Marcus *et al*, 1986

5.2.6 Zinc

Zinc as in other Western diseases was shown to be protective. The mechanism is not well understood.

5.2.7 Drinks (incl. alcohol)

Alcohol consumption was inversely associated with the risk of GSs. This is in line with a number of previous studies which have also reported alcohol as being protective[1] [2] [3]. This might explain the lower prevalence in men as opposed to women, as women generally consume less alcohol than men. Thornton and colleagues[4] suggested that the protective mechanism of alcohol might be due to the HDL cholesterol-raising effect of alcohol and the associated reduction in bile cholesterol saturation. That means that the protective effect of alcohol would be via the liver, by increasing the conversion of cholesterol into BAs[5], or by altering the enterohepatic circulation of bile acids such as DCA[6] which is normally raised in the bile of patients suffering from GSs.

Coffee consumption was not significantly different among cases and controls. A number of studies assessed the association between coffee consumption and GSs though with statistically non-significant results[7] [8] [9] [10] [11] [12] [13].

[1] Scragg *et al*, 1984
[2] Maclure e*t al*, 1990
[3] Leitzmann *et al*, 1999
[4] Thornton *et al*, 1983
[5] Nestel *et al*, 1976
[6] Yoshida *et al*, 1975
[7] Sahl *et al*, 1998
[8] Kratzer *et al*, 1997
[9] Basso *et al*, 1992
[10] Kono *et al*, 1991
[11] La Vecchia *et al*, 1991
[12] Pastides *et al*, 1990
[13] Jørgensen T, 1989

In only two studies the inverse association with coffee consumption was found to be statistically significant[1] [2]. In contrast decaffeinated coffee was not associated with a decreased risk of symptomatic GSs[15].

There are a number of physiological mechanisms that support the protective effect of coffee consumption from GSs. Coffee was found to increase cholecystokinin release[3], enhance GB contractility[4], and may increase colonic motility[5]. These factors were previously related to the development of GSs.

We found that **soft drinks** decreased the risk of GS formation. This may be due to the resultant increased water consumption, which was found to be a protective factor in other diseases[6] [7]. On the other hand some soft drinks are rich in simple/ refined sugars. There have been consistent reports on the positive association between the intake of refined sugars and GS formation, usually in the same studies when there was also a negative association with dietary fiber intake[8] [9] [10]. This observation points to the direction that it is difficult to differentiate which of those two factors have an independent effect. Furthermore, it has been speculated that high intake of refined sugars increase the risk of GS formation as a result the following: the insulin rises and the result is an increased cholesterol synthesis in the liver, part of which is probably released to the GB and leads to a supersaturation of cholesterol in the GB[4] [5] [6] [11] [12].

[1] Misciagna *et al*, 1996
[2] Leitzmann *et al*, 1999
[3] Douglas *et al*, 1990
[4] Keiner F, 1965
[5] Rao *et al*, 1998
[6] Shannon *et al*, 1996
[7] Lubin *et al*, 1997
[8] Daly *et al*, 1997
[9] Bennion *et al*, 1997
[10] Scragg *et al*, 1984
[11] DeLeon *et al*, 1978
[12] Thornton *et al*, 1983

5.2.8 Specific foods

Fish was surprisingly associated with an increased risk. It remained a risk factor even after adjustment for fat and protein. Therefore, maybe another component is responsible for the risk elevation. In Italy the opposite effect was observed[1]. A study done by Berr *et al*[2] found that supplementation of a Western diet with fish oil rich in n-3 PUFAs changed the fatty acid composition of biliary PLs which was associated with a lower cholesterol/PL molar ratio and lower cholesterol saturation of bile, most likely caused by lecithin species-dependent coupling of cholesterol for secretion. On the other hand the supplementation did not protect GS patients from nucleation of cholesterol crystals in supersaturated GB bile; nor did it improve emptying of the GB. The authors' conclusion was that their results were not sufficient to recommend its use for medical treatment or prophylaxis of GS.

One problem with interpreting our result for fish is that we did not differentiate between the different types of fish. That means that fatty fish and less fatty fish were all analyzed as one item which made a more correct interpretation impossible.

Chicken was also found to increase the risk of GS development. It is also important to mention that chicken consumption is relatively high in Israel, due to the fact that beef is expensive and pork is hardly eaten due to religious reasons. Again this could be associated with its fat and protein content. Though after adjustment for fat and protein, chicken remained an independent risk factor, which points to the direction that probably another component is responsible.

[1] Misciagna *et al*, 1996
[2] Berr *et al*, 1992

Olives were found to increase the risk in Israel-born subjects. Though we could not observe the same with olive oil, the team in Israel developed an hypothesis that might explain that risk elevation. Olive oil is known to lower cholesterol in the blood. There is a possibility that the serum cholesterol reduction leads to a cholesterol supersaturation of the bile and thereby to cholesterol crystallization. In Israel, it was found that young Druse Arabs have a high prevalence of GSs and they are known to drink olive oil every morning (Dr. M. Rubin, verbal communication). This is not supported by the results of this present study, where increased serum cholesterol was found to be a risk factor for GSs.

5.3 Non Diet-Related Factors

5.3.1 Medication-and supplement intake

Medication and supplement intake did not show any conclusive results mainly due to the fact that only a very small number was using them. 35 cases and 31 controls used chronic medication, and 33 cases and 39 controls used supplements. It was interesting that only the controls (n=7) used laxatives, whereas elsewhere the opposite was observed[1]. The opposite observation would be attributable to colonic motility as mentioned in chapter 2.

5.3.2 Family history

Family history of GS disease was strongly associated with the risk of GS development. This has been observed in many other studies in the past[2] [3] [4]. The authors concluded that the familial effect could be due to hereditary and environmental factors. We also found that 86% of the first-degree family members who were reported to have GSs were women. On the maternal side of the subjects we found 21 GS carriers compared to 7 on the paternal side. The large differences in reported GS carrying family members between cases and controls could be slightly biased, as GS patients might be more eager to inform themselves of other family members with GSs after diagnosis of GSs. We tried to minimize this by interviewing the subjects closely to their US examination.

[1] Misciagna *et al*, 1996
[2] Sarin *et al*, 1995
[3] Gilat *et al*, 1983
[4] Wheeler *et al*, 1970

A study based in India[1] also found that 65% of the positive relatives were women. In vegetarians the reporting of a family history was more predominant with the female cases[2]. Though this phenomenon is known, the gene responsible remains unknown to this day. Therefore environmental influences might also be responsible, especially as it is rather difficult to differentiate regarding family history whether it is due to genetics or due to similar lifestyle, incl. dietary habits.

5.3.3 Physical activity

Physical activity was not associated with GSs in this study. This confirms a number of previous studies which also could not find an association[3] [4] [5]. However, other studies found a negative association[6] [7] [8]. Several mechanisms were suggested to contribute to this association, including a direct effect of colonic motility[9], and the effect of reduction in insulin and insulin resistance[10].

5.3.4 Smoking

Though in this study no differences in smoking habits were found between cases and controls, another study performed on 96 cases and 118 age and gender matched controls[11] actually found smoking as well as jobs demanding hard labor to be protective. This could be explained by the increase of colonic motility after smoking.

[1] Sarin *et al*, 1995
[2] Pixley *et al*, 1985
[3] Sarles *et al*, 1969
[4] Sarles *et al*, 1978
[5] Jørgensen *et al*, 1989
[6] Misciagna *et al*, 1999
[7] Williams *et al*, 1980
[8] Kato *et al*, 1992
[9] Liu *et al*, 1993
[10] Eriksson *et al*, 1997
[11] Linos *et al*, 1989

On the other hand, in Australia[1] it was observed that current-smokers versus never-smokers had an increased risk of 1.3 in females and 1.6 in males. In Italy[2] smoking was found to be associated with risk of GSs. The results of various studies are thus contradicting.

5.3.5 Hormonal influences (females only)

With regard to the hormonal role, this study could not show any significant differences in female cases and controls in any of the parameters examined (age at menarche, use of contraceptive pills and/or had HRT, number of pregnancies and/or children) mainly because only a small number females used contraceptive pills and/or had HRT.

The same (non-)observation was made in Benha City[3], where neither age at menarche, duration of menstrual life, age at first pregnancy, multiparity nor duration of contraceptive pill use showed any differences between the female cases and controls studied. Neither could a study in vegetarian women[4]. In accordance with our study, others did also not find an association with HRT (estrogen) [5] [6] [7].

Nevertheless, most cohort and CCSs have shown a positive association between pregnancies and GSs[3] [4] [8] [9] [10] [11], fewer studies have not[5] [12].

[1] McMichael *et al*, 1992
[2] Misciagna *et al*, 1996
[3] Abdel-Rahman *et al*, 1993
[4] Pixley *et al*, 1985
[5] Jørgensen T, 1988
[6] Scragg *et al*, 1984
[7] Diehl *et al*, 1980
[8] Misciagna *et al*, 1996
[9] Bernstein *et al*, 1973
[10] Wheeler *et al*, 1970
[11] Friedman *et al*, 1966
[12] Layde *et al*, 1982

In pregnant women as compared to non-pregnant females the lithogenic index of the bile was higher[1], GB volume was larger, and emptying of the GB was frequently impaired[2] [3] [4]. Impaired GB emptying is well documented in cholelithiasis. It has been shown to exist in patients with GSs, persisting after stone dissolution[5]. Jørgensen[6] described significant associations with young age at menarche and abortions. Young age at menarche increases the fertility period. It was found that during the fertility period the lithogenic index in bile increases[7] [8]. A confounding factor could be the observation of the positive association between obesity and early menarche[9].

In Australia[10] it was observed that contraceptive use increased the risk of GS development in young women, whereas it decreased it in the older females. However many other studies did not find this association[11] [12]. The lithogenic index in bile was shown to increase during intake of oral contraceptives[13] [14] [15]. This was observed with progestin, rather than with estrogen in normal doses[16].

[1] Royal College of General Practitioners, 1982
[2] Everson *et al*, 1992
[3] Ylostalo *et al*, 1982
[4] Braverman *et al*, 1980
[5] Festi *et al*, 1990
[6] Jørgensen T, 1988
[7] von Bergmann *et al*, 1986
[8] Bennion *et al*, 1977
[9] Garn *et al*, 1986
[10] Scragg *et al*, 1984
[11] Abdel-Rahman *et al*, 1993
[12] Pixley *et al*, 1983
[13] Kern *et al*, 1982
[14] Bennion *et al*, 1980
[15] Bennion *et al*, 1976
[16] Down *et al*, 1983

5.3.6 Serum lipids

Regarding the analysis of all subjects we found that higher levels of total **serum cholesterol** as well as **serum TGs** significantly elevated the risk of GS formation. The literature is inconclusive regarding the associations with serum lipids. Nevertheless, there are more studies that confirm elevated triglyceride levels in GS patients[1] [2], than hypercholesteremia in GS carriers[3]. In Adelaide, total serum cholesterol was found to have an inverse relation with GS development[5].

A Japanese study[4] could not confirm any relation, neither positive nor negative, between serum TGs and total serum cholesterol and GS disease. Hypertriglyceridemia can be induced by oral contraceptives[5] and was shown in subjects sensitive to sucrose[6], and therefore in the past has been said to only be an additional marker for GS disease in those patients who were exposed to other factors, such as certain diets and use of oral contraception[7]. In this study neither a positive relation with oral contraceptives nor with sucrose intake was observed. Therefore it seems to be an independent factor.

The fact that the Israel-born subjects had a higher levels of serum lipid is not entirely clear. At least with serum cholesterol one would expect a lower level in people who consume a Mediterranean diet, which is rich in olive oil. Olive oil has been shown to lower cholesterol in the blood.

[1] Barbara *et al*, 1987
[2] Tandon *et al*, 1996
[3] Cavallini *et al*, 1987
[4] Kono *et al*, 1988
[5] Wynn *et al*, 1979
[6] Reiser *et al*, 1979
[7] Scragg *et al*, 1984

5.3.7 Family status

Marriage turned out to be a risk factor. As this cannot be a risk factor in itself we sub-analyzed pure married pairs, ie. only those pairs where case and control were married. The risk factors that were found for all subjects were also observed in the married pairs, which confirms that other factors and not being married in itself are responsible for an elevated risk of developing GSs. A study done in Melbourne also found that more cases were married than controls[1]. There the authors concluded that a change in marital status could have an influence on dietary intake, therefore on the subcutaneous fat.

[1] Wheeler *et al*, 1970

6. CONCLUSIONS

The overall results of the study suggest that nutritional factors are strongly associated with GS disease, as risk factors as well as protective factors. The strong association with fat and protein of animal origin, suggests that foods of this origin increase the risk of GS development.

Dietary fiber, zinc and liquids were found to be protective. This suggests that a fruit and vegetable rich diet could be helpful.

In the future research should differentiate between younger and older subjects, as the time-related exposure to potential risk factors might play an important role.

Our study did not relate to the mechanisms of these dietary influences. As many food components are not secreted into bile it is likely that the effects are metabolic or affect minor components in bile. For instance, pro-and anti-nucleating proteins modulate cholesterol crystallization at minute concentrations. If and when dietary studies arrive at agreed results, the mechanisms of the eventually identified dietary components can be studied.

Although at present no food recommendations can be made to reduce the risk of GS development, this study as well as previous studies suggest that a diet rich in complex carbohydrates, low in fat and proteins, especially of animal origin might be beneficial. The same recommendations can be found for a number of other diseases common in Western countries[1] [2].

[1] Diehl *et al*, 1987
[2] Kune G, 1996

7. SUMMARY

The prevalence of cholesterol GSs has substantially increased in the last century, especially in affluent countries. Genetic and environmental factors have been implicated in the pathogenesis of GSs. Yet, the role of many specific environmental factors remains unclear.

Aim

To study the role of diet and lifestyle habits in the etiology of asymptomatic GS disease.

Methods

Subjects with asymptomatic, GB stones detected by abdominal US, after a fast of = 8 hours, were enrolled. A total of 103 newly diagnosed GS patients (75 females, 28 males) were matched by age, gender and ethnic origin to 103 GS -free subjects. All subjects underwent abdominal US and were extensively interviewed on their dietary habits using a FFQ, and on their lifestyle habits. Major food items were analyzed for their PL content and included in the food composition tables.

Results

The paired analysis revealed the following dietary risk factors for the development of GS:

High BMI ($p<0.001$), total fat intake ($p=0.038$), especially fat of animal origin ($p=0.001$), total protein ($p<0.001$), protein of animal origin ($p<0.001$), PS ($p=0.016$), chicken ($p<0.001$), high BMI at age 18 in females ($p=0.007$), in younger subjects ($p=0.029$) and in Israel-born ($p=0.006$), high energy intake in females ($p=0.023$), beef in young subjects ($p=0.029$), fish in older subjects ($p=0.002$), and olives in Israel-born ($p=0.031$).

The following factors were found to be negatively associated:

Dietary fiber (p=0.009), alcohol (p=0.039), starch in older subjects (p=0.030), zinc in Israel-born (p=0.028), dried fruit in young subjects (p=0.022), and soft drinks in Israel-born subjects (p=0.016).

In addition, being married (p=0.002), family history of GS (p<0.001), high serum cholesterol (p< 0.05) and high serum TGs (p< 0.05) were found to correlate with the presence of GSs.

Conclusions

As indicated earlier there are a number of previous studies that dealt with the etiology of GSs, though the results are conflicting. One factor that might have led to the variability of study results is the problem of study design as discussed in chapter 1. This study, however, had many strengths due to its carefully planned study design, e.g. determination of GB status in all studied subjects by US examination, inclusion of recently diagnosed asymptomatic patients only, interview not longer than three months after first diagnosis and the use of a comprehensive, validated FFQ. Nevertheless, there is always a chance of recall bias and observer bias in those kind of studies and this has to be accounted for.

Concluding, the results of the study suggest that nutritional factors are strongly associated with GS disease, as risk factors as well as protective factors. The strong association with fat and protein of animal origin, suggests that foods of this origin increase the risk of GS development. The risk and protective factors which have been identified in this study are very similar to those found in a group of diseases which have been characterized as typical Western diseases[1] [2].

[1] Diehl *et al*, 1987
[2] Kune G, 1996

8. ZUSAMMENFASSUNG

Das Vorkommen von Cholesterin Gallensteinen (GS) hat sich im letzten Jahrhundert deutlich erhöht, insbesondere in den entwickelten Industriestaaten. Eine Vielzahl von genetischen und umweltspezifischen Faktoren wurden in der Vergangenheit im Hinblick auf die Pathogenese als Entstehungsfaktoren von GS genannt. Dennoch blieb die Rolle vieler spezifischer Umweltfaktoren bis dato ungeklärt.

Ziel:

Zielsetzung und Aufgabe der Studie ist die Untersuchung des Einflusses von Ernährung und Lebensstil auf die Etiologie und Entstehung der asymptomatischen GS-Erkrankung.

Methodik:

Patienten, bei denen nach einer 8-stündigen Nahrungsmittelkarenz asymptomatische Gallensteine durch eine abdominale US entdeckt wurden, wurden in die Studie aufgenommen. Insgesamt wurden 103 neu-diagnostizierte GS Patienten (75 Frauen, 28 Männer) basierend auf Alter, Geschlecht und Ethnischem Ursprung 103 GS-freien Subjekten zugeteilt. Alle Studienteilnehmer wurden einer abdominalen US Untersuchung unterzogen und mit Hilfe eines FFQ in extensiven Interviews zu ihrer Ernährungsweise und ihrem Lebensstil befragt. Die wichtigsten Nahrungsmittel wurden nach ihrem Phospholipid (PL) Inhalt untersucht und in die Nahrungsmittel-Zusammensetzungstabellen integriert.

Ergebnisse:

Die Paarweise Analyse enthüllte die folgende positive Beziehung zwischen Ernährungsfaktoren und der Entwicklung von GS:

Hoher BMI ($p<0.001$), gesamt Fetteinnahme ($p=0.038$), insbesondere Fett tierischen Ursprungs ($p=0.001$), gesamt Protein ($p<0.001$), Protein tierischen Ursprungs ($p<0.001$) und PS ($p=0.016$), Huhn ($p<0.001$), Gesamtenergie bei Frauen ($p=0.023$), Fisch bei älteren Untersuchungsteilnehmern ($p=0.002$), Rindfleisch bei jüngeren Untersuchungsteilnehmern ($p=0.023$), Oliven bei in Israel- geborenen Untersuchungsteilnehmern ($p=0.031$).

Bei folgenden Faktoren wurde eine negative Assoziation gefunden:

Ballaststoffe ($p=0.009$) und Alkohol ($p=0.039$), Zink bei in Israel geborenen Untersuchungsteilnehmern ($p=0.028$), Stärke bei älteren Untersuchungsteilnehmern ($p=0.030$) und Trockenobst bei jüngeren Untersuchungsteilnehmern ($p=0.022$).

Desweiteren wurden folgende, von der Ernährungsweise unabhängigen Faktoren, als positiv mit dem Entstehen von GS assoziert: Familiensstatus ($p=0.002$), familiäre Krankheitsgeschichte von GS ($p<0.001$), erhöhter Gesamt-Blut-Cholsterinspiegel ($p<0.05$) und erhöhter Triglycerinspiegel ($p<0.05$).

Abschließende Bemerkungen:

Wie schon zu Beginn angesprochen, gibt es einige ältere Studien die sich mit der Entstehung von GS befasst haben.

Die Ergebnisse dieser Studien sind jedoch größtenteils unstimmig und teilweise konträr. Diese unstimmigen und unterschiedlichen Ergebnissen könnten durch den jeweiligen Untersuchungsaufbau verursacht worden sein. In dieser Untersuchung wurde daher versucht die Probleme der vergangenen Studien soweit wie möglich und gezielt zu vermeiden.

Insbesondere wurde durch einen sorgfältigen Studienaufbau auf folgende Dinge geachtet:

1.) Ultraschall Untersuchung aller Studienteilnehmern

2.) Einbeziehung nur der asymptomatischen Patienten

3.) Ein Interviewer für die gesamte Studie

4.) Durchführung der Interviews innerhalb von 3 Monaten nach Stellung der Erstdiagnose

5.) Nutzung eines verständlichen und akzeptierten Fragebogens (FFQ).

Der Verfasser weist darauf hin, daß die Existenz von 'recall' bias und Beobachter-Bias in solchen Untersuchungen nie ganz ausgeschlossen werden kann. Dieses Risiko muß und wurde daher auch in den statistischen Analysen einkalkuliert.

Die Ergebnisse der Untersuchung zeigen, daß Ernährungsfaktoren einen starken Einfluß auf das Entstehen von GS haben, sowohl als Risikofaktoren als auch als Präventionsfaktoren. Die starke Korrelation mit Fett und Protein tierischen Ursprungs deutet darauf hin, daß möglicherweise Lebensmittel diesen Ursprungs das Risiko der GS Entstehung erhöhen. Die in dieser Studie gefundenen Risiko- und Präventationsfaktoren ähneln einer Gruppe von Krankheiten die als 'typisch westliche Industrie-Krankheiten' charakterisiert wurden[1] [2].

[1] Diehl *et al*, 1987
[2] Kune G, 1996

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10. APPENDIX

10.1 FOOD FREQUENCY QUESTIONNAIRE

Exclusion criteria: I would like to know whether you have ever suffered or suffer from the following diseases:

DISEASE	**no = 0 yes = 1**	**Since when (years)**	**Drugs / Medication**	**Operations**
-Diabetes			_____	_____
-Inflammatory bowel disease			_____	_____
-Renal failure			_____	_____
-Heart failure			_____	_____
-Malignant tumour			_____	_____
Blood tests:				
-Cholesterol _____mg%				
-Triglycerides _____mg%				
Other medical disorders:	**no = 0 yes = 1**	**Since when (years)**	**Drugs / Medication**	**Operations**
-Thyroid disease			_____	_____
-Hypertension			_____	_____
-Constipation (<1 in 72 hrs)			_____	_____
-Diarrhoea (>3 within 1 day)			_____	_____

What is the reason for having this ultrasound examination? _____
_____
Have you had an ultrasound examination done before? _____
If yes, what for? _____
When did the last one take place? _____
Have gallstones been found before, and when? _____

Results of the present examination *(Copy of the results? No=0; Yes=1)*:

-Gallstones: _____

-Number of gallstones: _____

-Size of the big stone: _____

-Units of H.U. in C.T.: _____

INTERVIEW DETAILS:

Date of interview: Time of interview:

Serialnumber:
Place of interview: Home 1

Other (specify):_____

My name is Eylath Kranz. I would like to ask you a few questions about your life-style. In specific: food intake, physical exercise, illness and disease in the previous year.

_____

A. I WILL START WITH GENERAL QUESTIONS.

1. First name and family name:_____
2. Name of father:_____

3. ID: 4. Year of birth: 19

5. Country of birth:_____ 6. Year of immigration:19

7. Father's country of birth:_____

8. Mother's country of birth:_____

9. Current address:_____

Street Place Tel. No.

B. FAMILY STATUS:

married	1
single	2
divorced	3
widowed	4

C. DO YOU CONSIDER YOURSELF AS BEING:

Secular	1
Traditional	2
Religious	3
Orthodox	4

Area Codes *(Place of birth):*	
Western Europe, North America, South Africa, Australia, New Zealand:	01
Eastern Europe:	02
North Africa, Asia:	03
Israel:	04

D: EATING HABITS

Adults may change their dietary habits because of health consciousness, chronic disease, etc. Did you change your nutritional habits in the past, and / or due to your present symptoms?
no 0
yes 1

Change	Reason	Since when?	Comments
Have you changed your eating habits due to teeth or chewing problems? (N=0; Y=1)			
Are you eating (swallowing):1. fast; 2. normal; 3. slow			
Change in eating patterns?			

Interviewer: For each item ask as follows:

- ♦ Do you eat every day, several times a week, several times a month, never?
- ♦ How many (slices, portions, units) do you eat a day?
- ♦ What size (use pictures)?
- ♦ Have you changed your eating habits of (name item) for any reason during the past 15 years?

if yes:

- ♦ How many years ago? (if less than one year change is omitted)
- ♦ How many times a week, how many times a month, did you eat_____ before this change occurred?
- ♦ How many slices, portion, units?
- ♦ What size (use pictures)

I would like to know more about your eating habits. For every food item I would like to know whether you consume it every day, several times a week / month, or never. How many portions / units and portion sizes. Lets start with how you eat each item now. If you changed your eating habits during the last 15 years, please tell me how did you eat then.

Frequency: 7= every day; 1-6= depending on no. of times per week; A= once a month; B= twice a month; C= three times a month; 8= altogether size a week; 9= altogether size a month.

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
	Bread *(P.1)*	Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
0104	Wholemealbread		,			_____		,	
0101	White bread, Chala		,			_____		,	
0103	Rolls *(small)*		,	1		_____		,	1
0105	Pita		,	1		_____		,	1
0106	Crackers, matzos		,	1		_____		,	1
01__	Other_____		,	1		_____		,	1
	Spreads & fats *(P.2)*								
0202	Butter *(spread)*		,			_____		,	
0201	Margarine *(spread)*		,			_____		,	
0211	Butter *(cooking)*		,	1		_____		,	1
0210	Margarine *(cooking)*		,	1		_____		,	1
0216	Olive oil (*teaspoon*)		,	1		_____		,	1
0205	Salad oil:_____		,	1		_____		,	1
0206	Oil (*frying*)		,	1		_____		,	1
0204	Salad mayonnaise (*teaspoon*)		,	1		_____		,	1
	Eggs								
0301	Eggs *(cooked)*		,	1		_____		,	1
0306	Eggs *(fried)*		,	1		_____		,	1
0302	Egg white *(only)*		,	1		_____		,	1

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
	Cakes, cookies & sweets*(P.3 & 4)*	Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
0715	Cake*(sand, chocolate)*		,			_____		,	
0718	Cheese cake		,			_____		,	
071__	Cake:_____		,			_____		,	
0721	Biscuits, cookies *(dry)*		,	1		_____		,	1
0731	Crackers *(salty)*		,	1		_____		,	1
0711	Honey, jam *(teaspoon)*		,	1		_____		,	1
0704	ice cream *(dairy)*^		,			_____		,	
0732	ice cream *(parve)*^		,			_____		,	
0713	Halva *(P.6)*		,			_____		,	
0733	Milk chocolate*		,			_____		,	
0708	Bitter chocolate*		,			_____		,	
1008	Diet-chocolate*		,			_____		,	
0413	Whipped cream *(spoon #)*		,	**1**		_____		,	**1**
07__	Other sweets_____		,	1		_____		,	1
[1=piece; 2=bar]* *[^ 1=scoop; Artic=2 scoops; 3=P.5]* *[# on cake=2 teaspoons]*									
	Hot Drinks *(Mugs)*								
050__	Coffee:_____		,	1		_____		,	1
0701	Cocoa		,	1		_____		,	1
0503	Tea		,	1		_____		,	1
0702	Sugar *(teaspoon(s)/day)*		,	1		_____		,	1
1002	Artificial sweetener		,	1		_____		,	1

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
	Milk products ***(1= portion [250g]=18 teaspoons; 2=teaspoons)***	Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
04__	Milk *(glasses)* ___%		,	1		_____		,	1
0401	Cheese *(yellow) (P.6)*		,			_____		,	
0403	Cheese *(white, 9 %)*		,			_____		,	
0404	Cheese *(white, 5 %)*		,			_____		,	
0426	Low-fat white cheese		,			_____		,	
0406	Salty + melted Cheese *(P.6)*		,			_____		,	
0407	'Leben', Yoghurt		,			_____		,	
0410	Yoghurt (fruit, sweet)		,			_____		,	
0412	Sour cream		,			_____		,	
04___	Other_____		,			_____		,	
	Poultry, Meat								
1203	Schnitzel *(poultry), (P.7)*		,			_____		,	
1201	Chicken, *¼*		,	1		_____		,	1
1202	Chicken *(grilled), ¼*		,	1		_____		,	1
1206	Meatballs *(chicken); (P.8,9)*		,			_____		,	
1205	Turkey *(red), (P.10)*		,			_____		,	
1901	Meat *(cattle), (P.10)*		,			_____		,	
1903	Meatballs *(beef), (P.8,9)*		,			_____		,	
2001	Lamb (shishlik) *(P.10)*		,			_____		,	
2303	Liver *(P.10)*		,			_____		,	
____	Other:_____		,			_____		,	

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
	Cooking style-meats	Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
2219	Poultry & beef, fried		,	1		_____		,	1
2230	Poultry & beef,cooked		,	1		_____		,	1
2240	Poultry & beef, grilled		,	1		_____		,	1
	Sausages								
2201	Salami *(slices)*		,	1		_____		,	1
2203	Turkey breast *(slices)*		,	1		_____		,	1
2202	Hot sausages, *(whole)*		,	1		_____		,	1
	Fish								
1302	Gefillte fish		,	1		_____		,	1
1301	Zoo carp		,	1		_____		,	1
1311	Deep sea fish (*Bakla*)		,	1		_____		,	1
1304	Sardines *(slices)*		,	1		_____		,	1
1305	Tuna, mackerel *(tin*)*		,	1		_____		,	1
1306	Smoked fish *(slices)*		,	1		_____		,	1
1307	Herring *(slices)*		,	1		_____		,	1
13__	Other_____		,	1		_____		,	1
	[tin=170g*]*								
	Cooking style-fish								
1319	Fish fried		,	1		_____		,	1
1330	Fish cooked		,	1		_____		,	1
1340	Fish grilled		,	1		_____		,	1

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
	Cereals ***(1=spoon; 2=P.11)***	Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
0109	White rice		,	2		_____		,	2
0124	Brown rice		,	2		_____		,	2
0112	'Burgol' *(spoon)*		,	1		_____		,	1
0113	Oats [Quaker] *(spoon)*		,	1		_____		,	1
0114	Semolina *(spoon)*		,	1		_____		,	1
0110	Semolina pearls *(soup)*		,	1		_____		,	1
0115	Spaghetti, macaroni *(P.12)*		,	2		_____		,	2
0111	'Kossemet' *(spoon)*		,	1		_____		,	1
0116	Noodles *(soup)*		,	1		_____		,	1
0117	'Shkedej Marak'		,	1		_____		,	1
2401	Bran [Subin] *(spoon)*		,	1		_____		,	1
0121	Corn Flakes *(spoon)*		,	1		_____		,	1
2402	'Granola' *(spoon)*		,	1		_____		,	1
	Ethnic dishes								
0603	Pizza *(regular size)*		,	1		_____		,	1
0604	'Tschulend' *(chamin) (P.11)*		,	2		_____		,	2
0607	'Lewiwot, Knisches, *(& potato dishes)*		,	1		_____		,	1
0608	'Kube' *(piece)*		,	1		_____		,	1
0610	'Falafel' *(½ port., 3 balls)*		,	1		_____		,	1
0611	'Humus salad' *(spoon)*		,	1		_____		,	1
0612	'Tehina salad' *(spoon)*		,	1		_____		,	1
0613	'Chilbe' *(teaspoon)*		,	1		_____		,	1

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
		Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
0420	Cheese dishes: 'Pashtedot',Blintzes		,	1		_____		,	1
0620	'Malauach, Jachnun'		,	1		_____		,	1
	'Pitzuchim' ***(2=100g; 1=single)***								
1401	'Garinim' [Sunflower]		,			_____		,	
1403	Almonds		,			_____		,	
1404	'Egosim' [Nuts]		,			_____		,	
1402	'Botnim' [Peanuts]		,			_____		,	
1405	'Fistuk Chalavi'		,			_____		,	
	Legumes								
1110	'Tivol' *(1=Shnitzel; 2=sausage)*		,			_____		,	
1101	Dried 'Shuit' *(1=spoon; 2=P.11)*		,			_____		,	
1103	Dried lentils *(spoon)*		,	1		_____		,	1
1104	Dried peas *(spoon)**		,	1		_____		,	1
1102	Dried 'Humus' *(spoon)*		,	1		_____		,	1
	*[*In soup = 2 spoons]*								
	Vegetables *(P.13)*								
1701	Olives *(single)*		,	1		_____		,	1
0935	Pickles *(single)*		,	1		_____		,	1
0937	'Resek Agwaniot'		,	1		_____		,	1
0901	Avocado, *¼*		,	1		_____		,	1
0902	Tomatoes		,	1		_____		,	1
0903	Cucumber		,	1		_____		,	1

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
		Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
0904	Radish		,	1		_____		,	1
0932	Onion		,	1		_____		,	1
0905	Green Onion		,	1		_____		,	1
0906	Lettuce *(P.13)*		,	2		_____		,	2
0907	Green pepper, paprika		,	1		_____		,	1
0909	Carrots *(1=one; 2=P.13; 3=P.5)*		,			_____		,	
0940	Broccoli		,	2		_____		,	2
0916	Cauliflower		,	2		_____		,	2
0912	Cabbage (red/white)		,	2		_____		,	2
0913	'Shuit' (green/yellow)		,	2		_____		,	2
0915	'Bamia' [Okra]		,	2		_____		,	2
0929	Fresh Peas		,	2		_____		,	2
0924	Corn, maize *(1=one; 2=P.13)*		,			_____		,	
0918	Eggplant salad *(1=spoon); 2=P.14)*		,			_____		,	
0917	Eggplants *(fried)*		,	1		_____		,	1
0919	Spinach		,	2		_____		,	2
0921	'Lefet' [Turnip]*(single)*		,	1		_____		,	1
0922	Celery *(spoon)*		,	1		_____		,	1
0923	Artichoke *(single)*		,	1		_____		,	1
0910	Zucchini *(1=single; 2=P.13)*		,			_____		,	
0927	'Selek' [Beet root] *(1=one; 2=P.13)*		,			_____		,	
0931	Chips *(P.11)*		,	2		_____		,	2
0930	Potatoes *(1=one; 2=P.13)*		,			_____		,	

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
		Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
0938	Parsley, Dill *(portion)*		,	1		_____		,	1
0920	'Ful' [Broad bean] *(P.13)*		,	2		_____		,	2
0934	Garlic *(tooth)*		,	1		_____		,	1
0615	Ketchup *(spoon)*		,	1		_____		,	1
0___	Other:_____		,	1		_____		,	1
	Cooking style vegetables								
0942	Vegetables fried with onions		,	1		_____		,	1
0941	Fried / cooked with (little) oil		,	1		_____		,	1
	Fruits *(1= single); [seasonal]*								
0801	Oranges		,	1		_____		,	1
0802	Grapefruits *(½)*		,	1		_____		,	1
0803	Clementines		,	1		_____		,	1
0804	Lemons		,	1		_____		,	1
0805	Freshly pressed juice		,	1		_____		,	1
0807	Apples		,	1		_____		,	1
0809	Pairs		,	1		_____		,	1
0810	Bananas		,	1		_____		,	1
0813	Grapes *(Cluster)*		,	1		_____		,	1
0811	Strawberry*		,			_____		,	
0812	'Afarsemon' [Persimmon]		,	1		_____		,	1
0814	Honey melon *(½ small)*		,	1		_____		,	1
*(*single=1; 100g=2; 3=P.5)*									

CODE	FOOD ITEM	LATELY					PREVIOUSLY		
		Frequency	No. of portions	Portion size	Time changes	Comment	Frequency	No. of portions	Portion size
0815	Watermelon *(¼)*		,	1		_____		,	1
0816	'Afarsek' [Peach]		,	1		_____		,	1
0817	Apricots		,	1		_____		,	1
0818	Plums		,	1		_____		,	1
0819	'Shessek' [Loquat]		,	1		_____		,	1
0820	Fresh figs		,	1		_____		,	1
0825	Mango		,	1		_____		,	1
1817	Dried apricots		,	1		_____		,	1
1818	Dried plums		,	1		_____		,	1
1820	Dried figs		,	1		_____		,	1
1802	Dried dates		,	1		_____		,	1
_____	Other:_____		,			_____		,	
	Drinks *(glasses)*								
1505	Preserved Juice		,	1		_____		,	1
0500	Water / Soda		,	1		_____		,	1
1503	Lemonade		,	1		_____		,	1
1507	Coca Cola		,	1		_____		,	1
1601	Wine *(100g)*		,	1		_____		,	1
1603	Beer *(glass)*		,	1		_____		,	1
1605	Cognac/Whisky		,	1		_____		,	1
	Other Foods or Drinks worth noting								
_____	_____		,			_____		,	

E. SUPPLEMENTS

Have you, in the past year, taken the following supplements:

Supplement	1=Yes, 0=No	Since when? (year)	If in the past: When? (year)	If in the past: For how long? (months)
Calcium				
Vitamin D, Multivitamin				
Fish oil				
'Tachshirei Subin'				

Have you got a food allergy?_____ Since when?

F. DRUGS

Have you taken, or still take, drugs for a period of 3 months or longer? (15-20 years back)

Reason (Disease)	Name/Kind of drugs	Regularly? 0=No; 1=Yes	Period of intake Months Years
_____	_____		_____
_____	_____		_____
_____	_____		_____
_____	_____		_____
_____	_____		_____

G. FOR WOMEN ONLY

Age at first pregnancy	Age at first menarche	Age at menopause	No. of "life" births	No of pregnancies

Pill (1=Yes; 0=No)	Oestrogen (1=Yes; 0=No)	Others (1=Yes; 0=No)

Months of lactation:

H: PHYSICAL ACTIVITY

Please give details on the number of hours during a day/week/month you are spending on the following activities:

Frequency: 7= every day; 1-6= depending on no. of times per week; A= once a month; B= twice a month; C= three times a month; 8= altogether size a week; 9= altogether size a month.

Degree of activity	Kind of activity	Frequency	No of hours
Resting	Sleeping, lying	7	
Sitting	At work		
	While watching TV, reading		
	During meals	7	
	While driving		
Light activity	At work		
	At home (incl. cooking)		

		In the past few years			Before the change	
		Frequency	No of hours	If there has been a change in activities, how many years ago?	Frequency	No of hours
Moderate / Heavy Activity	At work: _____					
	Gardening					
	Cleaning					
	Other:_____					
Voluntary activity	Distance walking					
	Jogging					
	Gymnastics					
	Swimming					
	Dancing					
	Other:_____					

How many times per day are you using stairs?

How many floors?

I. EDUCATION

Years of education: _____

Profession: _____

Husband's profession: _____

Please give details on the places where you have worked so far:

Name of workplace	No of years	Your job? (Profession)	What is produced?	Have you been in contact with any chemicals? Specify.
_____		_____	_____	_____
_____		_____	_____	_____
_____		_____	_____	_____
_____		_____	_____	_____
_____		_____	_____	_____

01 = Lead (oferet)	02 = Pesticides	03= Organic solvents
04 = Rays	05 = Colour	__ = Other:_____

K. SMOKING

1.Are you smoking? No 0
Yes 1 *(continue with 3)*

2.Have you ever smoked? No 0 *(continue with L)*
Yes 1

3.How many cigarettes/day?

4.Have you ever smoked more? No 0 *(continue with 6)*
Yes 1

5.How many did you smoke then?

6.When did you start?

7.When did you quit?

8.How many years did you smoke?

L. WEIGHT

During the past years: Most of your life:

At age of 18*:

{* If not known: 001=thinner; 002=fatter; 003=No change}

M. HEIGHT

cm

N. FAMILY HISTORY

Please specify on diseases/illnesses that have occurred in your family, also on deceased family members:

Member	**Dead=0 Alive=1**	**Disease codes and names**
Mother		
Father		
Grandmother (mother)		
Grandfather (mother)		
Grandmother (father)		
Grandfather (father)		

Member	**How many?**	**Disease codes and names** **1. 2. 3. 4. 5.**
Daughters		
Sons		
Sisters		
Brothers		

Member	**How many?**	**Disease codes and names** **1. 2. 3. 4. 5.**
Aunts (mother)		_____
Uncles (mother)		_____
Aunts (father)		_____
Uncles (father)		_____

24 Hour Recall

Time	What was consumed?	Portion	Position