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Iron, Folate and Vitamin B12 Status of Apparently Healthy Irish Adult Women Attending General Practitioners in Inner-city Dublin

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IRON, FOLATE AND VITAMIN B_{12} STATUS OF APPARENTLY HEALTHY IRISH ADULT WOMEN ATTENDING GENERAL PRACTITIONERS IN INNER-CITY DUBLIN.

By

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Table of contents.

Abstract.

There is substantial nutrition related morbidity and mortality among men and women in developed countries. Certain events during the course of a woman's life such as menstruation, pregnancy, lactation and the menopause may compromise haematinic nutrient status. The purpose of this study was to investigate iron, folate and vitamin B_{12} status among a sample of apparently healthy, non-pregnant, Irish adult women attending general practitioners in inner-city Dublin. Dietary, socio-economic, medical and lifestyle factors contributing to status of these haematinic nutrients were extensively examined.

The initial part of the work validated the methods in a representative sample of inner-city Dublin women (n=35, mean age 40.3 years, SD 5.5 years). The Food Intake Questionnaire was developed and validated against the Diet History and biomarkers of haematinic nutrients in blood samples. An 'Interview Questionnaire' was also developed and tested for reliability.

The main study examined iron, folate, vitamin B_{12} and homocysteine levels in a sample of inner-city Dublin women (n=104, mean age 32.8 years, SD 11.2 years). Iron deficiency anaemia and low iron stores were discovered in 2.8% (n=3) and 14.4% (n=15) of the women respectively. Folate deficiency was found in 1.9% (n=2) of the female volunteers and a further 48% (n=50) had sub-optimal folate levels for women of childbearing age. Plasma vitamin B_{12} levels were all within the normal range. Plasma homocysteine levels were elevated in 12.5% (n=13) of the sample.

Attitudes to nutrition and determinants of food choice were also assessed among the female volunteers (n=104, mean age 32.8 years, SD 11.2 years). Accuracy of self-reported dietary perceptions with regard to adequacy of nutrient intakes and food consumption was examined. The relationship between self-reported physical activity level and BMI confirmed that leisure time physical activity is an important determinant of body weight.

Body image concerns and slimming practices were assessed among the female volunteers (n=134, mean age 34.7 years, SD 10.7 years). Dissatisfaction with body weight was pervasive within the group at 82% (n=110). Many had used unsafe slimming practices such as smoking (30%, n=33) and diet pills (15%, n=16) in an attempt to lose weight. The results of this study highlight important nutritional issues for women in a primary healthcare setting.

Chapter 1

Nutritional and lifestyle factors in women's health in developed countries.

1.1 Gender differences in health and disease.

All over the world women live longer than men do, however even in developed countries they do not necessarily live better, healthier lives (Craft 1997). Women appear to suffer from different disorders at different stages of their lives than men. Certain physiological events during the course of a woman's life, such as menstruation, pregnancy, lactation and the menopause, place her at greater risk of poor nutritional status than males of the same age (Hallberg and Rossander-Hulten 1991).

It is well established that there is substantial nutrition related morbidity and mortality among men and women in industrialised countries where eating patterns contribute to several of the leading causes of death and disability, including cardiovascular disease, some cancers, stroke and diabetes mellitus (Glanz *et al* 1997). Dietary guidelines for the prevention of chronic disease are similar in developed countries and are applied equally to men and women (DHSS 1991, Food Safety Authority of Ireland 1999). Research on populations has traditionally been restricted to males and many of our models of human physiology and of the pathophysiology of disease are therefore skewed (Murphy *et al* 1977, Manson *et al* 1990, Legato 1998). This lack of differentiation by sex in medical investigation has given rise to the assumption that what is true for males is equally applicable to females, although in many cases this has not been scientifically verified (Legato 1998). Women were traditionally seen as more difficult to study accurately than men (Legato 1998). One of the major reasons for the exclusion of women from research studies has been the menstrual cycle, which introduces a potentially confounding variable into the analysis of data (Healy 1991, Craft 1997, Legato 1998). Several authors have pointed out the complex ethical questions involved in testing the pre-menopausal female (Healy 1991, Craft 1997, Legato 1998). Experimentation on women may expose foetuses to unknown risk and excluding women who may be pregnant involves intrusive questioning of volunteers or invasive testing (Ressler 1998, Craft 1997).

In recent years it has been recognised that gender is a critically important variable in disease and that male and female normal physiology are significantly different in almost every system of the body (Legato 1998). Some diseases are unique to women; others, such as cardiovascular disease may have different effects on women (Healy 1991, Craft 1997) and as the body of knowledge on women's health increases, the need for gender-specific care becomes apparent. Greater emphasis on nutrition counselling in primary health care could substantially reduce health care costs, improve quality of

life and promote greater longevity for both men and women (Glanz *et al* 1997). The future of nutrition counselling may lie in the provision of gender-specific therapy for male and female patients in an attempt to reduce some of the chief causes of death and disability in developed countries.

1.2.1 The impact of cardiovascular disease on women's health.

The differences in how men and women experience disease have been most comprehensively studied in the area of cardiovascular medicine. Cardiovascular disease has long been recognised as the leading cause of death among middle-aged men, but it is an equally important cause of death and disability among older women (Rich-Edwards *et al* 1995). By the age of sixty years only one in seven women in the United States has had a coronary event compared with one in five men (Rich-Edwards *et al* 1995). After the age of sixty years, however, cardiovascular disease is the primary cause of death among American women (Rich-Edwards *et al* 1995). In the United Kingdom for women of any given age the death rate for cardiovascular disease is approximately equivalent to that seen in men ten years earlier (WHO 1989, Williams 1997). Women have a greater mortality rate post myocardial infarction (Walsh & Grady 1995). This may be related to their older age at occurrence or the higher prevalence of diabetes and hypertension among women who experience cardiovascular events (Williams 1997). Women are also less likely to be offered intensive surgery or drug therapy after a myocardial infarction than men (Isles et al 1992) This less proactive treatment of females may also be linked to the age of the patient when they suffer their first cardiovascular event (Williams 1997).

1.2.2 Epidemiology of cardiovascular disease.

In Ireland vascular diseases, such as stroke and cardiovascular disease, accounted for forty two percent of all deaths in 1998 (WHO 1997-1999). Vascular diseases explained thirty five percent and twenty nine percent of mortality among Irish men and women respectively in 1998 (WHO 1997-1999). Cardiovascular disease is no longer perceived as an exclusively male disease, but as a significant cause of mortality in both sexes (Rich-Edwards *et al* 1995).

1.2.3 Cardiovascular disease risk factors.

Research has uncovered several unique aspects of cardiovascular disease in women. These gender differences include anatomical characteristics: women's hearts are two-thirds the size of the male heart and women have smaller coronary arteries (Kilip 1993, Legato 1998, Krauss *et al* 2000). While the risk factors for cardiovascular disease are qualitatively similar in men and women, they appear to have different effects as a function of gender (Legato 1998). Various expert groups in developed countries have formulated dietary and lifestyle guidelines for populations based on available epidemiological evidence. These guidelines represent practices that all individuals can safely follow throughout the lifespan as a foundation for achieving and maintaining cardiovascular and general health (DHSS 1991, FAO/WHO 1994, Krauss *et al* 2000). A large body of evidence has established hyperlipidaemia as a risk factor for cardiovascular disease (Williams 1997). This risk factor is modifiable by diet in most individuals. Dietary guidelines for the prevention of cardiovascular disease include several recommendations on fat intake (DHSS 1991, FAO/WHO 1994, Krauss *et al* 2000). The aim is an optimal level of dietary fat, which will reduce obesity, total cholesterol and LDL cholesterol without elevating plasma triglycerides or reducing plasma HDL cholesterol (Gibney 1999).

Most expert committees have advocated a reduction in the proportion of dietary energy from fat (DHSS 1991, FAO/WHO 1994, Krauss *et al* 2000). A review of several well-controlled studies showed that this results in a reduction in total and LDL cholesterol (Kris-Etherton & Yu 1997). When total fat intake is reduced intakes of atherogenic saturated fatty acids will automatically decrease but, depending on the overall dietary fat profile, the protective HDL cholesterol may be reduced (Kris-Etherton & Yu 1997, Gibney 1999). Also, it is argued that a reduction in total fat intake associated with an increase in carbohydrate intake leads to elevation of plasma triglycerides and a reduction in plasma HDL cholesterol (Katan *et al* 1997, Gibney 1999). This represents a particularly atherogenic profile in women (Katan *et al* 1997). This evidence suggests that the level of dietary fat is not the critical factor, rather the composition of the dietary fatty acid profile. This blood lipid profile of raised plasma triglycerides and low HDL cholesterol is frequently observed in non-insulin dependant diabetes (Knopp *et al* 1993). Diabetes has been found to be twice as important a risk factor for cardiovascular disease risk factor in women compared to men (Knopp *et al* 1993). The

results of a recent prospective study examining the effects of dietary glycaemic load on cardiovascular disease risk in women suggest that a prolonged high dietary glycaemic load from refined carbohydrate independently increases the risk of cardiovascular disease (Liu *et al* 2000). A cohort of 75,521 women aged between 38 and 63 years with no previous diagnosis of diabetes, myocardial infarction, angina, stroke or other vascular disease in 1984 was followed for ten years (Liu *et al* 2000). Each participant's dietary glycaemic load, based on glycaemic index, cardohydrate type and frequency of intake of individual foods, was calculated retrospectively from data collected using a validated food frequency questionnaire at baseline (1984) and in 1986 and 1990 (Liu *et al* 2000). During the ten year follow-up period 761 cases of cardiovascular disease were documented (Liu *et al* 2000). Dietary glycaemic load was found to be directly associated with cardiovascular disease risk after controlling for age, smoking, BMI, family and personal history of cardiovascular disease, raised cholesterol, menopausal status, aspirin use, energy intake and vitamin supplement use (Liu *et al* 2000). The association between glycaemic index and cardiovascular disease risk was most evident in women with a BMI greater than $23kg/m^2$ and is thought to be a function of insulin increasing resistance (Liu *et al* 2000). Low glycaemic index foods may therefore play a role in the primary prevention of insulin resistance and associated metabolic abnormalities (Bell 1997, Liu *et al* 2000).

1.2.4 Gender-specific guidelines for cardiovascular disease prevention.

While a reduction in dietary saturated and *trans* fatty acid intakes is desirable in both sexes for the reduction of LDL cholesterol (Kris-Etherton & Yu, Gibney 1999), the above findings underline the need for more gender-specific advice for the prevention of cardiovascular disease. Obesity is associated with hypertension (Kannel *et al* 1996), insulin resistant glucose intolerance (Feskens & Van Dam 1999) and dyslipidaemia (Mann & Chisolm 1999). With the increasing prevalence of overweight and obesity in industrialised societies (Flegal *et al* 1998, IUNA 2001) strategies for the prevention and treatment of excess body fat should be central to public health campaigns to prevent cardiovascular disease. The association between obesity and insulin resistance is particularly detrimental to the female cardiovascular risk profile as it contributes to hypertriglyceridaemia (Katan *et al* 1997). The hypertriglyceridaemia of obesity is characterised by the overproduction of VLDL particles and increased amounts of

triglyceride per particle (Mann & Chisolm 1999). Reducing caloric intake and increasing physical activity to achieve even a modest weight loss can improve insulin resistance and the concomitant metabolic abnormalities (American Diabetes Association 1999). In light of recent evidence it may also be prudent to consume low glycaemic index carbohydrate foods frequently in order to reduce the overall daily glycaemic load which may be important in the development of insulin resistance (Liu *et al* 2000). Diets rich in high soluble fibre carbohydrate foods may produce improved insulin sensitivity (Bell 1997, Liu *et al* 2000). Effects of fibre on glycaemic control are likely to be caused by delayed gastrointestinal carbohydrate absorption (Bell 1997).

Consumption of moderate to substantial amounts of alcohol also gives rise to hypertriglyceridaemia and alcohol reduction rapidly leads to a reduction in plasma triglyceride levels (Crouse & Grundy 1984). Women may metabolise alcohol less efficiently as an identical dose produces higher serum levels in women compared to men (Renaud & DeLorgeril 1992, Baraona *et al* 1994). Adherence to current guidelines for alcohol consumption may, therefore, be particularly important for women in view of its effect on plasma triglycerides. The use of certain fruits, vegetables and wholegrain cereals as the main dietary sources of carbohydrate may be beneficial in reducing the risk of carbohydrate induced hypertriglyceridaemia (Sonnenberg *et al* 1996, Bell 1997, Krauss *et al* 2000). The inclusion of a portion of dairy food at two meals per day has also been shown to lower the overall daily glycaemic index by promoting better glycaemic control at subsequent meals (Wolever 2000).

The benefits of regular physical activity (Williams 1997, Krauss *et al* 2000) and a moderate intake of monounsaturated fatty acids combined with a reduction in trans fatty acid intake (Kris-Etherton & Yu, Krauss *et al* 2000) for maintaining plasma HDL cholesterol levels, together with the hypotriglyceridaemic properties of polyunsaturated fatty acids of the n-3 series (Burr *et al* 1989, Roche 1999, Krauss *et al* 2000) and the effect of dietary glycaemic load on insulin sensitivity (Liu *et al* 2000) are dietary factors particularly important to women. These gender differences must be considered in the formulation of dietary guidelines for the prevention of cardiovascular disease in populations.

1.3.1 Epidemiology of cancer.

Several dietary and lifestyle factors have been implicated in the aetiology of cancer, however the precise nature and magnitude of the relationship between nutrient intakes has not yet been established. Most of the research carried out to date investigates the possible role of diet in the aetiology of breast and colon cancer. Breast cancer is second only to lung cancer as the most common cause of cancer mortality in American women (M^{c}Keown 2000). In terms of the total American population, colon cancer is the second most common cause of cancer mortality (Shike 1996, Kim 2000). In Ireland cancers accounted for 23% of all deaths in 1998 (WHO 1997-1999). Of these fatal cases of neoplastic disease, 8% were breast cancer and 31% involved the digestive organs (WHO 1997-1999).

1.3.2 Nutrition and colon cancer.

Up to 50% of colon cancers have a well-recognised genetic component, however dietary and lifestyle factors may play an essential role in the carcinogenic process (Shike 1996). Obesity, combined with low physical activity, has been associated with elevated risk of colon carcinoma (Kohl *et al* 1988, Lee *et al* 1991). Although there is no conclusive evidence linking increased body mass index with colon cancer, obesity may be a marker for increased risk through other dietary and lifestyle factors. Clarification of the complex relationship between overweight and colon cancer may contribute to the definition of healthy weight ranges. There is evidence to suggest that diets high in saturated fat and energy and low in fruit, vegetables and dietary fibre predispose individuals to the development of colon cancer (Shike 1990, Potter *et al* 1993). Red meat consumption has also been implicated in colorectal carcinogenesis. Suggested mechanisms by which meat increases colorectal cancer risk include the formation of hetracyclic amines in meat when it is cooked (Bingham 1999). Meat may also enhance the production of *N*-nitroso compounds in the colon and these compounds possibly act as carcinogens (Bingham 1999). Population studies of meat and colorectal cancer, reviewed by Hill (1986) generally show a strong correlation between red meat intake and colorectal cancer. These similar results arise through the use of broadly the same database (Hill 1999). Case control studies provide little evidence of a link, as they tend to show only a weak, if any, correlation between meat intake and colorectal cancer risk (DHSS 1998, Hill 1999). Only one cohort study to date (Willett *et al* 1990) has shown a statistically significant dose-response relationship between red meat and

colorectal cancer; another cohort study (Giovannucci *et al* 1994) showed an elevated risk only at the highest intake (129g/day) (Hill 1999). Several studies have shown no relationship (Thun *et al* 1992, Bostick *et al* 1994, Hill 1999). A large body of research evidence points to the intake of protective factors such as fruit, vegetables and wholegrain cereals as the main determinants of colorectal cancer risk (Franceschi *et al* 1997, Gaard *et al* 1996, Hill 1999). During the last thirty years the intake of red meat has fallen steadily to 75% of its 1965 value (Hill 1997, Hill 1999), while during this time the incidence of colorectal cancer has increased by almost 50% (Levi *et al* 1993). The World Cancer Research Fund (1997) published a global report on diet and cancer, this report recommended that if eaten at all red meat intake should be limited to 80g per week. However in light of the available evidence this recommendation appears to be erroneous. The unnecessary exclusion of red meat, an excellent source of bioavailable dietary iron, can compromise haematinic status particularly among vulnerable groups such as menstruating and pregnant women, adolescents and toddlers. Greater emphasis should be placed on the avoidance of overweight through adequate physical activity, avoidance of smoking and the consumption of fruit, vegetables and wholegrain cereals for cancer prevention and for general health.

1.3.3 Nutrition and breast cancer.

Body mass index may be associated with risk of breast carcinoma. Analysis of a cohort of 2956 women, involved in the Nurses' Health Study who developed breast cancer, showed a positive association between breast cancer risk and body mass index in women who had never used hormones and who experienced weight gain after eighteen years of age (Huang *et al* 1997). Research is ongoing and as yet no conclusive evidence has been found linking dietary fat with the aetiology of breast cancer. An increasing ratio of n-3 polyunsaturated fatty acids to total n-6 fatty acids in the adipose tissue has been associated with a trend toward a decreasing risk of breast carcinoma in one study (Simonsen *et al* 1998). Within the EURAMIC study an increase in breast cancer risk was observed in women with greater stores of *trans* fatty acids in the adipose tissue (Kohlmeier *et al* 1995). Dietary antioxidants such as vitamin C, vitamin E and carotenoids have been suggested as protective agents against the development of breast cancer (Byers *et al* 1995). A recent study involving 2697 women involved in the Nurses' Health Study who had developed breast cancer over fourteen years of follow-up found no association between overall risk of breast cancer and dietary intakes of

vitamin C and vitamin E (Zhang *et al* 1999). This study did find an inverse relationship between carotenoid intake and risk of breast cancer among pre-menopausal women with a positive family history of the disease and among women who consumed more than 15g of alcohol per day (Zhang *et al* 1999). Recent studies have evaluated a possible role for increased dietary calcium and vitamin D in reducing the risk of mammary cancers (Xue *et al* 1996, Xue *et al* 1999). Results from these animal studies suggest that increasing dietary calcium and vitamin D may inhibit the development of breast cancer (Xue *et al* 1996, Xue *et al* 1999, Lipkin *et al* 1999). Mechanisms through which vitamin D and calcium may inhibit mammary carcinogenesis include the differentiation inducing properties on cancer cell lines of biologically active hormone-like forms of vitamin D (1,25 dihydroxycholecalciferol) (Lipkin *et al* 1999). This differentiation inducing property may involve the regulation of epidermal growth receptors and morphological changes in the cells (Lipkin *et al* 1999). The role of vitamin D in raising intracellular calcium concentrations through facilitating gastrointestinal calcium absorption replenishes calcium lost via binding to free fatty acids and is another suggested anti-cancer mechanism (Lipkin *et al* 1999). Conjugated linoleic acid (CLA), a naturally occurring *trans* fatty acid found in ruminant meat and dairy products has recently been shown to potentially have anti-cancer properties (Zock *et al* 2001). Animal and in-vitro studies have revealed that varying supplemental doses of CLA can inhibit breast cancer cell lines (Zock *et al* 2001). There is, however a dearth of human studies examining the effect of CLA on cancer incidence. Despite extensive research the role of diet in the aetiology of breast cancer remains controversial. The majority of available evidence is derived from cohort studies, which although they highlight trends do not show cause and effect relationships as would be seen in intervention studies. Dietary recommendations for the prevention of chronic disease reflect current thinking about the role of diet in the aetiology of cancer.

1.4.1 Obesity prevalence in developed countries.

Maintenance of body weight within the normal range is conducive to long-term good health. Obesity occurs when there is a sustained imbalance between energy intake and expenditure (M^{c}Crory *et al* 2000). Involuntary weight gain worsens all elements of the cardiovascular risk profile, including dyslipidaemia, hypertension, insulin resistance, hyperuricaemia and increased fibrinogen (Kannel *et al* 1996). Recently a raised body mass index has also been associated with increased cancer risk (Shike 1996). Despite

being one of the most important modifiable risk factors for a number of life-threatening diseases and for serious morbidity, obesity prevalence is on the increase in all age groups in developed countries (M^{c}Crory *et al* 2000). In general western populations obesity, or a Body Mass Index (BMI) of greater than 30kg/m^2, is more common in women than in men, ranging from approximately 15% of females in the U.K. (Bennet *et al* 1995) to around 35% of women in the United States (Lovejoy 1998). More than half of the American population is overweight or obese (Flegal *et al* 1998). This problem is particularly prevalent in certain regional and ethnic subgroups (Kuczmarski *et al* 1994). Obesity is most common among minority women with nearly 50% of African American women and 47% of Mexican American women having a body mass index in the obese range (Lovejoy 1998). Although obesity in Britain has been rising since the 1960s the most rapid increases have been seen since 1980 (Prentice & Jebb 1995). A similar trend of rising levels of overweight and obesity has been observed in Ireland (Irish Nutrition and Dietetic Institute (INDI) 1990, Irish Universities Nutrition Alliance (IUNA) 2001, McCarthy *et al* 2001). The Irish National Nutrition Survey (INNS) (1990) found that 50.8% and 28.4% of men and women respectively were overweight with 7.8% and 12.9% of men and women respectively having a BMI in the obese range (INDI 1990). Comparisons between data from the recent North South Ireland Food Consumption Survey (NSIFCS) (2001) and the INNS revealed that the prevalence of obesity has increased by 67% in the population (INDI 1990, IUNA 2001, McCarthy *et al* 2001). The NSIFCS revealed that 39% of a representative sample (n = 1379) of the Irish adult (18-64 years) population were overweight and a further 18% were obese (IUNA 2001, McCarthy *et al* 2001). The NSIFCS found that 46.3% and 32.5% of men and women respectively were overweight with 20.1% and 15.9% of men and women respectively having a BMI in the obese range (IUNA 2001, McCarthy *et al* 2001). The greatest incidence of obesity was observed in older women (51-64 years) as 29.6% of this population group had a BMI in the obese category (IUNA 2001, McCarthy *et al* 2001).

1.4.2 Obesity, body fat distribution and disease risk.

Obesity is a well-established risk factor for non-insulin dependent Diabetes, hypertension and cardiovascular disease (Lean *et al* 1995, Feskens & Van Dam 1999). Expert groups predict that the spiralling increase in the prevalence of obesity in

developed countries is likely to precipitate an epidemic of non-insulin dependent diabetes in the near future (Feskens & Van Dam 1999, IUNA 2001). The major metabolic cardiovascular risk factors all aggregate independently with both body mass index and waist:hip ratio (Bjorntorp 1987). Individuals with a high waist:hip ratio or an abdominal body fat distribution have been found to be at greater cardiovascular risk than those with a gluteal-femoral fat distribution (Lean *et al* 1995). Waist:hip ratio cut-off points (greater than 0.80 in women and 0.95 in men) were established (Lean *et al* 1995) to enable health professionals to identify those at increased disease risk at any given level of adiposity and therefore most in need of weight management. Using established cut-off points the NSIFCS identified 24% of men and 24% of women to be at 'increased risk' and an additional 23% of men and 23% of women to be at 'high risk' of cardiovascular disease (IUNA 2001, McCarthy *et al* 2001). This risk increased with age in both men and women (IUNA 2001, McCarthy *et al* 2001). The use of waist:hip ratio as a predictor of cardiovascular disease risk may partially explain the gender differences in the incidence of this condition. An abdominal body fat distribution is frequently observed in males throughout their lifespan whereas premenopausal women almost exclusively have the more cardio-protective gluteal-femoral body fat distribution (Bjorntorp 1993). An important issue with regard to women's cardiovascular health in the postmenopausal years is the shift towards an abdominal body fat distribution (Lovejoy 1998). Studies using CT scans in addition to anthropometric measurements have found a postmenopausal increase in visceral fat, the type of fat most associated with health risk in both genders (Zamboni *et al* 1997). Decreased lipoprotein lipase activity in the femoral adipocytes and loss of the high lipolytic responsiveness of abdominal and mammary adipocytes are suggested mechanisms for the shift to abdominal body fat distribution observed in postmenopausal women (Lindberg *et al* 1990). It has also been shown that oestrogen replacement therapy in postmenopausal women has beneficial effects on body fat distribution (Lindberg *et al* 1990). The identification and treatment of individuals with abdominal obesity is central to cardiovascular disease prevention.

Body fatness and body fat distribution are recognised as important determinants of health and longevity (Lean *et al* 1995, Lee *et al* 1999, Feskens & Van Dam 1999) however the effect of cardiorespiratory fitness on disease risk and mortality at all levels of adiposity remains unclear (Lee *et al* 1999). A study examining the effect of cardiorespiratory fitness, body composition and all-cause and cardiovascular mortality

in 21925 American men concluded that cardiorespiratory fitness is a significant determinant of longterm health at all levels of body fatness (Lee *et al* 1999). These researchers found, after adjustment for age, cigarette smoking, alcohol intake and family history of ischaemic heart disease, that unfit (defined by maximal exercise testing) lean men had double the risk of all-cause mortality of lean fit men (Lee *et al* 1999). Unfit lean men had a higher risk of cardiovascular mortality than fit men who were overweight or obese (Lee *et al* 1999). Unfit men had a higher risk of all-cause and cardiovascular mortality than did fit men in all body fat categories (Lee *et al* 1999). Similarly unfit men with low waist girths had greater risk of all-cause mortality than did fit men with high waist girths (Lee *et al* 1999). There is a dearth of information on the effect of physical fitness on chronic disease risk and all-cause mortality in women. Blair *et al* (1989) examined the influence of cardiorespiratory fitness, determined by maximal exercise treadmill testing, on all-cause mortality in 10,244 men and 3,120 women. During a mean follow-up period of eight years all-cause mortality rates were significantly higher among underweight, normal weight and over weight women with low cardiorespiratory fitness compared to those with moderate and high fitness in each BMI category (Blair *et al* 1989, Blair & Brodney 1999). These researchers reported similar findings for males (Blair *et al* 1989, Blair & Brodney 1999). Active women may have a lower incidence of non insulin dependant diabetes than sedentary women (Manson *et al* 1991, Blair & Brodney 1999). A study of 87,253 American nurses examined the effect of self-reported physical activity level on incidence of non insulin dependant diabetes (Manson *et al* 1991, Blair & Brodney 1999). Diagnosis of non insulin dependant diabetes was significantly lower among the active participants and this association was stronger among those in the higher BMI categories (>27kg/m^2) (Manson *et al* 1991, Blair & Brodney 1999). It is widely acknowledged that any public health initiative to combat obesity should incorporate guidelines for increasing physical activity. In light of recent evidence awareness of the importance of regular physical activity must be raised among all members of the community not just among those in need of weight management.

1.4.3 Contributory factors to female obesity in developed countries.

The interaction between diet and a sedentary lifestyle appears to be the principal contributory factor in the spiralling increase in obesity prevalence observed recently in developed countries (Prentice and Jebb 1995, M^{c}Crory *et al* 2000, IUNA 2001,

Livingstone *et al* 2001). Lack of physical activity appears to be central to the obesity issue. The NSIFCS found that television watching is correlated with BMI in men and women of all ages (IUNA 2001, Livingstone *et al* 2001). Vigorous recreational activity is inversely associated with BMI in all sectors of the population (IUNA 2001, Livingstone *et al* 2001). Recent research from the United States supports these Irish findings. Crespo *et al* (2001) found that television watching in adolescent females is positively associated with obesity even after controlling for age, ethnicity, social class and energy intake. Public health campaigns designed to improve physical activity levels in the population will promote healthy BMIs and may also reduce micronutrient deficiencies through the avocation of less dietary restraint particularly in certain subsections of the population, notably teenage girls and women.

Certain hormonal changes during the child-bearing years may contribute to the greater rate of obesity observed among women in developed countries (Lovejoy 1998). Weight retention after childbearing may account for the development of obesity in at least a portion of the female population. The average weight retained due to pregnancy usually ranges from 0.5kgs to 2.4kgs (Ohlin & Rosner 1990), however it appears that 10% to 20% of women gain significant amounts of weight (> 15kgs) as a result of pregnancy (Lederman 1993). Suggested factors that are associated with weight gain during pregnancy include frequent snacking, reduced physical activity and stopping smoking (Ohlin & Rosner 1990). Another important contributory factor to weight retention postpartum appears to be the timing of weight gain during pregnancy (Ohlin & Rosner 1990, Muscati *et al* 1996, Lovejoy 1998). An American study of 371 Caucasian women during their pregnancies found that 86% of the weight gained during the first 20 weeks was retained by the women at least six weeks postpartum (Muscati *et al* 1996). Women who retained most weight had gained on average 6.2kgs in the first 20 weeks compared to 3.3kgs gained by the women found not to retain excess weight (Muscati *et al* 1996). Obesity has been found to increase with parity, and failure to return to baseline weight postpartum before conceiving subsequent pregnancies is a suggested reason for this (Ohlin & Rosner 1990). Pregnant women need to be aware that although adequate nutrient intake is essential during pregnancy excessive energy intakes early in pregnancy are unnecessary for infant growth and may lead to postpartum obesity.

1.5 Smoking; an increasingly important issue in women's health.

The NSIFCS found that 33% of men and 32% of women surveyed were current smokers and 28% of men and 25% of women smoked daily (IUNA 2001). In men and women the incidence of smoking decreased with increasing age (IUNA 2001). Forty one percent of younger men and 42% of younger women (18-35 years) were current smokers compared with 27% and 17% of older (51-64 years) men and women respectively (IUNA 2001). The Department of Health in the United States reported that smoking rates among men have declined by 46% since 1965, whereas smoking rates among women have dropped by only 31% (DHHS 1987, Califano 1995). In the early 1990s smoking among American teenage girls reversed its fifteen-year downward trend (Califano 1995). It is thought that 75% of adult smokers are addicted by the age of twenty-one years and that 90% have tried unsuccessfully to stop smoking (Califano 1995). Cigarette smoking is a major risk factor for cardiovascular disease, lung cancer and chronic obstructive pulmonary disease (Tverdal *et al* 1993). Cigarette smoke contains substances, which interact with host intracellular and extracellular constituents such as lipids, protein and DNA (Cross *et al* 1999). Dietary intakes of fruits and vegetables, and antioxidant micronutrients are decreased in smokers (Dallongaville *et al* 1998, Cross *et al* 1999). This together with the increased utilisation of vitamin C and Vitamin E due to oxidative stress contributes to the low plasma antioxidant concentrations observed in many smokers (Dallongaville *et al* 1998, Cross *et al* 1999). One of the many reasons why individuals, particularly young women, smoke is the perception that smoking helps to control body weight. There is documented evidence that a lifetime of smoking is associated with a 2.3kg to 3.2kg difference in body weight compared to non-smokers (Klesges *et al* 1989). A recent American study of 5115 men and women aged between 18 and 30 years found that weight gain attributable to cessation of smoking was 4.2kgs in Whites and 6.6kgs in Blacks (Klesges *et al* 1998). Although smokers tend to be leaner than their non-smoking counterparts (Flegal *et al* 1998) the negative effects of smoking on health far outweigh any weight control benefits and weight gained after cessation of smoking poses less of a health risk than smoking itself (Flegal *et al* 1998). Unfortunately individuals, particularly women, tend to find the immediate prospect of weight gain of more concern than the possibility of premature death from tobacco related conditions (Califano 1995). Specific advice and support is required for those attempting to stop smoking and it may be more appropriate for health professionals to emphasise the benefits of stopping smoking more than the

dangers of weight gain particularly in the light of the significant increases in smoking initiation by young women. Maternal smoking during pregnancy has been strongly associated with low birth weight and increased perinatal mortality (Rantakallio *et al* 1995). Mothers who smoke during pregnancy also have poorer prognosis themselves, being 2.3 times more likely to die form respiratory tract and oesophageal cancer and cardiovascular disease than those who stop smoking prior to pregnancy (Rantakallio *et al* 1995). Maternal smoking may also have other long-term consequences for child health (Rantakallio *et al* 1995). In families where the mother is more often ill, is likely to die earlier and is less likely to take care of her own health developmental delay, poorer school performance and retarded growth have been documented in children (Rantakallio *et al* 1995). Increased smoking among teenage girls and women portends disastrous consequences not only for women's health in the future but, because women produce the next generation, for the health of populations as a whole.

1.6.1 Prevalence of body image concerns among apparently healthy adult women.

Despite the increasing prevalence of overweight and obesity in developed countries preoccupation with body image has never been greater particularly among women (Biener & Heaton 1995). This weight dissatisfaction is not limited to overweight and obese individuals, normal weight individuals are also reported to engage in weight loss practices (Williamson *et al* 1992, Biener & Heaton 1995). An American national study reported that the proportion of normal weight adults on weight reduction diets at any time is between 29% and 38% of women and 13% and 18% of men depending on the standards used for normal weight (Williamson *et al* 1992). Several studies have commented on the high rate of sub-clinical cases of eating disorders in general practice. One study investigated the prevalence of undiagnosed eating disorders in three UK general practices, and among a group of 540 women aged between 16 and 35 years (Whitehouse *et al* 1992). These investigators found 5.4% (29 cases) of sub-clinical bulimia nervosa (Whitehouse *et al* 1992). Although these conditions do not fulfil formal diagnostic criteria patients are preoccupied with weight, food intake and losing weight and present a chaotic eating pattern (Whitehouse *et al* 1992).

1.6.2 Age of onset of body weight dissatisfaction in women.

The desire for an unrealistically slim appearance has been promoted widely by the media and fashion industries (Biener & Heaton 1995). Onset of dissatisfaction with body weight appears to occur mainly during adolescence (Ressler 1998). Female adolescence, usually beginning between the ages of nine and thirteen years, represents one of the most rapid periods of growth and development experienced by women during their lifetime (Flynn 1997). Often these changes can be overwhelming for the female struggling with food and weight issues (Ressler 1998) and adolescent girls who reach menarche at least one year before their peers have been found to be prone to developing depression (Nolen-Hoeksema *et al* 1992). Impressionable young girls are particularly susceptible to external images of female beauty (Ressler 1998) and on average up to 70% of adolescent girls are thought to have attempted to lose weight (Flynn 1997). Whilst avoidance of obesity is recognised as a healthy practice, the inappropriate weight loss practices, such as smoking (Ryan *et al* 1998), known to accompany fear of weight gain in adolescent girls may be a greater threat to their long-term health than obesity (Flynn 1997). Studies investigating the body image concerns and slimming practices of adult women report that the average age of onset of dieting behaviour is sixteen years of age (Grunewald 1985, Ressler 1998), suggesting that, once established, this fear of weight gain continues into adulthood (Biener & Heaton 1995).

1.6.3 Implications of body image concerns and unsafe slimming practices for women's health.

The lifelong use of unsafe slimming practices may have significant adverse effects on long-term health. Although the consensus among health professionals remains that overweight and obese individuals should attempt to reduce their weight, dieting may expose normal weight individuals to unnecessary health risks (Biener & Heaton 1995). Normal weight dieters may suffer impairment of performance on cognitive tasks (Rogers & Green 1993). Weight cycling can have negative effects on self-esteem and mood (Garner & Wooley 1991). Lifelong dieting has been found to be a risk factor for the development of eating disorders (Wilson 1993).

A significant prevalence of inadequate intakes, defined as intakes below the average requirement (AR) determined by the EU Scientific Committee for Food (1993), of iron,

calcium, zinc and copper was observed in Irish women by the NSIFCS (IUNA 2001, Hannon *et al* 2001). Forty eight percent of women in the eighteen to fifty year age group had inadequate iron intakes and 23% of women failed to meet calcium requirements (IUNA 2001, Hannon *et al* 2001). Lower intakes of micronutrients have been reported in the diets of slimmers (Gendall *et al* 1995, Biener & Heaton 1995). Low energy diets mean lower food intakes and such diets usually provide fewer vitamins and minerals unless food intakes are well planned to include nutrient dense food choices. The avoidance of staple foods, such as meat and dairy produce, is a common weight loss practice, especially among adolescent females (Flynn 1997). Vegetarianism is increasing in popularity as a possible means of weight control (Gilbody *et al* 1999). Ryan (1997) in a study of 420 Irish adolescent females found that 75% of reduced meat eaters wanted to be slimmer compared with 61% of meat eaters and that more of the reduced meat eaters had tried to lose weight. O'Connor *et al* (1987) found that 54% of patients with anorexia nervosa avoided meat and that adopting a vegetarian diet was associated with a longer duration of anorexia nervosa. Meat avoidance eliminates an important source of haem iron from the diet and therefore is potentially deleterious to haematinic nutrient status (Hallberg & Rossander Hulthen 1991). This is of particular importance in women of childbearing age who have high iron requirements and appear to be most susceptible to body image concerns. Approximately 20% of women diagnosed with anorexia nervosa will die from related health problems, a much higher percentage of women with sub-clinical eating disorders will suffer from long-term medical and psychological complications (Theander 1985, Ressler 1998). While it is well documented that individuals with anorexic and bulimic tendencies have lower dietary intakes of micronutrients (Gendall *et al* 1997), the impact of sub-clinical eating disorders on nutrient intake is less well understood. Sub-clinical eating disorders are thought to give rise to significant but inconspicuous morbidity in primary care (Whitehouse *et al* 1992).

The use of smoking as a method of weight control is of special concern. Female smokers frequently report using cigarettes as a weight control strategy (Austin & Gortmaker 2001). Studies suggest an association between dieting and smoking initiation (Klesges *et al* 1998, Austin & Gortmaker 2001). Dieters are more likely than non-dieters to report retrospectively that they initiated smoking to lose weight or prevent weight gain (Austin & Gortmaker 2001). Fear of weight gain is a significant

barrier to cessation of smoking among women (Austin & Gortmaker 2001), even during pregnancy (Pomerleau *et al* 2000). This increase in smoking among women, which appears to be related to weight concerns is likely to impact negatively on the health of these women and that of their families.

Pregnancy also gives rise to concerns over body weight (Ressler 1998). Eating disorders, both overt and sub-clinical, affect menstruation, ovulation and hence fertility (Frank & Walton 1993). Excessive caloric restriction during pregnancy can lead to inadequate weight gain, miscarriage and decreased uterine size (Frank & Walton 1993). The negative impact on the foetus includes intrauterine growth retardation, low birth weight, congenital abnormalities, pre-maturity, peri-natal mortality and delayed child development (Frank & Walton 1993). The offspring of mothers who restrain their dietary intakes during pregnancy are less likely to be breast-fed (Barnes *et al* 1997, Flynn 2000). Women who slim reject reliable contraceptive methods due to fear of weight gain (Hellerstedt & Storey 1998, Flynn 2000).

The emphasis on obesity prevention by health professionals may be contributing to the relentless pursuit of thinness by women in developed countries, however it appears that what is considered normal weight for good health is not what is considered aesthetically pleasing. Greater emphasis should be placed on the quality of women's diets and physical activity should be encouraged in preference to excessive caloric restriction for maintenance of a healthy weight.

1.7.1 Women's bone health in developed countries.

Hip fracture incidence rates due to osteoporosis are predicted to increase dramatically in the first half of the 21st century (Melton 1993, Anderson 1999). Although genetics are a major determinant of long-term bone health certain lifestyle factors including smoking, weight-bearing exercise and mineral intake also play an important role (Melton 1993, Anderson 1999). Bone density peaks in women between ages 30 and 39 years and the pattern of bone turnover is different in men and women (Langton & Langton 1997). The smaller vertebral bodies of women compared with those in men may partly explain the higher incidence of osteoporotic vertebral fractures in elderly women (Langton & Langton 1997). Reducing the risk of osteoporosis requires cessation of smoking, regular weight-bearing exercise, the correct hormonal balance and good nutrition (Weaver *et al* 1999).

1.7.2 Factors contributing to women's bone health in developed countries.

Fear of weight gain may be linked to compromised bone health. Body image concerns, starting in adolescence and continuing into adulthood, which lead to avoidance of staple foods have serious implications for the future functional independence and quality of life of increasingly elderly populations. Several studies demonstrate that osteoporosis is a feature of anorexia nervosa (Riggotti *et al* 1999). Individuals with bulimia nervosa have been found to consume only between half and two thirds of the reference nutrient intakes of calcium, iron and zinc (Gendall *et al* 1997). Sub-clinical eating disorders may also contribute to the development of osteoporosis since the detrimental conditions may be present although less obviously so. The earlier the onset of body weight dissatisfaction and caloric restriction the more serious the potential complications are likely to be as maintenance of body mass index below a critical limit of 16.5kg/m^2 prevents any increase in bone mineral density (Hotta *et al* 1999). Adolescent girls with anorexia nervosa have been found to have lower circulating levels of insulin-like growth factor-1, which may contribute to low bone mass since insulin like growth factor-1 stimulates osteoblasts, collagen synthesis and longitudinal bone growth (Hock *et al* 1988, Skottner *et al* 1990, Soyka *et al* 1999). Hypoestragenaemia has been proposed as a contributing factor to low bone density in anorexia nervosa due to secondary ammenorrhea (Soyka *et al* 1999). Diminished gonadal androgens may also be an important feature in osteopoenia associated with anorexia nervosa (Soyka *et al* 1999). Higher circulating levels of cortisol observed in anorexia nervosa may contribute to osteoporosis through the mediation of bone resorption (Soyka *et al* 1999). Adequate dietary calcium is a prerequisite for maximising peak bone mass during the first three decades of life and for minimising subsequent bone loss (Weaver *et al* 1999). For most individuals liberal consumption of dairy produce is the most effective way to achieve adequate dietary calcium. This is of particular importance during adolescence to achieve peak bone mass and avoidance of dairy produce by teenage girls in the mistaken belief that they are fattening can severely compromise calcium intake (Flynn 2000). The NSIFCS found inadequate calcium intakes in 23% of adult females (IUNA 2001, Hannon *et al* 2001).

During pregnancy there is an additional requirement for calcium in order to build the foetal skeleton (DHSS 1991, Food Safety Authority of Ireland 1999). Failure to meet these increased requirements results in resorption of the maternal skeleton to provide

bone minerals for the infant (Frank & Walton 1993). The increasing numbers of older people in the populations of many nations are largely responsible for the emerging epidemic of osteoporosis (Anderson 1999). A review by Anderson (1999) concluded that adequate dietary calcium and physical activity remain extremely important for older women as female bone density begins to decline dramatically after the menopause when the protective effect of circulating oestrogen is lost.

1.8.1 Iron deficiency and iron deficiency anaemia.

Iron deficiency is a state in which body iron is depleted to such an extent that there are no stores and the supply of iron is insufficient to meet the requirements of different body tissues (Hallberg & Rossander-Hulthen 1989). When the body is iron replete the major portion of body iron (greater than 70%) is classified as functional iron, the remainder is storage or transport iron (Bothwell 1995). More than 80% of functional iron in the body is found in the red blood cells as haemoglobin, the remainder is found in myoglobin and in intracellular respiratory enzymes such as cytochromes (Bothwell 1995). Iron is stored primarily as ferritin, but some is stored as haemosiderin (Bothwell 1995). Iron is transported in the blood by the protein transferrin and total body iron is determined by intake, loss and storage of this essential mineral (Bothwell 1995). Iron deficiency can be established by showing an absence of stainable iron in the reticulo-endothelial cells in the bone marrow or when there is low serum ferritin (<15μg/l) (Hallberg & Rossander-Hulthen 1989).

Iron deficiency anaemia occurs when an insufficient supply of iron to the bone marrow leads to a haemoglobin level below the 95th confidence limits of the population group (Hallberg & Rossander-Hulthen 1989). For women of childbearing age this is below 12g/dl (Hallberg & Rossander-Hulthen 1989). Iron deficiency anaemia occurs when transferrin saturation drops below a critical level (thought to be 16%) (Frewin *et al* 1997). When this occurs the red cell precursors in the bone marrow, which require a regular supply of iron to form haemoglobin, will be affected (Hallberg & Rossander-Hulthen 1989). This will rapidly result in anaemia characterised by hypochromic, microcytic red blood cells and impaired oxygen-carrying capacity (Hallberg & Rossander-Hulthen 1989).

1.8.2 Assessment of iron status.

Vitamin and mineral deficiencies can be detected in several ways, none of which is entirely unambiguous (Bates 1999). The advent of an assay to measure the soluble transferrin receptor in serum has provided a new 'gold standard' for the laboratory diagnosis of iron deficiency. In typical, uncomplicated iron deficiency the mean corpuscular volume, haemoglobin, serum ferritin and serum iron are low with elevated total iron binding capacity (Provan & Weatherall 2000). However, serum ferritin is an acute phase protein and can be falsely elevated in the presence of infection and previously the only true measure of iron status required a bone marrow aspirate (Provan & Weatherall 2000). The concentration of soluble transferrin receptor in serum is therefore useful in the diagnosis of iron deficiency, especially in patients with concurrent chronic disease where routine tests of iron status such as ferritin are compromised by the inflammatory process (Allen *et al* 1998). The uptake of iron by cells is mediated by a transferrin receptor expressed on their external surface (Allen *et al* 1998). Soluble transferrin receptors bind diferric transferrin and the receptor-transferrin complex is internalised into an endosome from which the iron is transferred into the cytosol (Allen *et al* 1998). Cells deficient in iron express greater numbers of soluble transferrin receptors so that they can compete more effectively for available iron (Allen *et al* 1998). The soluble transferrin receptor in serum seems to correlate well with the amount of receptor expressed at the cell membrane, which in turn reflects the cellular need for iron (Akesson *et al* 1998). Serum levels of soluble transferrin receptor increases with iron deficiency and with increased erythropoesis (Akesson *et al* 1998). As well as being unaffected by inflammatory, infectious or malignant disease, low biological and analytical variability has been reported for the soluble transferrin receptor (Akesson *et al* 1998). The ability of the soluble transferrin receptor to identify iron deficiency in the presence of inflammation was demonstrated convincingly in a recent study of 129 anaemic patients (of these patients: 48 had iron deficiency anaemia, 64 had anaemia of chronic disease and 17 had both conditions) (Punnonen *et al* 1997). These patients underwent bone marrow examination to determine their iron status (Punnonen *et al* 1997). Serum transferrin receptor levels and serum ferritin were also measured (Punnonen *et al* 1997). The soluble transferrin receptor performed better in identifying iron deficiency anaemia, as ferritin is an acute phase protein and can be falsely elevated even in the presence of mild infection or inflammation (Punnonen *et al*

1997). The ability of the soluble transferrin receptor to detect iron deficiency in the presence of infection represents a major advance in the population assessment of iron status (Cook 1999). The use of three variables (haemoglobin, serum ferritin and soluble transferrin receptor) appears to be desirable in field studies of anaemia prevalence (Cook 1999). The soluble transferrin receptor, expressed in greater quantities in iron deficiency, is unaffected by inflammation and replaces serum ferritin as the primary predictor of iron status (Provan & Weatherall 2000).

1.8.3 Prevalence of iron deficiency and iron deficiency anaemia.

Iron deficiency and iron deficiency anaemia are the commonest nutritional deficiencies in industrialised countries and are frequently observed in general practice (Frewin *et al* 1997). Iron deficiency is thought to effect between twenty and thirty percent of the world population (Bruner *et al* 1996), (Provan & Weatherall 2000). Iron deficiency (serum ferritin <15µg/l) is the commonest cause of anaemia (haemoglobin <12g/dl) accounting for up to 500 million causes annually world-wide (Provan & Weatherall 2000). The prevalence is higher in developing countries than in more industrialised societies, 51% versus 8% (Provan & Weatherall 2000). The World Health Organisation (1992) reported that iron deficiency anaemia affects approximately 43% of non-pregnant women and between 35% and 75% of pregnant women in developing countries (Allen 1997). The problem is not limited to developing countries, iron deficiency anaemia affects approximately 12% of non-pregnant women in developed countries (WHO 1992, Allen 1997) and approximately 18% to 20% of pregnant women (WHO 1992, Allen 1997, Van den Broek 1998). A study of 444 Dutch adults found low iron stores in 5% of women aged between twenty and forty-nine years (Brussard *et al* 1997). The third National Health and Nutrition Examination in the United States, conducted between 1988 and 1994, found that between 9% and 11% of adolescent girls and women of childbearing age were iron deficient with between 2% and 5% having iron deficiency anaemia (Looker *et al* 1997). The prevalence of iron deficiency anaemia in low income pregnant women enrolled in public health programmes in the United States has been found to be 9%, 145 and 375 in the first, second and third trimesters respectively (Perry *et al* 1995). A recent Irish study examining haematinics in adolescent girls found that in a sample of 318 girls, 28% were iron deficient with 3% having frank clinical anaemia (Flynn & Ryan 1998-personal communication). Although

prevalence figures vary slightly, the available information indicates that menstruating women and teenagers are at particular risk of iron deficiency and subsequent anaemia (Hallberg *et al* 1995).

1.8.4 Factors contributing to iron deficiency and iron deficiency anaemia among women in developed countries.

Iron losses in women are comprised mainly of menstrual losses and losses due to the desquamation of epithelial cells (Hallberg *et al* 1966). The amount of iron lost through desquamation is thought to be quite constant at around 0.6mg daily (Hallberg *et al* 1966). The variation in iron losses between individual females is due mainly to the variation in menstrual losses between different women (Hallberg *et al* 1966). The amount of blood lost through menstruation can be translated into iron losses. In a classic study of menstrual blood loss and iron deficiency among 476 Swedish women the amount of menstrual blood which could be lost without inducing iron deficiency was approximately 40 to 60mls (Hallberg *et al* 1966). A menstrual blood loss of 40mls corresponds to a daily loss of 0.6mg of iron, while a blood loss of 60mls corresponds to a loss of 1.0mg of iron per day (Hallberg *et al* 1966). With the addition of basal iron losses, by desquamation of cells, the total critical daily loss of iron in most women will be 1.2mg to 1.6mg (Hallberg *et al* 1966). In this study women with the highest Total Iron Binding Capacity (TIBC) had menstrual blood loss in the range of 60 to 80mls (Hallberg *et al* 1966). Plasma iron and serum ferritin were also significantly lower in women with the higher menstrual losses and 30% of the women surveyed were iron deficient (Hallberg *et al* 1966). A more recent study of iron balance in 203 menstruating Swedish women found a sharp decrease in serum ferritin when iron requirements reached the range 1.6 to 1.8mg per day. Of the women surveyed 33% were found to be iron deficient (Hallberg *et al* 1995). High iron requirements were associated with higher menstrual blood losses and women with the highest losses would require approximately 18mg of iron per day to maintain iron balance (taking average bioavailability of iron in western diets into account) (Hallberg *et al* 1995). The menstrual loss of iron is therefore the main factor affecting iron balance in women (Hallberg *et al* 1966, Hallberg *et al* 1995). The Irish RDA for iron for females aged between 19 and 54 years is 14mg (Food Safety Authority of Ireland 1999), however this may not be sufficient for all women. This evidence suggests that a substantial minority of menstruating women are at risk of iron deficiency secondary to higher than

average menstrual blood loss. This extra requirement was acknowledged by the working group who reviewed the Irish RDA for iron (Food Safety Authority of Ireland 1999). This expert group concluded that the requirements of up to 25% of menstruating women will not be achieved through dietary iron and that these females will require supplementation (Food Safety Authority of Ireland 1999).

Careful consideration should be given to iron status and menstrual history by general practitioners providing family planning advice for women of childbearing age. The oral contraceptive pill reduces menstrual blood loss by approximately half (Nilsen *et al* 1967, Hallberg & Rossander-Hulten 1991, Hallberg *et al* 1995) while intrauterine contraceptive devices, such as coils, have been found to increase menstrual blood loss (Guillebaud *et al* 1976, Hallberg & Rossander-Hulten 1991, Hallberg *et al* 1995). The likely impact of the prescribed contraceptive method on menstrual losses and hence iron status must be taken into account in primary care.

Chronic occult gastrointestinal bleeding has been associated with iron deficiency and iron deficiency anaemia (Rockey 1999). Patients with gastrointestinal blood loss of 100mls per day may have stools that appear normal (Ahliquist *et al* 1985). Thus, occult bleeding may not become apparent until it results in iron deficiency anaemia (Rockey 1999). Inflammatory conditions such as oesophagitis, gastritis and ulcers are among the commonest causes of gastrointestinal bleeding identified in general practice (Rockey 1999). Occult gastrointestinal bleeding is often attributable to therapy with anticoagulants such as heparin or warfarin and non-steroidal anti-inflammatory drugs such as asprin or ibuprofen (Blackshear *et al* 1996). Chronic excessive use of alcohol and non-steroidal anti-inflammatory drugs is a risk factor for the development of gastritis (Blackshear *et al* 1996). Patients on anticoagulant therapy are likely to be monitored closely for gastrointestinal blood loss (Rockey 1999), however, the unsupervised use of 'over the counter' non-steroidal anti-inflammatory preparations is of concern. The unsupervised use of these drugs for the management of rheumatic symptoms may be an important contributory factor to iron status in the elderly (Rockey 1999).

Menstruating women require an adequate supply of bioavailable dietary iron to meet their high iron requirements (Hallberg & Rossander-Hulten 1991). The percentage of dietary iron absorbed can vary from less than 1% to greater than 50% (Hallberg 1981). The main factor controlling iron absorption is the amount of iron stored in the body

(Bothwell 1995). The gastrointestinal tract increases iron absorption when the body's iron stores are low and decreases absorption when stores are sufficient (Bothwell 1995). An increased rate of red blood cell production can also stimulate iron uptake several fold (Bothwell 1995). Among adults the absorption of dietary iron averages approximately 6% for men and 13% of non-pregnant women of childbearing age (Hallberg 1981). Iron absorption increases in iron deficiency and during pregnancy although the extent of this increase is not well defined (Hallberg 1981, Allen 1997).

Several other factors affect the bioavailability of dietary iron. The amount of iron absorbed from the diet is not only determined by the amount of iron present but also by the source of the iron and meal composition (Hallberg & Rossander-Hulten 1989). Haem iron derived mainly from meat accounts for approximately 10 to 15% of total iron intake in typical Western diets (Hallberg & Rossander-Hulten 1989). Haem iron is nutritionally important due to its relatively high bioavailability of up to 25% in meat containing meals (Hallberg & Rossander-Hulten 1989). Non-haem iron, derived from cereals and vegetable sources, is much less bioavailable and its absorption is strongly influenced by other dietary components (Hallberg & Rossander-Hulten 1989). Meat markedly enhances the absorption of non-haem iron from meals in addition to providing haem iron (Hallberg & Rossander-Hulten 1989). Vitamin C is also a promoter of iron absorption (Hallberg & Rossander-Hulten 1989, Robinson *et al* 1998). Even doses of vitamin C as small as 25mg have a positive effect on iron absorption (Hallberg *et al* 1982*a*) and when the ascorbic acid in a meal is destroyed, for example by prolonged cooking, iron absorption is significantly reduced (Hallberg *et al* 1982*a*). Alcohol may have a promotional effect on iron absorption (Hallberg & Rossander-Hulten 1981, Robinson *et al* 1998). The main inhibiting factors are phytates, found mainly in cereals and beans, which chelate to divalent cations rendering them difficult to absorb (Hallberg & Rossander-Hulten 1989). Iron binding polyphenols such as tannins also inhibit iron absorption (Hallberg & Rossander-Hulten 1989). Tea taken with a meal markedly reduces iron absorption (Hallberg *et al* 1982*b*, Robinson *et al* 1998). Dietary calcium has been reported to be an inhibitor of iron absorption (Hallberg *et al* 1991, Hallberg 1998, Robinson *et al* 1998) and inhibition by milk is directly related to its calcium content (Hallberg *et al* 1991, Hallberg 1998).

As the bioavailability of iron in the diet decreases, the amount of food required to meet iron requirements must increase. Energy intakes in men and women have declined

significantly with industrialisation as daily life has become much less energy demanding (Hallberg & Rossander-Hulten 1981). Currently the average female daily energy intake is 8.4 mega joules or 2000 calories in industrialised countries, however a daily intake of 14.4 mega joules or 3440 calories would be required to ensure that iron requirements are met by 95% of menstruating women consuming a typical Western-type diet (Hallberg & Rossander-Hulten 1991). The NSIFCS reported a mean daily energy intake of 7.6 mega joules among Irish women (IUNA 2001). Forty eight percent of women in the eighteen to fifty year age group in the NSIFCS had iron intakes below the AR (EU Scientific Committee for Food 1993, IUNA 2001). Low energy intakes are an important contributory factor to iron deficiency in industrialised countries (Hallberg & Rossander-Hulten 1991). This may be compounded among females in industrialised countries due to body image concerns and unsafe slimming practices.

Iron deficiency and iron deficiency anaemia are more common in pregnant women than in any other population group (Allen 1997). During pregnancy plasma volume expands by 46% to 55% whereas red cell volume expands by 18% to 25%. The resulting haemodilution has been termed the physiological anaemia of pregnancy (Van den Broek 1998). In most published studies the minimum normal haemoglobin level in healthy pregnant women living at sea level is between 11 and 12g/dl, however 20% of pregnant women in industrialised countries have haemoglobin concentrations below this level (Johnson-Spear & Yip 1994, Van den Broek 1998). During the first trimester of pregnancy iron requirements are met by most women due to the iron sparing effect of the cessation of menses (Kaufer & Casaneuva 1990). When blood volume and red cell mass increase at around week sixteen of gestation, iron requirements increase substantially (Kaufer & Casaneuva 1990). The need for iron then increases almost linearly until term (Kaufer & Casaneuva 1990). The total amount of iron required for an average pregnancy is 840mg, of this 350mg is transferred to the foetus and placenta, 250mg mg is lost in blood at delivery and 240mg is basal losses (Kaufer & Casaneuva 1990). As in iron deficiency, iron absorption in the gut increases during pregnancy particularly during the last two trimesters (Kaufer & Casaneuva 1990), however adequate maternal iron stores are necessary to meet the demands if iron transfer to the foetus. Many of the women that experience anaemia during pregnancy have depleted or no iron stores prior to pregnancy (Allen 1997). Even in industrialised countries, a

substantial number of women enter pregnancy with depleted stores (Allen 1997). Every pregnancy places demands on a woman's iron stores, therefore anaemia may be more likely when there are numerous frequent, pregnancies between which insufficient time elapses to replenish iron stores (Allen 1997). A British study of determinants of iron status in early pregnancy in 576 women found that serum ferritin concentrations were significantly lower in multiparous women (Robinson *et al* 1998). These researchers also observed that iron status during early pregnancy declined with age (Robinson *et al* 1998). Pregnancies beginning at an early age place further demands on nutritional status as the demands of growth combine with those of pregnancy. Hence, pregnant adolescents are a particularly vulnerable group (Scholl *et al* 1992, Allen 1997). As it is uncertain whether pregnant women can maintain iron balance with normal dietary practices, iron prophylaxis may be necessary. Supplementation may also be beneficial for non-pregnant women with above-average menstrual losses.

1.8.5 Impact of iron deficiency and iron deficiency anaemia on public health.

Iron deficiency and iron deficiency anaemia have adverse effects on sufferers and their offspring. Iron deficient and anaemic patients complain of lethargy and dyspnoea (Frewin *et al* 1997) and impaired physical endurance and work capacity (Bruner *et al* 1997). Changes in immune function have been documented in iron deficiency (Bruner *et al* 1996), (Walter *et al* 1997). Two immune system abnormalities have been documented in iron deficient humans, namely an impaired T lymphocyte response to mitogens and a reduction in the bactericidal activity of neutrophils (Walter *et al* 1997). It is well established that iron deficiency and iron deficiency anaemia are common during pregnancy (Allen 1997, van den Broek 1998, Robinson *et al* 1998, Provan & Weatherall 2000). Iron deficiency and iron deficiency anaemia during pregnancy have been associated with poor maternal immune status (Kandoi *et al* 1981, Allen 1997), intrauterine growth retardation (Steer *et al* 1995, Allen 2000), pre-term delivery (Steer *et al* 1995, Allen 2000) and increased peri-natal mortality (Scholl *et al* 1992, Allen 2000). Evidence also suggests that maternal iron deficiency during pregnancy reduces infant iron stores in the first year of life (Gaspar *et al* 1993, Allen 2000). Iron deficiency during the first two years of life can significantly impair mental and motor development (Bruner *et al* 1996, Cook 1999). Iron deficiency is thought to be associated with impaired learning ability well beyond infancy (Pollitt 1997). Iron

deficiency and iron deficiency anaemia have been associated with impaired cognitive development and poor educational performance in children (Pollitt 1997). A recent population based study examined the association between early childhood anaemia and mild or moderate mental retardation at ten years of age in 5411 American children (Hurtado *et al* 1999). These researchers observed an increased likelihood of mild or moderate mental retardation associated with early childhood anaemia independent of birth weight, maternal education, sex, ethnicity and maternal age (Hurtado *et al* 1999). Although evidence is sparse, it is thought that these functional deficits may occur in iron deficiency as well as in iron deficiency anaemia (Bruner *et al* 1996). A recent study of American adolescent girls found significantly poorer cognitive function among girls with serum ferritin less than 12μg/l (Bruner *et al* 1996).

Iron deficient and anaemic mothers produce larger placentas and low birth-weight babies (Barker *et al* 1993, Barker 1998). Babies who are small at birth or during infancy have increased rates of cardiovascular disease and non-insulin dependant diabetes as adults (Barker *et al* 1990, Barker 1998). Roseboom *et al* (2000) investigated the effect of intrauterine malnutrition on the plasma lipid profiles of adults born during the Dutch famine (1944-1945). Persons exposed to famine during gestation had a more atherogenic lipid profile than those who were not exposed to intrauterine undernutrition (Roseboom *et al* 2000). Research involving 69526 participants in the Nurses' Health Study concluded that low birth weight is associated with increased risk of non-insulin dependent diabetes during adulthood (Rich-Edwards *et al* 1999). This association is independent of adult BMI, social class and family history of diabetes (Rich-Edwards *et al* 1999). The intrauterine environment is also important in the development of hypertension in adults (Barker *et al* 1990, Barker 1998). The highest blood pressure appears to occur in adults who were small babies with large placentas (Barker *et al* 1990, Barker 1998). The prevention and treatment of iron deficiency and iron deficiency anaemia in women has implications not only for the sufferers themselves but also for the health of future generations.

1.9.1 Sources and bioavailability of different types of folate.

Folic acid and its role in health and disease have prompted much research over recent decades. Folate is the generic term for a water-soluble B-complex vitamin, which is an important cofactor in the transfer of one-carbon moieties and plays a key role in DNA

synthesis, repair and methylation (Wagner 1996). Folic acid is the most oxidised and stable form of folate and rarely occurs naturally in food, however it is used in vitamin supplements and fortified food products. Folic acid consists of a *p*-aminobenzoic acid molecule, a pteridine ring and one glutamic acid molecule. Naturally occurring food folates contain up to six glutamic acid molecules as a polyglutamate side chain and are significantly more chemically labile than folic acid (Scott 1999). The instability of naturally occuring folates causes a significant loss of activity during harvesting, storage, processing and preparation (Scott 1999). Up to 75% of folate activity may be lost during these procedures (Scott 1999). This poses problems for the estimation of dietary folate intakes using food tables, which take an average of six measurements. Food folates are hydrolysed to monoglutamic forms in the gut prior to absorption by the γ-glutamylhydrolase enzyme and the monoglutamate form of folate is then actively transported across the small intestine. Pharmacalogical doses of monoglutamate folic acid are absorbed by passive diffusion. The mechanism of absorption appears to contribute significantly to the bioavailability of the different types of folate. Sauberlich *et al* (1987) found that the bioavailability of food folates was only around 50% that of folic acid. A more recent study also demonstrated that compared with the use of fortified food and supplements, consumption of extra naturally-occurring food folate is relatively ineffective at improving folate status (Cuskelly *et al* 1996, McNulty *et al* 2000).

1.9.2 Assessment of folate status.

The most frequently selected indicator of folate status is the erythrocyte folate level. Other indicators include serum and plasma folate levels (Jacques *et al* 1993). The main limitation of serum and plasma folate levels is that they reflect transient changes in folate intake (Jacques *et al* 1993). The circulating folate concentration may be reduced in situations where there is no alteration in overall status such as acute alcohol ingestion (Jacques *et al* 1993). Folate is taken up only by the developing erythrocyte in the bone marrow and not by the circulating erythrocyte during its 120 day lifespan (Wu *et al* 1975). Erythrocyte folate concentration is therefore an indicator of long term folate status (Wu *et al* 1975). The microbiological assay for the analysis of erythrocyte folate is considered the 'gold standard' by investigators in the assessment of folate status (Molloy & Scott 1997). A chloramphenicol-resistant strain of *Lactobacillus casei*

maintained by cryopression is the microorganism most frequently used (Molloy & Scott 1997). The traditional microbiological assay has been modernised and automated to improve speed, precision and cost effectiveness (Molloy & Scott 1997). Red cell folate can also be measured by radioassay (Molloy & Scott 1997). Plasma homocysteine concentration increases when inadequate quantities of folate are available for the remethylation of homocysteine to methionine (Selhub *et al* 1993). There is evidence to suggest that plasma homocysteine increases in the presence of reductions in indicators of folate status (Selhub *et al* 1993). Plasma homocysteine may therefore be another indicator of folate status (Selhub *et al* 1993).

1.9.3 Plasma homocysteine.

Plasma homocysteine, a sulphur-containing amino acid that is the demethylated derivative of methionine, is a normal constituent of human plasma (Scott 1999). Homocysteine is formed in the methylation cycle and can be converted to either cysteine via cystethionine β synthetase or remethylated to methionine (Scott 1999). The enzymes involved in controlling plasma homocysteine levels require B complex vitamins as cofactors. Cystathionine β synthetase requires vitamin B_6. Methionine synthetase requires vitamin B_{12} and folate; 5,10 methyltetrahydrofolate reductase indirectly controls plasma homocysteine levels and is folate dependant (Scott 1999). Under normal circumstances the majority of homocysteine is reconverted to methionine in the folate- and vitamin B_{12}-dependant reaction (Brussard *et al* 1997). A defect in any of these enzyme pathways leads to an accumulation of homocysteine in the plasma (Picciano 2000). Patients with the inborn error of cystathione β synthetase have marked elevations in plasma (200-300μmol/l) and urinary homocysteine, mental retardation and die prematurely from atherosclerosis (Brattstrom & Wilcken 2000, Picciano 2000). Untreated women who are heterozygous for this inborn error of metabolism experience a foetal loss of approximately 50% (Picciano 2000). Causes of milder (>15μmol/l) (Brattstrom & Wilcken 2000) forms of hyperhomocysteinaemia include the thermolabile variant of 5,10 methyltetrahydrofolate reductase. This MTHFR C667T mutation results in a 50% reduction in enzyme activity and hyperhomocysteinaemia (Picciano 2000). Other reasons for mild hyperhomocysteinaemia include deficiency of folate, vitamin B_{12} and vitamin B_6.

1.9.4 Implications of folate deficiency, sub-optimal folate status and elevated plasma homocysteine.

Due to its role in DNA synthesis and repair the effects of folate deficiency are observed in rapidly proliferating tissues (Wickramasinghe 1995). Deficiency of folate induces megaloblastic changes in the bone marrow (Wickramasinghe 1995). Among the first abnormalities observed in the peripheral blood are hypersegmented neutrophils, as folate deficiency progresses mean cell volume increases above normal and macrocytic cells are produced (Wickramasinghe 1995). Megaloblastic anaemia has been found to occur when erythrocyte folate levels fall to 150μg/l. Initially the megaloblastic anaemia of folate deficiency may be asymptomatic, however moderate to severe deficiencies produce symptoms of weakness, fatigue, poor concentration, irritability and shortness of breath (Wickramasinghe 1995). An increase in plasma homocysteine is observed before clinical deficiency of folate occurs (Selhub *et al* 1993).

Understanding of the role of folate has greatly evolved from the prevention of macrocytic anaemia (Kim 2000). Several authors have reported a link between low maternal folate status and neural tube defects (NTDs) such as spina bifida and anencephaly (MRC 1991, Cuskelly *et al* 1996, Brussard *et al* 1997, Daly *et al* 1997, Scott 1999, Kim 2000). Women who have had a previous neural tube defect (NTD) affected pregnancy are at a high risk of having a subsequently affected pregnancy (Rush 1994). Low red cell folate (Kirke *et al* 1993) and high plasma homocysteine levels (Mills *et al* 1995) have been documented in NTD affected pregnancies. Vollset *et al* (2000) reported that hyperhomocysteinaemia may also be an important biological marker for other pregnancy complications such as preeclampsia, premature delivery, very low birth weight and clubfoot. There is more than an eightfold difference in the risk of an NTD affected pregnancy between women with red cell folate levels less than 150μg/l and those with levels of 400μg/l or higher suggesting that red cell folate levels below 400μg/l are sub-optimal for women who could become pregnant (Kirke *et al* 1993, Daly *et al* 1995). NTD affected pregnancies have been observed in women with red cell folate levels above the clinically deficient range (Kirke *et al* 1993, Daly *et al* 1995). Between 50% and 70% of NTDs are thought to be preventable through folate (Cuskelly *et al* 1996). An intake of 400μg per day of folates would produce a sufficient increase in red cell folate levels to reduce the incidence of NTDs by 47% (Cuskelly *et al* 1996). It is acknowledged that this is greater than the current British reference

nutrient intake of 200μg per day (DHSS 1991) and the Irish recommended dietary allowance of 300μg per day of folates for adult women, (Food Safety Authority of Ireland 1999). Supplemental folic acid has been shown to prevent NTDs if taken in the periconceptual period (MRC 1991, Cuskelly *et al* 1996, Brussard *et al* 1997, Daly *et al* 1997, Scott 1999, Kim 2000). The critical periconceptual period for NTD prevention is short (Scott 1999) and may pass before the woman is aware of a pregnancy. The discovery that folic acid reduced the risk of neural tube defects led to the recommendation by Departments of Health in America, Australia and Europe that women of childbearing age should consume an extra 400μg of folic acid per day as a supplement or in fortified food (Cuskelly *et al* 1996). Although the RDA for Irish women was set at 300μg of folates, the working group that reviewed the Irish RDA recommended that all women of childbearing age should consume at least 400μg of folic acid per day (Food Safety Authority of Ireland 1999).

Homocysteine is the amino acid that has been implicated in the pathogenic processes observed in folate deficiency (Brussard *et al* 1997). An increased homocysteine concentration has been found to be an independently associated with risk of cardiovascular disease (Pancharuniti *et al* 1994). Almost 30% of people with cardiovascular disease have been reported to have hyperhomocysteinaemia and the risk of premature occlusive vascular disease is thought to be thirty times greater for people with hyperhomocysteinaemia (>15μmol/l) than for controls (Pancharuniti *et al* 1994). The highest risk of cardiovascular disease is associated with the lowest plasma folate levels (Selhub et al 1993). Two hypotheses have been suggested to explain the pathogenic effects of homocysteine on cardiovascular disease. Direct toxicity to epithelial cells has been observed in laboratory studies, although at much higher concentrations than would be found in nature (Taylor *et al* 1991, Pancharuniti *et al* 1994). Homocysteine may promote the oxidation of LDL cholesterol (Stampfer *et al* 1992, Pancharuniti *et al* 1994, Bellamy *et al* 1999 Brattstrom & Wilcken 2000). Sulphur containing amino acids in the presence of a transition metal such as iron or copper may cause oxidation of LDL cholesterol (Stampfer *et al* 1992, Pancharuniti *et al* 1994). Oxidised LDL is scavanged by macrophages forming foam cells, which contribute to the formation of atheromatous plaques (Fitzgerald & Barry 1997). Plasma folate and vitamin B_{12} levels are inversely related to plasma homocysteine levels, and supplements of folic acid have been shown to reduce plasma homocysteine

concentrations (Scott 1999). Despite extensive epidemiological evidence that mild hyperhomocysteinaemia is an independent risk factor for vascular disease, whether modest increases in plasma homocysteine are causally related to atherosclerosis has recently come up for debate (Brattstrom & Wilcken 2000). A recent review of available epidemiological evidence proposed that mild hyperhomocysteinaemia (>15umol/l) is an effect rather than a cause of atherosclerosis (Brattstrom & Wilcken 2000). Elevated plasma homocysteine has been shown to be strongly positively associated with major cardiovascular disease risk factors such as age, male gender, smoking, hypercholesterolaemia and a sedentary lifestyle (Nygard *et al* 1995, Brattstrom & Wilcken 2000). Raised plasma homocysteine is also associated with hypertension (Nygard *et al* 1995, Brattstrom & Wilcken 2000) which is a well-established risk factor for atherosclerosis and nephrosclerosis resulting in impaired renal function (Nygard *et al* 1995, Brattstrom & Wilcken 2000). Also atherosclerosis is thought to contribute to nephrosclerosis (Ruilope 1999, Brattstrom & Wilcken 2000). Serum creatinine, a marker of decline in renal function is a strong determinant of plasma homocysteine concentrations (Arnadottir *et al* 1996, Brattstrom & Wilcken 2000). In renal disease creatinine and homocysteine concentrations increase and in end-stage renal disease plasma homocysteine concentrations may become three to five times higher than normal (Arnadottir *et al* 1996, Brattstrom & Wilcken 2000). In light of this Brattstrom & Wilcken (2000) suggest that elevated plasma homocysteine concentrations observed in vascular disease patients may be due to inefficient clearance of homocysteine through the kidneys secondary to hypertension and atherosclerosis. At the very least hyperhomocysteinaemia is a marker of increased cardiovascular disease risk but there is also some evidence of a causal link through direct epithelial toxicity (Taylor *et al* 1991, Pancharuniti *et al* 1994) and increased oxidative stress (Stampfer *et al* 1992, Pancharuniti *et al* 1994, Bellamy *et al* 1999 Brattstrom & Wilcken 2000). Folate supplementation reduces plasma homocysteine concentrations and it has been suggested that folate also exhibits a direct antioxidant effect (Bellamy *et al* 1999 Brattstrom & Wilcken 2000). The role of plasma homocysteine in pathogenesis requires further elucidation however optimal folate status is still likely to be very important in attenuating disease risk associated with this amino acid.

Research is ongoing, and recent evidence has implicated sub-optimal folate status in other pathogenic processes. Although it is well established that depleting folate retards the growth of, and occasionally kills, established cancers it appears that folate depleted

tissues are no less likely to undergo neoplastic transformation (Kim 2000, Wiemels *et al* 2001). Recent evidence suggests that folate deficiency in normal epithelial tissues may predispose individuals to neoplasms arising from these tissues (Mason *et al* 1996, Kim 2000, Wiemels *et al* 2001). A study involving 3483 participants in the Nurses' Health Study who developed invasive breast cancer during the sixteen year follow up period found a significant inverse association between total folate intake and breast cancer risk among women consuming more than 15g of alcohol per day. This inverse relationship between a total folate intake of at least 600µg per day and risk of breast cancer was observed in pre and post-menopausal women (Zhang *et al* 1999). The role of folate in carcinogenesis has been most widely studied for colorectal cancer. The majority of published epidemiological studies indicate that dietary folate intake is inversely associated with the risk of adenomatous polyp formation and colorectal cancer in a dose-dependant fashion (Mason *et al* 1996, Kim 1999, Kim 2000, Wiemels *et al* 2001, Riboli & Norat 2001). Several case control studies have found a lower relative risk of cervical neoplasia among women consuming higher amounts of folate (Mason 1995). There is also some early epidemiological evidence of a role for folate in the prevention of bronchial squamous cell, oesophageal and stomach cancer (Mason *et al* 1996, Kim 1999, Kim 2000, Wiemels *et al* 2001).

There is also some recent evidence to link hyperhomocysteinaemia with microvascular disease and dementia in the elderly (Snowden *et al* 2000). A study involving elderly nuns found a strong negative correlation between serum folate and severity of post-mortem atrophy found in the brain neocortex of subjects with significant numbers of Alzheimer disease lesions (Snowden *et al* 2000). This does not prove a causal relationship but suggests that when a pathogenic process inducing dementia such as Alzheimer's disease is present, impaired folate metabolism may exacerbate the condition (Snowden *et al* 2000).

1.9.5 Causes of deficient and sub-optimal folate status.

Dietary recommendations are based on the evidence that certain definable ranges of specific nutrients are required to prevent deficiency in normal healthy people (Bates 1999, Punnonen *et al* 1997). Poor dietary folate intake is thought to be the most common cause of deficiency. The INNS found an average intake of folates of 244µg/day and 183µg/day in men and women respectively (INDI 1990). The NSIFCS

found that only 2% of women aged between eighteen and thirty five years and 5% of women aged thirty six to fifty years achieved the COMA recommended intake of folates of 600μg per day for women of reproducative years (IUNA 2001, O'Brien *et al* 2001). All of the women who met the recommendation were using folic acid containing supplements (IUNA 2001). Among women consuming supplements mean intake of folates was 489μg per day (233μg folate from food and 248μg folic acid from supplements) (IUNA 2001). Of the women who did not take supplements none had mean intakes approaching this level (IUNA 2001). Particularly low intakes of folates have been observed in adolescent females and in elderly subjects from low income subgroups (Sauberlich 1995, McNulty 1997). In addition to low intakes, the destruction of food folate during food preparation and cooking may also contribute to sub-optimal status (McNulty 1997, Scott 1999). Drugs, which interact with folates and hence impair folate status include the oral contraceptive pill (Senti & Pitch 1985, McNulty 1997) and anti-convulsant drugs (McNulty 1997). Alcohol is also known to negatively effect folate status possibly through intestinal malsbsorption, altered hepatobiliary metabolism or increased folate excretion or catabolism (Halsted 1995, McNulty 1997). Sulphasalazine, used to treat ulcerative colitis, impairs folate absorption, transport and metabolism (Lashner *et al* 1989, McNulty 1997). Smoking has been associated with low erythrocyte and serum folate levels although it is unclear as to whether this is due to lower intakes or increased requirements among smokers (Sauberlich 1995, McNulty 1997).

The role of folates in DNA synthesis results in an increased requirement for folates during periods of rapid tissue growth and cell replication such as pregnancy (McPartlin *et al* 1993, McNulty 1997). This increased requirement has potentially serious implications for women entering pregnancy with low stores combined with a low dietary intake.

The dietary reference values for folates, as for other nutrients, are targeted at the general, supposedly normal, population (Molloy *et al* 1997). It does not take into account those with genetic or metabolic abnormalities. Approximately 5%-15% of the general population is homozygous for a thermolabile variant of 5,10 methylenetetrahydrofolate reductase (MTHFR), which causes mild hyperhomocysteinaemia (>15μmol/l) and is positively associated with low red cell folate levels, cardiovascular disease and neural tube defects (Molloy *et al* 1997). The

presence of this genetic variant may also be a determinant of pregnancy outcome and risk of dementia in later life (Vollset *et al* 2000). The prevalence of this metabolic block suggests that a substantial minority of individuals in general populations have increased requirements for folates. It is therefore vital for individuals with this variant to consume adequate folates from folate rich foods, folic acid supplements, folic acid fortified foods or a combination of these. A recent study on the relationship between the presence of the common mutation MTHFR C677T and folate status in 82 NTD affected and 260 control mothers (Molloy et al 1998) revealed that a significant number of the NTD affected women had red cell folate levels below 200μg/l that were not explained by the MTHFR C677T mutation (Molloy *et al* 1998). This study confirms that low maternal folate status is a major determinant of NTD risk, and that other folate-dependant genetic variants may exist and increase NTD risk by reducing folate levels (Molloy *et al* 1998).

In addition, folate malabsorption and deficiency are common in untreated coeliac disease, as the proximal small intestine is predominantly affected (Dahele & Ghosh 2001). Evidence to support a dominant genetic mechanism underlying the relatively high prevalence of coeliac disease in the west of Ireland has been documented (Hernandez *et al* 1991). It has been suggested that undiagnosed coeliac disease may be relatively common in geographical areas where this genotype occurs (Hernandez *et al* 1991). It is estimated that 50% of women with untreated coeliac disease experience miscarriage or an unfavourable pregnancy outcome (Martinelli *et al* 2000). The risk of unfavourable pregnancy outcome is greatly reduced among the coeliac women who adhere to a gluten free diet (Martinelli *et al* 2000). Furthermore, it is suggested that coeliac disease is considerably more common in certain geographical areas than other diseases for which women with a poor reproductive history are routinely screened (Martinelli *et al* 2000). In these geographical areas undiagnosed coeliac disease and concomitant sub-optimal nutrition may contribute to unfavourable birth outcomes and may require consideration for inclusion in ante-natal screening programmes (Martinelli *et al* 2000).

1.9.6 Food fortification as a population strategy for improving folate status.

Consuming sufficient quantities of foods fortified with folic acid is a way of ensuring an adequate intake. In the United States the Food and Drug Administration issued a

regulation, which came into effect in 1998, requiring all flour and uncooked cereal grains to be fortified with folic acid to a level of 140μg per 100g (Daly *et al* 1997). Because of safety concerns the American Food and Drug Administration has chosen a level of fortification that will increase the average woman's intake by approximately only 100μg per day (Daly *et al* 1997). Although higher doses of folic acid would be more effective (Daly *et al* 1997) it is thought that the current fortification dose taken over a long period will produce a significant reduction in NTDs. A recently published evaluation study reported that the current level of folic acid fortification in the United States has produced a 19% reduction in the incidence of NTDs over the last two years (Honein *et al* 2001). Another study measuring the changes in folate consumption and the prevalence if NTDs in Britain and Ireland during the past two decades also found a reduction in NTDs in response to folate consumption (Murphy *et al* 2000). Average daily dietary folate consumption for Britain for the period 1980 to 1996 was compared to annual NTD prevalences for the same period (Murphy *et al* 2000). Dietary folate increased on average 1.65% in Scotland and 1.4% in England during the study period. The annual rate of decline in NTD prevalence averaged 10.4% in the Irish population, 8.2% in Glasgow and 5.2% in England (Murphy *et al* 2000). The decline in NTD prevalence observed in Britain and Ireland since the 1970s may be related to the fortification of cereals with folic acid which, produced measurable increases in daily consumption of folates (Murphy *et al* 2000). Mandatory food fortification programmes may be introduced in Europe in an attempt to improve folate status in vulnerable groups.

Although food fortification will certainly benefit population groups with high requirements for folates it may pose problems for others. The greatest concern with respect to the safety of folic acid fortification is its well-established role in complicating the diagnosis of vitamin B_{12} deficiency (Firth *et al* 1998). Other possible adverse effects of folic acid fortification include the interference with anticonvulsant drugs and steroids (Firth *et al* 1998). Consumption of large quantities of folate fortified foods may give rise to unmetabolised folic acid in the serum (Kelly *et al* 1997), this is unphysiological and initial research suggests that this may have pathological effects (Kelly *et al* 1997). Therefore, great care must be taken when choosing foods to fortify with folic acid in order to avoid as much as possible the adverse effects.

1.10.1 Vitamin B_{12}.

Vitamin B_{12} or cobalamin is the largest of the B complex vitamins, with a molecular weight of over 1000. It consists of a porphyrin ring with a cobalt molecule in the centre. All vitamin B_{12} arises initially from synthesis in micro-organisms and enters the human food chain by being incorporated into food of animal origin (Weir & Scott 1998, Scott 1999). Therefore meat, especially liver, milk, eggs, butter and cheese are among the best sources of cobalamin in human diets (Weir & Scott 1998, Scott 1999). Plants do not contain the necessary enzymes to make the vitamin and as there are no vegetable sources of vitamin B_{12} vegetarians require supplementation. The absorption of vitamin B_{12} occurs in the terminal ileum. Vitamin B_{12} in food is bound to proteins, which are removed by gastric hydrochloric acid (Weir & Scott 1998, Scott 1999). The free cobalamin is then bound to glycoproteins known as haptocorrins, which protect it from denaturation by the gastric acid (Weir & Scott 1998, Scott 1999). The parietal cells in the stomach also secrete a glycoprotein known as intrinsic factor which binds to the vitamin B_{12} in the duodenum and facilitates absorption by phagacytosis at specific ileal receptor sites (Weir & Scott 1998, Scott 1999). Vitamin B_{12} status may be assessed using a microbiological assay (Kelleher & O'Broin 1991).

1.10.2 Vitamin B_{12} deficiency.

Vitamin B_{12} is a cofactor for two enzymes in human tissues: methionine synthase and methylmalonyl CoA mutase (Weir & Scott 1998, Scott 1999). Methionine synthase is involved in the regulation of plasma homocysteine concentrations and deficiency of vitamin B_{12} is associated with an elevation this amino acid in plasma (Weir & Scott 1998, Scott 1999). Vitamin B_{12} deficiency gives rise to a megaloblastic anaemia similar to that seen in folic acid deficiency. It can be reversed by administering oral or intramuscular doses of vitamin B_{12}, depending on how the deficiency has occurred. However vitamin B_{12} deficiency is also associated with a neuropathy due to subacute degeneration of the spinal cord. It has not been conclusively established whether the demyelination is the result of a build up of methyl-malonic acid due to the limitation of methylmalonyl CoA mutase or due to the impaired functioning of methionine synthase (Scott 1999). The nerve damage, unlike the anaemia, is irreversible once it has progressed past a certain stage. The neuropathy presents as tingling of the fingers,

ataxia and loss of memory in the early stages, eventually progressing to paralysis and eventually death if untreated (Carmel 1995).

1.10.3 Causes of Vitamin B_{12} deficiency.

Deficiency of vitamin B_{12} can arise in various ways. Dietary deficiency is rare, as this vitamin is abundant in the diets of most healthy free-living humans. It has however been documented in individuals following strict vegetarian or vegan diets (Carmel 1995, Haddad *et al* 1999). Sub-optimal plasma vitamin B_{12} levels and macrocytosis have been documented in male and female vegans (Haddad *et al* 1999). Sub-optimal cobalamin may affect cognitive function even in the absence of haematological signs (Louwman *et al* 2000). Psychological test performance of 48 adolescents who consumed a strict vegan diet up to the age of six years, subsequently followed by lacto-vegetarian or omnivorous diets, was compared to that of a control group of adolescents who consumed a mixed diet from birth onwards (Louwman *et al* 2000). Almost two thirds (65%) of the previously vegan group were cobalamin deficient (Louwman *et al* 2000). The control group performed significantly better on most psychological tests than the previously vegan subjects (Louwman *et al* 2000). This evidence suggests that vegans represent a subgroup of the population at risk of vitamin B_{12} deficiency and its concomitant neurological manifestations.

The NSIFCS reported vitamin B_{12} intakes that were more than adequate in both Irish men and women (IUNA 2001, O'Brien *et al* 2001). Vitamin B_{12} deficiency can also arise through malabsorption. This vitamin requires gastric acid, pepsin and the glycoprotein intrinsic factor in order to be absorbed. A number of elderly people develop achlorhydria and a reduced secretion of intrinsic factor (Charlton *et al* 1997). This has been reported to increase the risk of vitamin B_{12} deficiency in the elderly population (Charlton *et al* 1997).

A further complication occurs in some older people (generally those aged >50 years) known as pernicious anaemia. This results in the autoimmune destruction of the parietal cells in the stomach, which secrete intrinsic factor, the glycoprotein necessary for the absorption of vitamin B_{12}. Treatment of pernicious anaemia requires B_{12} injections, as oral supplements would be ineffective due to the inability of sufferers to absorb them (Carmel 1995). A recent study examining the haemopoetic nutrient status of an elderly South African population found that 3.1% of women over the age of 65 years had serum B_{12} levels of <100pg/ml compared to 0% of men (Charlton *et al* 1997).

Some authors suggest that the aetiology of vitamin B_{12} deficiency in the elderly is probably multifactorial (Charlton *et al* 1997). A combination of low dietary intake (due to poverty, reduced energy requirements, the consumption of foods with low nutrient density and the physical inability to prepare food), decreased absorption and chronic polypharmacy may be responsible for the greater incidence of vitamin B_{12} deficiency in the elderly (Charlton *et al* 1997). All over the world populations are getting older and modern geriatric medicine aims at achieving optimal function and independence in the elderly (Charlton *et al* 1997). Malnutrition is known to have significant adverse effects on quality of life and life expectancy in older people. Those living alone from lower socio-economic groups are particularly at risk (Charlton *et al* 1997). As the proportion of the population > 65 years increases, it will be necessary to raise awareness of nutrient deficiencies that affect elderly people among all health professionals involved age related health care.

The growing trends in food folic acid fortification also pose potential risks for an increasingly elderly population through masking of B_{12} deficiency anaemia (Tucker *et al* 1997). Currently the Food and Drug Administration in America feels that the benefits of increasing folic acid intake outweigh the risks (Tucker *et al* 1997). However there have been representations to Health Authorities regarding the addition of vitamin B_{12} to folic acid fortified foods (Tucker *et al* 1997). This should be considered in any European initiative to fortify the food supply with folic acid.

1.11.1 Socioeconomic status and health.

Practising a healthy lifestyle and consuming a healthy diet is associated with higher socio-economic status (Hjartaker & Lund 1998). It has been suggested that educational campaigns are more successful in higher socio-economic classes and that lower socio-economic groups benefit less from these efforts (Hjartaker & Lund 1998). Studies in England and Scotland have linked lower socio-economic groups with higher mortality (Smith and Egger 1993). Variations in environmental factors such as diet may explain some of the variation in mortality rates in different socio-economic groups. Higher intakes of dietary fibre, fruit and vegetables have been observed in women of higher socio-economic class and those who have spent longer in formal education (Hjartaker & Lund 1998). Higher socio-economic classes place more emphasis on diet for health (Hjartaker & Lund 1998).

1.11.2 Measurement of socio-economic class.

As socio-economic class and level of education appear to be important determinants of health and longevity the accurate measurement of this variable is essential in research studies. Four occupational classifications are commonly used in Ireland for research purposes. The British Registrar General's Social Class Scale groups occupations into social class categories in terms of their standing in the community and takes account of education and economic environment (O'Hare 1982). Although this scale has been used effectively in research in Ireland it does have certain disadvantages in an Irish setting for example it does not satisfactorily divide the agricultural sector, which accounts for a large proportion of Irish society (O'Hare 1982). The Market Research Scale (1970) uses occupation as the determinant of class and a sample of occupations is used to aid coding. It categorises individuals into eight groupings, which can be combined under broader groupings (O'Hare 1982). The main disadvantage of this method is the use of prestige rather than economics to categorise individuals (O'Hare 1982). The Hall Jones Prestige Scale is again a British model, however a modified version of this scale has been devised by sociologists to make it applicable to the Irish situation (MacGreil 1977). The Irish census socio-economic group classification is a nominal grouping of occupations (O'Hare 1982). The social grouping of individuals is decided based on their occupation, retired and unemployed individuals are classified according to former occupations. This scale consists of eleven categories of socio-economic status. A modified version of this scale was proposed for use in epidemiological health research (O'Hare 1982). This version of the scale consists of six categories, which can be further combined for analysis. It is applicable to the Irish situation as it takes detailed account of the agricultural sector. The social class category of each person aged 15 years or over is classified according to their occupation, unemployed and retired persons are classified as above and housewives and students are classified according to the occupation of those on whom they are dependant (O'Hare 1982). The main disadvantage of this scale for research in women is the downward social mobility associated with child rearing. The classification of women by their current occupation alone may not reflect their level of education and does not take account of former employment with a higher socio-economic classification.

1.12.1 Demographic determinants of food and lifestyle choices

In the European Union most countries issue health-related nutrition guidelines (Kearney & McElhone 1999). These guidelines exhibit a high level of agreement across different countries (Cannon 1992). Generally these healthy eating guidelines have been derived based on available epidemiological evidence without taking the attitudes and perceptions of consumers into account (Kearney & McElhone 1999). These dietary guidelines, although ideal, appear to be unattainable to the majority of the population (Kearney & McElhone 1999). Studies in the UK (MAFF 1994) and in Holland (Hulshof *et al* 1993) found that less than 1% of the population was achieving all the recommendations (Kearney & McElhone 1999). Although the role of diet and lifestyle in health is well recognised, there is relatively less understanding of the factors that influence an individual's food choice, physical activity patterns, alcohol consumption and smoking behaviour (Vaandrager & Koelen 1997). For health promotion to be successful it must address the needs and aspirations of the target audience, while taking into account their current level of knowledge, attitudes, perceptions and behaviours (Buttriss 1997). Extensive nutrition education programmes commencing in the 1970s have attempted to communicate the principals and benefits of a healthy diet to the public (Vaandrager & Koelen 1997). Several studies have revealed that information and knowledge are important but not sufficient factors to promote behavioural change (Vaandrager & Koelen 1997). Food choice is influenced by social, cultural economic and political factors (Vaandrager & Koelen 1997) and research has identified several barriers to healthy eating among consumers.

Glanz *et al* (1998) examined the self-reported importance of taste, nutritional value, cost, convenience and weight control on personal dietary choices among 2967 American adults. Respondants reported taste as the most important influence on their food choices, followed by cost (Glanz *et al* 1998). Higher socio-economic class individuals reported nutritional value as an important predictor of food choice (Glanz *et al* 1998). Johansson *et al* (1999) examined social determinants and lifestyle factors in relation to dietary habits among 3144 Norwegian adults. These researchers found that socio-economic class, length of education and location of residence were associated with indicators for a healthy diet (for example self-reported percentage dietary energy from fat) (Johansson *et al* 1999). However it was found that social status was a poor predictor of healthy dietary habits and nutritional adequacy (Johansson *et al* 1999). Although socio-economic class appears to be associated with indicators for a healthy

diet and better nutritional knowledge personal preference may be just as important for implementing healthy dietary practices (Glanz *et al* 1998, Johansson *et al* 1999).

1.12.2 Determinants of food choice in relation to the development of effective nutrition education programmes

It is increasingly recognised that understanding personal and socio-economic predictors of nutrition behaviour is necessary for the development of effective public health education programmes (Kearney *et al* 2000). In Britain in the early 1990s there was some evidence that people were beginning to adopt advice about fat intake (MAFF 1991, Buttriss 1997). However there are concerns that this may be happening at the expense of micronutrients such as iron and calcium (MAFF 1991, Buttriss 1997). There is evidence to suggest that in an attempt to reduce fat intake individuals were avoiding milk and meat and hence compromising their calcium and iron intakes (MAFF 1991, Buttriss 1997). The misconception that staple foods such as milk and meat are high in fat appears to be widespread particularly among women (Flynn 1997, Gilbody *et al* 1999, Flynn 2000). The misguided avoidance of these foods in an attempt to eat more healthily has significant implications for nutritional status and longterm health.

Probably the most important aspect of health promotion is ensuring that individuals have sufficient information to translate the messages into healthy dietary behaviour. In a study of 1700 members of the British general public 80% believed that they were well informed about healthy eating (Buttriss 1997). Women and higher socio-economic class individuals were more likely to claim to be well informed regarding nutrition (Buttriss 1997). However, when nutritional knowledge was assessed 42% failed to identify fibre rich foods, 90% could not identify foods containing saturated fatty acids, 97% failed to identify foods containing polyunsaturated fatty acids, 92% were unable to identify calcium rich foods and 90% failed to identify iron rich foods (Buttriss 1997). From this it can be concluded that many consumers do not have sufficient knowledge to translate health messages into food choices.

Various studies in developed countries have identified other barriers to adopting a healthier diet and lifestyle. The Institute of European Food Studies (IEFS) examined perceived influences on food choice in a nationally representative sample (n = 1009) of Irish adults (Kearney *et al* 2000). Cost was selected by 30% of the sample (40% of lower socio-economic class subjects) (Kearney *et al* 2000).

A pan-EU consumer attitudinal survey found that the most frequently mentioned perceived barriers to healthy eating concerned time and taste factors (Kearney & McElhone 1999).

An important barrier affecting food choice may be related to the general public's perceptions of their own diets (Kearney *et al* 2000). The IEFS survey reported a low level of perceived need among Irish consumers to change their diet for health reasons (Kearney *et al* 2000). Seventy percent of subjects surveyed in the pan-EU study believed that their diets were already healthy (Kearney & McElhone 1999). The NSIFCS reported that 52% of the 1379 respondents considered that they do not need to change their diet for health reasons (IUNA 2001, Kearney *et al* 2001). People in the older age group (51-64 years) were more likely to perceive that they did not need to change their diet for health reasons despite the fact that 24.4% of men and 29.6% of women in this age group were obese (IUNA 2001, Kearney *et al* 2001). Interestingly, 50% of this age group felt that their weight was 'fine for their age' (IUNA 2001, Kearney *et al* 2001).

1.12.3 Sources of nutrition information utilised by consumers: implications for health promotion campaigns

Studies have observed varying degrees of nutritional knowledge among consumers (Buttriss 1997, Vaandrager & Koelen 1997, Hiddink *et al* 1997, Glanz *et al* 1998). Consumers receive nutrition information from a variety of sources and these sources appear to be viewed with varying degrees of credibility by members of the public (Buttriss 1997). The media represents a major source of nutrition and health information (Buttriss 1997). Media sources are seen as largely unreliable, the information that these sources provide being cited as inconsistent (Buttriss 1997, Vaandrager & Koelen 1997, Hiddink *et al* 1997, Glanz *et al* 1998). Physicians and other health professionals are perceived as the most credible source of health information (Hiddink *et al* 1997) therefore, primary care personnel can potentially play a key role in providing accurate, effective nutrition information. It is estimated that in 14-28% of patient consultations with general practitioners (GPs) diet is discussed (Hiddink *et al* 1997). However GPs perceive a lack of knowledge and skill in their ability to counsel patients on nutritional matters (Hiddink *et al* 1997, Lazarus 1997). Nutrition education is provided in very limited amounts in most training programmes for GPs (Hiddink *et al* 1997, Lazarus 1997) and many family physicians feel that

valuable opportunities to provide individual dietary advice are under-utilised by them (Hiddink *et al* 1997, Lazarus 1997). Dietary advice given by GPs appears to be rather generic (Hiddink *et al* 1997, Lazarus 1997). The key to effective health promotion appears to lie in providing individual practical advice for patients to enable them to implement dietary and lifestyle changes. Family physicians are ideally placed to offer this advice if provided with adequate training and support from a community based nutrition expert.

1.13.1 Establishing a link between diet and disease

It is generally believed but difficult to prove that diet plays a role in the aetiology of various chronic diseases in developed countries (Livingstone 1995). Dietary assessment of individuals is necessary in many areas of clinical medicine and in the formulation of dietary recommendations and agricultural policies (Bingham 1987). Studying the association between diet and disease requires reliable and valid methods for the assessment of diet in individuals and groups of individuals (Lee-Han *et al* 1989).

1.13.2 Dietary assessment methods.

Several methods exist for the assessment of dietary intake, which differ in the way in which information is collected, the time frame that is included in the inquiry and the type of information that is sought (Lee-Han *et al* 1989). The aim of a dietary survey is to establish the habitual intake of individuals (Livingstone 1995). Establishing long-term exposure to dietary variables is difficult, therefore most studies aim to assess dietary intake accurately over a specified time frame (Livingstone 1995). The dietary assessment instruments most frequently used in epidemiological research are based on either recall or recording methods (Livingstone 1995). Recall methods include the Diet History and food frequency questionnaires. All recall methods are prone to errors of memory or errors in reporting, also the recall ability of individuals may be dependant on age, socio-economic class or health status (Livingstone 1995). Recall methods require a skilled interviewer to obtain accurate information (Livingstone 1995).

The Diet History measures the typical food intake during one seven day period. Standardised food photographs of known portion weights, data collection and coding sheets are used. Information is obtained according to the usual meal and snack pattern of the individual, including usual foods consumed during the week and at weekends, with detailed descriptions of these foods including methods of preparation and portion

size. After the usual eating pattern has been described, the information is reviewed with emphasis on foods that may have been omitted such as take-aways. Amounts of food and drinks are then estimated using the photographic atlas and common household measures. The interview takes between one and two hours to complete depending on the stability of the subject's eating pattern and the amount of probing necessary to facilitate recall.

The Fat Intake Questionnaire is a semi-quantitative food frequency questionnaire, which was developed for use in Irish conditions (Cantwell *et al* 1997). It is a much shorter dietary assessment tool than the Diet History taking approximately twenty minutes to administer. It is a standardised, interview-assisted questionnaire, which includes eighty-eight commonly eaten foods in the Irish diet in a meal pattern format. The Fat Intake Questionnaire was previously validated against the Diet History to assess its ability to accurately estimate fatty acid intake among adults (Cantwell *et al* 1997).

Food recording represents another option for dietary assessment in research studies. Food recording methods measure current intake and require that all foods consumed be recorded at the time of consumption over a specific period of time (Livingstone 1995). This method relies on the assumption that dietary patterns will remain unchanged throughout the recording period. However subjects have been found to change their diets either to simplify recording or to provide what they consider to be an acceptable record of dietary intake (Livingstone 1995). This method also requires a degree of literacy and therefore may be inappropriate for use in lower socio-economic classes.

The strengths and weaknesses of each method are well understood and none of the known methods fulfil the requirements of a 'gold standard', as different types of error are associated with each (Boeing *et al* 1997).

1.13.3 Validation of dietary assessment methods.

The reliability and validity of methods of measurement are important considerations in all epidemiological applications (Lee-Han *et al* 1989). There is an understanding that surrogate measurement instruments of food and nutrient intake may be validated against more detailed, established methods before use in epidemiological studies (Boeing *et al* 1997). Several food frequency questionnaires have been developed and validated as measures of nutrient intake (Block *et al* 1992). However, most of the methods for assessing dietary intakes have rarely been independently validated because

of the absence of techniques to verify dietary survey methodology (Livingstone 1995). The majority of these validation studies have been tests of relative validity (Livingstone 1995). In relative validation studies an established dietary assessment tool, for example the Diet History (Livingstone 1992), is used as a 'gold standard' for evaluating the alternative dietary assessment method. Good agreement between the test and reference methods is not absolute proof of validity as the errors in the two methods may be correlated indicating that both are affected by similar bias. However, poor agreement suggests that at least one of the methods is invalid (Livingstone 1995). If the difference between the two methods is not sufficient to be problematic in clinical interpretation then the methods can be used interchangeably (Bland & Altman 1995). Validity of a dietary assessment method can be verified using a biological marker of nutrient intake measurable in body fluids. Ideally this biological marker should be easily accessible, sensitive to dietary intake and unaffected by physiological mechanisms (Livingstone 1995).

The measurement of the diet of individuals is one of the prime requisites of the nutritional sciences (Bingham 1987).

1.13.4 Factors affecting the quality of dietary assessment data.

Accurate assessment of dietary intake is essential for detecting and clarifying the relationship between exposure to dietary variables and disease outcome (Livingstone 1995). It is has been established that the majority of self reported dietary intakes are biased towards under-estimation of usual intake (Livingstone 1995). Subjects who report the highest energy intake tend to estimate their requirements more accurately than those who report low intakes (Livingstone 1995). Whichever method is chosen, under-reporting remains a major contributory factor to inaccuracy in dietary assessment. Under-estimation in self-reported intakes has been observed in obese adults (Prentice *et al* 1986), post-obese adults (Black *et al* 1991) and obese adolescents (Bandini *et al* 1990). The doubly labelled water method has provided a tool for assessing the accuracy of self-reported energy intake (Livingstone 1995). However, this method is too expensive and sophisticated for use in large-scale epidemiological studies (Livingstone 1995). An alternative method expresses energy intakes in terms of basal metabolic rate (Goldberg *et al* 1991). This checks the physiological acceptability of dietary intake data with particular emphasis on under-reporting (Livingstone 1995).

Therefore, all dietary surveys should include measurements of height and weight in order to allow investigators to predict the basal metabolic rate to calculate the energy intake to basal metabolic rate ratio.

1.14 Assessment of physical activity levels for research

Regular physical activity causes numerous and substantial performance improving and health enhancing effects (Lee *et al* 1999, Vuori 2001). Many of the biological effects of regular, moderate intensity physical activity translate into substantially reduced risk of vascular diseases, non-insulin dependent diabetes, overweight, obesity and osteoporosis (Lee *et al* 1999, Vuori 2001). As the health consequences of physical activity and inactivity are increasingly documented there is growing recognition of the importance of studying physical activity and the need for accurate techniques for doing so becomes apparent (Philippaerts *et al* 1999). Methods for assessing physical activity or energy expenditure include behavioural observation, questionnaires, activity diaries, mechanical and electronic motion detectors, heart rate monitoring and doubly labelled water (Philippaerts *et al* 1999). Physical activity is a complex behaviour incorporating occupational and leisure activity and hence is very difficult to quantify (Philippaerts *et al* 1999). All measurement techniques incorporate some methodological problems because of the lack of a universally acceptable validation standard (Philippaerts *et al* 1999).

Total daily energy expenditure in anthropological settings has generally been estimated by the factoral method in which the researcher or subject records the amount of time spent in various activities throughout the day. The activity times are then converted into energetic equivalents. Activity-specific energy costs can be determined by indirect calorimetry in the field or by using standard tables (Leonard *et al* 1997). In 1985, this approach was recommended by the WHO as the preferred method for estimating individual and population energy expenditure (FAO/WHO/UNU 1985, Leonard *et al* 1997). Although there are limitations associated with this method it is still widely used. The NSIFCS characterised usual levels of physical activity using a comprehensive self-administered questionnaire, which provided a detailed assessment of all daily physical activities (IUNA 2001, Livingstone *et al* 2001). Metabolic energy equivalents were then assigned to each activity (IUNA 2001, Livingstone *et al* 2001).

In the last decade the Doubly Labelled Water (DLW) technique has been increasingly considered as the gold standard in the assessment of physical activity in the field (Ritz

& Coward 1995, Philippaerts *et al* 1999). The DLW technique uses the principals of indirect calorimetry to measure total energy expenditure from the turnover rates of two staple isotopes; deuterium and oxygen 18 (Ritz & Coward 1995, Philippaerts *et al* 1999). This method, although accurate, is time consuming and expensive and is generally used in studies with a small sample size (Ritz & Coward 1995, Philippaerts *et al* 1999).

In contrast to DLW the questionnaire technique is relatively easy and inexpensive and appears to be the most suitable for use in large-scale studies (Philippaerts *et al* 1999). Several questionnaires exist which have been used to estimate physical activity with varying degrees of accuracy (Philippaerts *et al* 1999). Studies to validate these questionnaires have established reliability and validity for interview assisted questionnaires using DLW as a gold standard (Leonard *et al* 1997, Philippaerts *et al* 1999). These questionnaires are still subject to bias relating to subject characteristics and skill of individual investigators (Leonard *et al* 1997, Philippaerts *et al* 1999). However, when used in conjunction with other indicators of physical activity such as BMI and energy intake they represent an acceptable measure of physical activity for research (Leonard *et al* 1997, Philippaerts *et al* 1999).

BMI has been shown to correlate well with reported energy expenditure during leisure time (Martin-Almendros *et al* 2000). This association has been observed even where only less accurate measurement techniques for physical activity are feasible (Martin-Almendros *et al* 2000). Although the inherent limitations of questionnaires to determine self-reported physical activity levles must be acknowledged, these questionnaires appear to be able to broadly divide individuals into different physical activity categories (Martin-Almendros *et al* 2000). At the very least questionnaires allow researchers to identify trends associated with varying levels of self-reported physical activity.

1.15 Study objectives.

The objective of the current study is to establish the prevalence of undiagnosed anaemia and to investigate factors relating to anaemia in a representative sample of Irish adult women from advantaged and disadvantaged socio-economic classes attending general practitioners in inner-city Dublin. Comparable studies have been conducted in the United States and Britain, however there is a dearth of information on the haematinic status of Irish adult women. The current study will assess typical

dietary intake with emphasis on iron, folates and vitamin B_{12}. Biological markers of haematinic nutrients will be examined through the analysis of venous blood samples. The effect of supplement use, non-steroidal anti-inflammatory drug use and parity on these haematalogical parameters will be assessed. Body weight status, body image concerns and slimming practices will also be assessed together with nutritional knowledge of factors relating to anaemia. Determinants of food choice, attitudes to nutrition and lifestyle habits will also be examined in relation to actual dietary habits and nutritional adequacy. This study will also attempt to determine the effect of socio-economic class and education on all outcomes measured. This is the first comprehensive study to highlight nutritional anaemia in a group of apparently healthy Irish adult women in a primary health care setting. This project will attempt to identify vulnerable groups in order to enable primary care physicians to prevent and treat nutritional anaemias among their patients.

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Chapter 2

Development and validation of a semi-quantitative food frequency questionnaire and an interview questionnaire for use in a study of iron, folate and vitamin B_{12} status in Irish adult women.

2.1 Introduction:

Studying the association between diet and disease requires reliable and valid methods for the assessment of dietary intakes in individuals and groups of individuals (Lee-Han *et al* 1989, Flagg *et al* 1999). The method of choice will depend on the aim of the study, the population at issue and the amount of time and human resources available (Grootenhuis *et al* 1995). The most frequently used dietary assessment methods rely on recall or recording. Recall methods include the Diet History (Livingstone *et al* 1992) and food frequency questionnaires. These methods of dietary assessment can be affected by errors of memory, incorrect reporting, socio-economic class, age or state of health and are often time consuming (Livingstone 1995, Grootenhuis *et al* 1995). Recording methods assume that dietary intake remains the same over the recording period and require literate, motivated subjects (Livingstone 1995, Grootenhuis *et al* 1995). Some semi-quantitative food frequency questionnaires have been developed and validated, against other previously validated methods, as measures of food and nutrient intake (Block *et al* 1982, Flagg *et al* 1999). This is considered to be relative validity, where an established dietary assessment method is used as a reference to test the validity of a new method. Neither method is entirely error free, however validation studies establish that that the bias associated with each is similar and that they can be used interchangeably in epidemiological research.

The use of a biological marker of nutrient intake verifies the validity of dietary assessment methods more directly and should represent another tool in validation studies (Willett 1994, Livingstone 1995). Twenty-four hour nitrogen excretion has been used as a biological marker of nitrogen turnover to validate dietary assessment methods for estimating nitrogen intakes (Black *et al* 2000). Similarly the ability of dietary assessment methods to accurately determine energy intakes has been validated against energy expenditure using the doubly-labelled water technique (Black *et al* 2000). Fatty acid levels in adipose tissue stores obtained by biopsy have been used as biological markers to validate dietary assessment methods designed to assess fatty acid intakes (Cantwell *et al* 1997). Biological markers of micronutrient intake have also been employed to validate dietary assessment methods. Plasma β-carotene was assessed in 87 subjects as a biological marker of intake to successfully validate a food frequency questionnaire (Daures *et al* 2000). Although it is acknowledged that some random variations in levels of the biological marker will occur due to factors not

directly related to dietary intake (Black *et al* 2000, Daures *et al* 2000) the inclusion of a biological marker in validation studies is recommended (Willett 1994).

The two methods of dietary assessment examined in this validation study were the Diet History (Livingstone 1992) and a modified version of the Fat Intake Questionnaire (Cantwell *et al* 1997). The Diet History method is based on a questionnaire developed in 1947. It consists of an examination of usual eating patterns over a seven day period taking the weekend into account. It also incorporates a food frequency assessment. The Diet History is used in conjunction with a food portion size atlas to assist in the estimation of food portions consumed. The use of common household measures also aids in quantifying portion sizes of foodstuffs consumed. This method of dietary assessment has been validated against the doubly labelled water technique in children and adolescents (Livingstone *et al* 1992). The Diet History involves an extensive interview taking up to one hour to complete.

The Fat Intake Questionnaire (Cantwell *et al* 1997) is a much shorter dietary assessment tool taking approximately twenty minutes to administer. It is a standardised, interview-assisted questionnaire, which includes eighty-eight commonly eaten foods in the Irish diet in a meal pattern format. The Fat Intake Questionnaire, used in conjunction with a brand atlas of commonly eaten sources of dietary fat in the Irish diet, was previously validated against the Diet History and linoleic acid levels in fat biopsies to assess its ability to accurately estimate dietary fatty acid intake in Irish adults (Cantwell *et al* 1997). It was also found to assess intakes of other macronutrients and micronutrients in comparable terms with the Diet History (Cantwell et al 1997).

The purpose of the present study was to develop the Fat Intake Questionnaire for use in a study to assess iron, folate and vitamin B_{12} status in Irish adult women and to validate the new Food Intake Questionnaire against the Diet History and biological markers of haematinic nutrient intake in blood samples. It is well established that there are several non-dietary contributory factors to haematinic status, for example menstrual blood losses (Hallberg *et al* 1966, Hallberg *et al* 1995) and the use of the oral contraceptive pill (Hallberg & Rossander-Hulten 1991, Hallberg *et al* 1995). The development and validation of an interview assisted questionnaire to take account of these factors is essential in the assessment of iron, folate and vitamin B_{12} status in Irish adult women.

2.2 Subjects and Methods

2.2.1 Subjects.

Prior to the recruitment of subjects, ethical approval was sought and granted by the Irish College of General Practitioners ethics committee. Female volunteers were recruited from within a group of mothers of primary school children involved in a Dublin Institute of Technology research project. Three hundred and ninety three consent forms inviting participation were circulated to six primary schools (two fee-paying and four non fee-paying) in Dublin's south inner city. All participating female volunteers were aged between eighteen and sixty-four years and had no history of chronic disease. Pregnant women were excluded, as were women known to be anaemic. Women who were found to be dieting were also excluded. All female volunteers attended general practitioners in inner city Dublin. Each participating subject visited Mercer's Medical Centre (Department of General Practice, Royal College of Surgeons in Ireland) twice between February and April 1999. All female volunteers were reimbursed for travel expenses incurred.

2.2.2 Development and administration of the dietary assessment methods.

Traditionally only the Diet History method included the use of a photographic atlas of food portion sizes to enable subjects to estimate portion sizes of foods consumed. For the purpose of this study the Irish National Nutrition Survey (INDI 1990) portion size atlas was updated using photographs from the Irish Universities Nutrition Alliance dietary survey atlas (IUNA 2001) and the new food portion size photographic atlas was used in conjunction with both proposed dietary assessment methods to improve accuracy.

The accurate assessment of haematinic nutrient intake requires the measurement of fortified foodstuffs. The proposed methods of dietary assessment tested in the current study were modified to include information on folic acid fortified foods in the Irish diet using a photographic brand atlas which was used in conjunction with the Diet History and the Food Intake Questionnaire. This new atlas was developed using manufacturers' data on fortified milk, yogurt, breakfast cereals and breads on the Irish market which were purchased, photographed and compiled into a brand atlas for use in conjunction with each dietary assessment method. The use of this atlas with both

proposed dietary assessment methods allowed subjects to identify fortified foodstuffs consumed.

The Diet History and Food Intake Questionnaire were administered to each volunteer during separate interviews with the same investigator to eliminate inter-observer bias. Care was taken to ensure that approximately equal numbers of female volunteers from each socio-economic class completed each dietary assessment method at their first interview to minimise potential bias regarding any food during the investigation period. The length of time between interviews ranged from fourteen to twenty-one days.

The dietary information was coded using M^{c}Cance and Widdowson's food composition tables and their supplements (Holland *et al* 1988, Holland *et al* 1991, Holland *et al* 1995). The conversion from foodstuffs to nutrients was established using a computerised version of the Royal Society of Chemistry food and nutrient database WISP version 1.26 for Windows. This database was updated to include the amount of folic acid per 100g of each fortified food based on the manufacturers' data on foods fortified with folic acid on the Irish market used to compile the fortified food atlas. The dietary intakes were checked for under-reporting by comparing the reported energy intake with the calculated energy requirements defined as multiples of the basal metabolic rate (Goldberg *et al* 1991). An EI:BMR cut-off point of 1.46 was used to determine under-reporting for the current sample of female volunteers (Goldberg *et al* 1991).

2.2.3 Development and administration of the 'Interview Questionnaire'.

Information on age, time spent in formal education and occupation was collected using a standardised, interview assisted questionnaire designed for this study (Appendix II). Data on menstrual history (age at menarche, duration of menses, length of cycle and subjective estimation of level of menstrual blood flow), reproductive history (number of pregnancies and miscarriages, age at first pregnancy, birth weight and state of health of offspring and birth interval) and contraceptive use (type and duration of use) were obtained using this questionnaire. The use of non-steroidal anti inflammatory drugs (frequency of both over-the-counter and prescription non-steroidal anti inflammatory drugs) and vitamin and mineral supplements was investigated using this questionnaire, as were body image concerns, slimming practices and nutritional knowledge relating to iron and folic acid. Each multiple-choice question included a 'don't know' option.

The 'interview questionnaire' was administered to each subject twice by the same investigator, once at each visit to the research centre.

Using data from this questionnaire, socio-economic class was calculated for each subject using the census based Irish social class scale for epidemiological health research (O'Hare 1982). For meaningful statistical analysis in this small group of subjects O'Hare's social classes one and two, three and four and five and six were combined to form social classes one, two and three respectively.

2.2.4 Anthropometry.

Height to the nearest mm and weight to the nearest 0.01kg were measured barefoot, in light indoor clothing and used to calculate body mass index (kg/m^2). Waist measurements were taken midway between the lowest rib and the iliac crest (Lean *et al* 1995). Hip measurements were taken at the level of the great trochanters (Lean *et al* 1995) using a metal tape. Waist to hip ratio was calculated. All measurements were taken by the principal investigator to eliminate inter-observer bias.

2.2.5 Blood sampling and analysis.

Nine millilitres of blood was obtained from each female volunteer using two vacutainers (Beckton Dickinson). The vacutainers contained EDTA as an anticoagulant. Immediately after drawing the blood the vacutainers were refrigerated for transport to the Dublin Institute of Technology research laboratory within two hours of collection. Full blood count was measured in one anticoagulated sample using the Cobas Micros analyser and appropriate control samples (Boehringer Mannheim). For analysis of red cell folate 100µl of anticoagulated blood was aliquotted with 900µl of 1% ascorbic acid solution. This was stored in micronic tube strips at -20°C for transport to the Vitamin Research Laboratory, St. James's Hospital, Dublin 8. The remaining anticoagulated blood was centrifuged at 3500rpm for six minutes and part of the resulting plasma was aliquotted and stored in micronic tube strips at -20°C for transport to the Vitamin Research Laboratory, St. James's Hospital, Dublin 8 for analysis of vitamin B_{12} and homocysteine. One ml of the remaining plasma was aliquotted and stored at -20°C for analysis of plasma ferritin. Analysis of plasma ferritin was performed using the Enzymun-Test Ferritin kit (Boehringer Mannheim). This is an enzyme immunoassay for the quantitative determination of ferritin in vitro,

the principle of which is an ELISA one step sandwich assay using streptavidin technology. The appropriate samples were transported on ice to the Vitamin Research Laboratory, St. James's Hospital, Dublin 8 where red cell folate was analysed using a microbiological assay for serum, plasma and red cell folate using a cryopreserved microtitre plate method (Molloy & Scott 1997). Plasma vitamin B_{12} was determined using a microbiological assay for vitamin B_{12} performed in 96-well microtitre plates (Kelleher & O'Broin 1991). Plasma homocysteine was determined according to the method described by Nexo *et al* (2000).

2.2.6 Data management and statistical analysis.

Information obtained was coded and entered into version 8 of the Statistical Package for Social Sciences for Windows. The distributions of dietary intake generated by each method were checked for normality. Non-parametric values were logarithmically transformed prior to analysis. The ability of the Food Intake Questionnaire to estimate group means was investigated using paired t-tests. Agreement of mean intakes from the Food intake Questionnaire relative to the Diet History does not eliminate the possibility of non-constant bias as low intakes can be underestimated and high intakes overestimated and vice versa (Grootenhuis *et al* 1995). To examine the possibility of non-constant bias, the mean of and the differences between the two methods were calculated for each nutrient. The association between these parameters was examined using regression analysis (Bland & Altman 1995). The mean and standard deviation of the differences between the two methods were calculated to assess agreement between the methods at an individual level (Bland & Altman 1995). The 95% limits of agreement (defined as the mean difference $\pm$ 1.96 standard deviations of difference) were calculated for each nutrient (Bland & Altman 1995). The Pearson product moment correlation was used as a global measure of linear association between the two methods (Grootenhuis *et al* 1995). For biological markers of nutrient intake used in this study (plasma ferritin, red cell folate and plasma vitamin B_{12}) Pearson product moment correlation coefficients were calculated for total iron, haem and non-haem iron and iron obtained from supplements, total folates, total food folates, folic acid from fortified foods, food folate and folic acid obtained from supplements and for vitamin B_{12} intakes recorded by both dietary assessment methods.

The standardised 'interview questionnaire' was tested for reliability and reproducibility by comparing responses at baseline and second administration of the questionnaire for each subject. The questionnaire is considered to be reliable when responses at the second administration of the questionnaire are ≥95% in agreement with responses at the baseline administration. Proportional analysis was performed using the Chi-square test.

2.3 Results:

The response rate was 15% (*n*=59). Of these respondents thirty-five participated fully in the validation study, 60% (*n*=21) were recruited through non fee-paying schools and 40% (*n*=14) were recruited through the fee-paying schools. Mean age of the women was 40.3 years with a standard deviation of 5.5 years. Mean body mass index was within the normal range at 24.8kg/m^2 (standard deviation: 4.03kg/m^2). The measurement of body fat distribution used in this study was waist hip ratio, mean values for this was 0.82 with a standard deviation of 0.59. Of the 35 female volunteers 60% (*n*=21) were in socio-economic class one, 14% (*n*=5) were in socio-economic class two and 26% (*n*=9) were in socio-economic class three.

Of the total sample 40% (*n*=14) were using vitamin supplements at the time of the study. There was a socio-economic gradient apparent in the use of supplements since 71% (*n*=10) and 29% (*n*=4) of those using supplements were in socio-economic classes one and two respectively. None of the women in socio-economic class three reported using vitamin supplements.

Cigarette smoking was also assessed and was found to be more prevalent in the lower socio-economic classes: of the women in socio-economic class one, 22% (*n*=5) were current smokers compared with 60% (*n*=3) and 67% (*n*=6) of those in socio-economic classes two and three respectively.

Of the 35 female volunteers who completed the pilot study none were found to be clinically anaemic (haemoglobin <12g/dl) (Hallberg & Rossander-Hulthen 1989), however 26% (*n*=9) were found to have low iron stores defined as plasma ferritin of <15μl (Hallberg & Rossander-Hulthen 1989). Six percent (*n*=2) of the female volunteers had a red cell folate level in the deficient range (<150μg/l) and 31% (*n*=11) had suboptimal red cell folate levels (<400μg/l) (Daly *et al* 1995). Of the 63% (*n*=22) female volunteers with an optimal red cell folate level 50% (*n*=11) were found to use

vitamin and/or mineral supplements at the time of the study. Plasma homocysteine levels were elevated (>15μmol/l) in 9%(n=3) of subjects.

Responses to the 'interview questionnaire' at baseline and the second administration were found to be ≥95% in agreement. Percentage agreement figures were as follows: 100%, 95%, 99%, 100%, 100%, 95%, 97%, 95% for sections one to eight respectively.

The thirty five female volunteers who were included in the study were identified as having a ratio of energy intake to basal metabolic rate of ≥1.46, the lower limit of the 95% confidence interval, therefore their dietary records were included in the analysis (Goldberg *et al* 1991). Four subjects found to be dieting at the time of interview were excluded.

Table 1 summarises the results with regard to macronutrients and tables 2(a) and 2(b) presents the results regarding micronutrient intakes derived from diet and supplements. The haematinic status of the group of female volunteers is detailed in table 3 and the results of the analysis on the biological markers of haematinic nutrient intake are presented in table 4. Paired t-tests of group means of nutrients recorded by both methods of dietary assessment showed no significant differences (Tables 1, 2(a) and 2(b)). Regression analysis demonstrated that the differences between the individual pairs of intake estimates were not significantly related to the means. The 95% limits of agreement for nutrient intake estimated by the two methods did not differ significantly from zero (Tables 1, 2(a) and (b)). Correlation coefficients were found to be significantly positive for all nutrients (Tables 1, 2(a) and 2(b)). Correlation coefficients showed a weakly positive correlation between dietary sources of iron and plasma ferritin (Table 4). Dietary vitamin B_{12} intake recorded by the Diet History was significantly correlated with plasma vitamin B_{12}. The correlation coefficient for vitamin B_{12} intake recorded using the Fat Intake Questionnaire and plasma vitamin B_{12} was also positive but not significant. Correlations for red cell folate levels for total folate intake, folic acid from supplements and dietary folic acid from fortified foods were significantly positive for both methods of dietary assessment.

Table 1: A comparison of daily macronutrient intakes assessed by the Diet History (DHx) and Food Intake Questionnaire (FIQ).

Nutrient	**DHx** mean±SD	**FIQ** mean±SD	mean difference (95% CI)	r^2
EI:BMR	1.68 ± 0.40	1.69 ± 0.28	-0.71(-0.8;0.4)	0.814*
Energy (MJ)	9.9 ± 1.6	10.4 ± 2.1	-0.84 (-4.1;2.4)	0.869*
Energy (kcal)	2338 ± 382	2536 ± 497	-36 (-334;262)	0.869*
Protein (g)	86.5 ± 12.9	93.8 ± 16.8	-2.8 (-22.2;16.6)	0.755*
Protein (%)	14.9 ± 2.5	15.0 ± 2.2	-0.14 (-2.8;2.6)	0.781*
CHO (g)	279 ± 57.6	288.7 ± 69.2	-7.2 (-32.0;17.7)	0.772*
CHO (%)	43.5 ± 5.4	42.2 ± 6.7	1.3 (-7.5;11.4)	0.746*
Total Fat (g)	96.9 ±28.3	103.9 ± 27.7	-3.9 (-14.5;6.7)	0.881*
Total Fat (%)	37.2 ± 5.3	38.7 ± 5.2	0.5 (-3.6;2.6)	0.822*
SFAs (g)	35.9 ± 9.9	37.7 ± 10.7	-1.8 (-7.6;3.6)	0.862*
SFAs (%)	15.8 ± 2.4	16.3 ± 2.8	-0.82 (-5.9;4.3)	0.839*
MUFAs (g)	29.8 ± 8.6	32.2 ± 8.5	-2.8 (-15.1;9.1)	0.800*
MUFAs (%)	12.9 ± 2.8	13.5 ± 2.7	-1.2 (-7.5;5.1)	0.811*
PUFAs (g)	16.5 ± 5.7	18.9 ± 6.3	-1.2 (-6.7;4.3)	0.776*
PUFAs (%)	8.4 ± 2.6	8.4 ± 2.9	-1.0 (-2.9;2.9)	0.976*
Englyst fibre (g)	16.2 ± 4.6	18.5 ± 4.2	-2.3 (-7.2;2.6)	0.840*
Southgate fibre (g)	22.9 ± 6.1	26.3 ± 5.5	-3.0 (-10.2;4.2)	0.802*
Alcohol (g)	12.8 ± 11.0	11.7 ± 10.3	⊥0.71 (-3.2;4.7)	0.779*
Alcohol (%)	4.1 ± 2.6	3.2 ± 2.5	⊥0.11 (-1.2;1.4)	0.759*

*Correlation significant at level ($p<0.01$). ⊥: Logarithmically transformed variable. CI: Confidence interval. SD: standard deviation. CHO: carbohydrate. SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids. PUFAs: polyunsaturated fatty acids.

Table 2 (a): A comparison of daily haematinic micronutrient intakes assessed by the Diet History (DHx) and Food Intake Questionnaire (FIQ).

Nutrient	**DHx** mean±SD	**FIQ** mean±SD	mean difference (95% CI)	r^2
Total iron (mg)	18.3 ± 9.1	18.6 ± 8.6	⊥-0.02 (-0.17;0.12)	0.961*
Dietary iron (mg)	13.7 ± 3.2	14.3 ± 2.9	-0.62 (-5.1;3.9)	0.732*
Haem iron (mg)	3.1 ± 1.3	3.14 ± 1.6	⊥-0.03 (-0.3;0.3)	0.711*
Non-haem iron (mg)	11.7 ± 3.5	11.2 ± 2.9	0.36 (-4.5;5.3)	0.951*
Supplementary iron (mg)	4.3 ± 7.9	4.8 ± 8.2	⊥-0.03 (-0.2;0.2)	0.928*
Total folates (μg)	375 ± 77	390 ± 148	-6.3 (-26.1;13.5)	0.921*
Dietary folates (μg)	282 ±77	321 ±74	-9.6 (-41.3;22.2)	0.667*
Dietary folic acid (μg)	19.5 ± 26.7	20.2 ± 31	⊥-0.1 (-0.8;0.5)	0.908*
Dietary folate (μg)	266 ± 68	300 ± 61	-12.3 (-61.1;36.5)	0.591*
Supplementary folic acid (μg)	96 ± 125	74 ± 107	⊥0.3 (-0.2;0.3)	0.921*

*Correlation significant at level ($p<0.01$). ⊥: Logarithmically transformed variable. CI: Confidence interval. SD: standard deviation.

Table 2 (b): A comparison of daily intakes of other micronutrients assessed by the Diet History (DHx) and Food Intake Questionnaire (FIQ).

Nutrient	**DHx** mean±SD	**FIQ** mean±SD	mean difference (95% CI)	r^2
Calcium (mg)	947 ± 299	1053 ± 334	-32 (-145;82)	0.722*
Zinc (mg)	9.5 ± 2.3	10.1 ± 2.2	-0.7 (-4.8;3.4)	0.558*
Vit. B_1 (µg)	1.7 ± 0.5	1.8 ± 0.4	-0.1 (-0.6;0.6)	0.624*
Vit. B_2 (µg)	1.9 ± 0.7	2.1 ± 0.7	-0.2 (-1.1;0.7)	0.388*
Vit. B_3 (µg)	41.5 ± 8.8	45.3 ± 8.8	-3.8 (-21.2;13.6)	0.480*
Vit. B_6 (µg)	2.2 ± 0.5	2.5 ± 0.6	-0.3 (-1.1;0.5)	0.776*
Vit. B_{12} (µg)	3.9 ± 1.8	4.7 ± 1.7	⊥-0.08 (-0.5;0.3)	0.488*
Vit. C (mg)	96.7 ± 47.5	114 ± 49	-17 (115;81)	0.455*
Vit. D (µg)	2.3 ± 1.2	3.1 ± 1.54	⊥-0.2 (-0.8;0.4)	0.481*
Vit. E (µg)	7.2 ± 2.3	8.7 ± 3.3	-0.2 (-2.4;2.0)	0.390*
Retinol Equivalents	1169 ± 467	1382 ± 508	-41 (-702;620)	0.351*

*Correlation significant at level ($p<0.01$). ⊥: Logarithmically transformed variable. CI: Confidence interval. SD: standard deviation.

Table 3: Iron, folate, vitamin B_{12} and homocysteine status of the participating female volunteers (n=35).

Haematalogical parameter	mean ± SD	Normal range
Haemoglobin (g/dl)	13.2 ± 0.8	12-15g/dl
Mean Cell Volume (fl)	91.8 ± 4.5	75-95fl
Mean Cell Haemoglobin Content (pg/ml)	34.1 ± 1.8	27-38 pg/ml
Plasma Ferritin (µg/l)	52.5 ± 50.5	15-140µg/l
Red Cell Folate (µg/l)	363 ± 206	150-1000µg/l
Plasma vitamin B_{12} (ng/l)	718 ± 274	150-1000 ng/l
Plasma homocysteine (µg/l)	8.7 ± 3.6	5-15µg/l

SD: standard deviation.

Table 4: Pearson correlation coefficients (r^2) for haematinic nutrient intakes recorded using the Diet History (DHx) and Food Intake Questionnaire (FIQ) and relevant biological markers.

	Plasma Ferritin	
	DHx	FIQ
Total iron (mg)	0.180	0.133
Total dietary iron (mg)	0.129	0.101
Haem iron (mg)	0.176	0.133
Non-haem iron (mg)	0.135	0.117
Supplement iron (mg)	0.116	0.136
	Red Cell Folate	
	DHx	FIQ
Total folates (μg)	0.670*	0.754*
Total food folates (μg)	0.182	0.278
Food folic acid (μg)	0.583*	0.670*
Food folate (μg)	0.219	0.199
Supplement folic acid (μg)	0.707*	0.765*
	Plasma vitamin B_{12}	
	DHx	FIQ
Dietary vitamin B_{12}	0.341*	0.291

*Correlation significant at level ($p<0.01$).

2.4 Discussion:

In this study a new semi-quantitative food frequency questionnaire was developed and validated as a quick method of assessing dietary intakes and more specifically haematinic nutrients in a cross-over design in a sample of thirty five inner city Dublin women. The group of female volunteers was sufficiently large to provide a normal distribution of most variables, therefore the statistical analysis can be considered meaningful, non-parametric variables can be normalised for analysis of non-constant bias using logarithmic transformation (Grootenhuis *et al* 1995). Energy-adjusted correlation coefficients comparing nutrient intakes assessed by food frequency questionnaires with those measured by dietary records or recall methods have generally been in the range of 0.4 to 0.7 for a wide variety of populations and with use of questionnaires that varied greatly in length and detail (Willett 1994). The results of the dietary assessment analysis in the current study suggest that the modified Diet History and Food Intake Questionnaire are interchangeable for the purpose of this research. The highly significant correlation coefficients and little variation in the mean differences between the dietary intake measurements indicate that the Food Intake Questionnaire is able to rank participants into categories of nutrient intake comparable with the Diet History (Bland & Altman 1995). However, as none of the known methods of dietary assessment fulfil the criteria of a 'gold standard', the validity proven in this study is not absolute validity (Livingstone 1995). It can be described as relative validity as it has been established that the errors and bias associated with each method are probably similar.

Although the sample of female volunteers surveyed in the current pilot study was representative of those recruited for the main study, the results of this pilot study do not necessarily support the validity of the Food Intake Questionnaire in the Irish population as a whole. Re-examination of the method would be necessary prior to use in future studies, depending on the specific aims and objectives of the research. The 'interview questionnaire' was found to be reliable in this pilot study examination of test-retest reliability. Again although no changes were made to it prior to use in the main study this 'interview questionnaire' would require re-examination and possibly modification prior to use as a research tool with other population groups. Additional research would be required to develop and evaluate food frequency questionnaires that would be applicable to a range of ages, populations, settings and dietary factors (Willett 1994). Experts suggest that even well established methods of dietary assessment need to be

continually validated and modified because of rapidly changing patterns of food consumption and dietary composition within contemporary populations (Willett 1994, Livingstone 1995).

Traditional analysis in nutritional epidemiology typically examine diseases in relation to a single or limited number of nutrients or foods (Hu *et al* 1999). However this approach may be inadequate for taking into account complex interactions between nutrients in studies of free living people (Hu *et al* 1999). The analysis of dietary patterns may be important in examining the relationship between diet and disease (Hu *et al* 1999). Many food frequency questionnaires do not consider how foods and hence nutrients are consumed in combination (Hu *et al* 1999). A potential limitation of the Food Intake Questionnaire in the current study is its insufficient examination of dietary patterns. However, unlike other food frequency questionnaires, the Food Intake Questionnaire does present foods in a meal pattern format and thus takes some account of dietary patterns. This characteristic of the Food Intake Questionnaire is potentially advantageous in the current study as iron status in particular could be influenced by the consumption of other foodstuffs such as tea, citrus fruit or high fibre foods at the same meal as iron containing foods. However, it is acknowledged that dietary records represent a superior choice for assessing dietary patterns.

This study found consistently slightly higher mean estimates of almost all nutrients measured using the questionnaire compared to the Diet History, this has been the case in most relative validation studies where a semi-quantitative food frequency questionnaire is compared to the modified Diet History or food records (Grootenhuis *et al* 1995, Black *et al* 2000, Daures *et al* 2000, Flagg *et al* 2000). In the current study the differences between mean estimates of nutrient intake recorded using the Food Intake Questionnaire, although higher, were not significantly different from those recorded using the reference method.

Although iron intakes from diet and supplements were not significantly correlated with plasma ferritin, a weakly positive correlation was observed. A significant correlation between a biological marker of iron status and dietary iron intake would not be expected as iron status is influenced by several other factors. The most potent determinant of iron status in non-pregnant women in the fertile years is menstrual blood loss (Hallberg 1981). There is considerable variation in menstrual blood loss between women, and even within the same woman menstrual losses can fluctuate monthly (Hallberg 1981). It is therefore quite possible that a woman with high menstrual losses

can have low iron stores despite an apparently adequate intake of dietary iron. Furthermore, several factors within the diet markedly influence the absorption of iron from food (Hallberg 1981). This is particularly true of non-haem iron where several fold differences in absorption are seen depending on the composition of meals (Hallberg 1981). The absorption of iron from food is impaired by phytates, oxalates, tannins and calcium (Hallberg *et al* 1995). Vitamin C and alcohol enhance the absorption of dietary iron (Hallberg *et al* 1995). In addition, the biological marker of iron status used in this study was plasma ferritin, which is reduced in cases of iron deficiency and iron deficiency anaemia. However as ferritin is an acute phase protein it can be falsely elevated in the presence of inflammation or infection (Mast *et al* 1998). It is therefore possible that an individual with poor iron status and a low intake of dietary iron may escape detection based on a falsely raised plasma ferritin level. The recently discovered soluble transferrin receptor is increasingly recognised as the new 'gold standard' in the assessment of iron status and would be a more suitable biological marker for use in future validation studies as it is unaffected by the inflammatory process (Mast *et al* 1998).

The observed correlation coefficients for sources of folates suggest that both dietary assessment methods were able to place individuals into biologically meaningful categories of intake. The significantly positive correlations for sources of folic acid reflect its superior bioavailability compared to dietary folate from which glutamic acid side-chains must be cleaved prior to absorption. It has been established that that approximately 5%-15% of the Irish population is homozygous for a thermolabile variant of 5,10 methyltetrahydrofolate reductase, which impairs folate absorption at a cellular level. This genetic variant has a negative impact on folate status despite an apparently adequate intake of dietary folates (Daly *et al* 1997) and may compromise the ability of the biological marker of folate status to reflect dictary intake.

Although the Food Intake Questionnaire was the method chosen in the present study, certain limitations associated with it require consideration prior to use. In order to obtain accurate information, the questionnaire must be administered by an investigator trained in dietary assessment methodology. The use of a photographic atlas of food portion sizes was necessary to improve accuracy. Depending on the nutrient of interest in a particular research study, information on fortified foods may need to be included in the method either in the form of an atlas or by extending the questionnaire. This information is likely to vary between different countries and will require regular

updating. As with all dietary assessment methods based on recall, the Food Intake Questionnaire may be affected by errors of memory or incorrect reporting. However, the fact that the questionnaire is interview-assisted makes it particularly appropriate for use with lower socio-economic class subjects since degree of functional literacy of the subject is unimportant. In this study the shorter method of dietary assessment produced valid estimates of nutrient intake in a sample of inner-city Dublin women and, due to its significant time advantages, was chosen for use in the main study.

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Chapter 3

Iron, folate and vitamin B_{12} status of Irish adult women attending GPs in inner-city Dublin.

3.1 Introduction:

Iron is essential for the carriage of oxygen, for oxidative metabolism and for normal cellular growth (Provan & Weatherall 2000). The average adult contains between 3g and 5g of iron, of which two thirds is in the oxygen-carrying molecule haemoglobin (Frewin *et al* 1997). Due to its reactivity and toxicity, iron in the body is bound to various proteins for transport and storage. The principal proteins involved in the transport and storage of iron are transferrin, transferrin receptors and ferritin (Provan & Weatherall 2000). The maintenance of positive iron balance is dependent on intake, loss and storage of this mineral (Bothwell 1995). Iron deficiency refers to a spectrum of disorders ranging from depleted iron stores to frank clinical iron deficiency anaemia. Iron deficiency leads to an anaemia characterised by microcyric, hypochromic erythrocytes (Frewin *et al* 1997). Iron deficiency and iron deficiency anaemia impair several vital body functions and can lead to substantial morbidity (Bothwell 1995, Hercberg *et al* 2001).

Iron deficiency and iron deficiency anaemia are the most prevalent nutritional disorders worldwide (Hallberg & Rossander-Hulthen 1991). An analysis of studies of iron deficiency in Europe reported depleted iron stores in approximately 10% to 30% of menstruating women (Hercberg *et al* 2001). The prevalence of iron deficiency anaemia in this group is estimated at between 1.5% and 14% in Europe (Hercberg *et al* 2001). In European adult men the prevalence of low iron stores ranges from 0% to 3% (Hercberg *et al* 2001). Researchers have reported iron deficiency in 9-11% of American adolescent girls and women of childbearing age (Looker *et al* 1997). It is estimated that serum ferritin levels are on average three times lower in women of reproductive age than in their male counterparts (Hercberg *et al* 2001) Therefore, in developed countries menstruating women and girls appear to be most at risk of poor iron status (Hallberg & Rossander-Hulthen 1991, Hercberg *et al* 2001).

Symptoms of iron deficiency and iron deficiency anaemia include lethargy, dyspnoea and impaired physical endurance and work capacity (Frewin *et al* 1997). Depleted iron stores and iron deficiency anaemia have been associated with impaired immune response (Walter *et al* 1997). Iron deficiency and iron deficiency anaemia during pregnancy have been associated with low birth weight, prematurity and increased peri-natal mortality (Allen *et al* 2000, Steer 2000). Foetal under-nutrition and a high placental-foetal ratio have been linked to cardiovascular disease, stroke (Barker 1998, Godfrey & Barker 2001), impaired glucose tolerance (Rich-Edwards *et al* 1999) and hypertension in adulthood (Barker 1993, Barker 1998, Godfrey & Barker 2001). A

suggested mechanism for the development of hypertension during adult life in the low birth weight infant is a possible alteration in the foetal renin-angiotension system (Godfrey & Barker 2001). In response to an inadequate supply of nutrients through the placenta the foetus diverts blood flow to maintain brain function, reducing blood flow to the foetal kidneys resulting in sensitisation of the renin-angiotension system throughout life (Godfrey & Barker 2001). Low maternal iron stores may lead to iron deficiency anaemia in infancy and early childhood which has been associated with behavioural abnormalities and impaired cognitive development (Pollitt 1997, Hurtado *et al* 1999). Menstrual losses account for the greatest losses of iron in women of childbearing age (Hallberg 1995). Therefore adult women require an adequate supply of bio-available dietary haem iron to meet their high iron requirements. Iron intake and absorption can be impaired in several ways.

Restrained eating due to body image concerns is increasingly common in women in developed countries (Gilbody *et al* 1999, Hercberg *et al* 2001). A study of British adolescent girls found that iron deficiency anaemia is three times more common among teenage girls who had tried to lose weight during the previous twelve months than among those who had not attempted to diet (Nelson *et al* 1993). Vegetarianism is increasingly being adopted as an attempt to control weight (Ryan *et al* 1998, Gilbody *et al* 1999, Hercberg *et al* 2001). The lack of haem iron in such diets accompanied by greater amounts of iron binding phytates and oxalates may act to compromise iron status (Gilbody *et al* 1999, Hercberg *et al* 2001). Other factors which increase iron losses include certain types of intrauterine contraceptive devices which increase menstrual losses (Hallberg 1995) and the prolonged use of non-steroidal anti-inflammatory drugs which may cause gastro-intestinal bleeding (Provan & Weatherall 2000).

Folate is the generic term applied to a B-complex vitamin which exists in many chemical forms (Wagner 1996). Naturally-occurring food folate is the highly labile polyglutamate form of the vitamin, containing up to six side chains (Wagner 1996, Scott 1999). These glutamic acid side chains must be enzymatically cleaved by γ-glutamylhydrolase to give the monoglutamate form prior to absorption (Wagner 1996, Scott 1999). Folic acid, used in fortified food and supplements, is the stable monoglutamate form of the vitamin (Scott 1999), hence bioavailability of folic acid is far greater. Folate acts as a coenzyme in single-carbon transfer reactions in the metabolism of nucleic and amino acids (Wagner 1996). Folate mediated transfer of single carbon units is of particular importance in rapidly dividing cells.

The recommendations for folates for adults (DHSS 1991, Safety Authority of Ireland 1999) are targeted at the normal population and do not take account of genetic or metabolic abnormalities (Molloy *et al* 1997). However, between 5% and 15% of general populations are homozygous for a thermolabile of 5,10 methyltetrahydrofolate reductase (Molloy *et al* 1997). This variant has been associated with hyperhomocysteinaemia secondary to impaired folate metabolism and poor folate status. The minimum effective dose of folic acid to prevent the majority of neural tube defects is 400μg per day (Daly *et al* 1997) suggesting that a substantial minority of people have elevated requirements. This has been recognised by expert groups which recommend that women of child bearing age consume an extra 400μg of folic acid per day to reduce the incidence of neural tube defects in the population (Food Safety Authority of Ireland 1999).

Research conducted over recent decades has produced substantial evidence to support the role of folic acid in health and disease (Kim 2000). Hyperhomocysteinaemia has been implicated in the various pathogenic processes associated with poor folate status (Daly *et al* 1997). Folic acid is recognised as a protective agent against neural tube defects (MRC 1991, Daly *et al* 1997, Scott 1999, Molloy & Scott 2001), coronary, cerebral and peripheral vascular disease (Pancharuniti *et al* 1994, Scott 1999, Cortese & Motti 2001), some cancers (Kim 2000, Kawakami *et al* 2001), birth defects (Picciano 2000) and possibly dementia in the elderly (Snowden *et al* 2000). Peri-conceptual folic acid supplementation can effectively prevent the majority of neural tube defects (Molloy *et al* 1997). However despite education campaigns, knowledge and use of folic acid among Irish women of childbearing age remains poor (Sayers *et al* 1997). The North/South Ireland Food Consumption Survey (NSIFCS) (2001) reported that only 2% of women aged 18 to 35 years and 5% of women aged 36 to 50 years achieved the recommended intake of folates (Scientific Committee for Food 1993) for the prevention of neural tube defects (IUNA 2001, O'Brien *et al* 2001). All of the women who achieved the recommendation (Scientific Committee for Food 1993) were using folic acid containing supplements (IUNA 2001, O'Brien *et al* 2001). In the United States, fortification of all grain products with folic acid was introduced in 1998 in an attempt to improve the folate status of the population. Since the introduction of mandatory fortification of grain products in the United States the birth prevalence of neural tube defects has declined by 19% (Honein *et al* 2001).

Vitamin B_{12} is the largest of the B-complex vitamins with a molecular weight of over 1000 (Scott 1999). In mamalian cells Vitamin B_{12} is a necessary cofactor for two

enzymes namely methionine synthase and methylmalonyl Co-A mutase (Scott 1999). Vitamin B_{12} plays a role in the regulation of plasma homocysteine through its role as cofactor in the remethylation reaction. A glycoprotein, intrinsic factor, synthesised by the gastric parietal cells facilitates the absorption of vitamin B_{12} in the terminal ileum. Vitamin B_{12} is stored in the liver and a replete adult has sufficient stores to last for three to five years (Scott 1999). Dietary vitamin B_{12} deficiency is uncommon in free living adults consuming a mixed diet. The NSIFCS (2001) found more than adequate intakes of vitamin B_{12} among Irish men and women (IUNA 2001, O'Brien *et al* 2001). Charlton *et al* (1997) documented vitamin B_{12} deficiency in 1% (2) of a sample of 200 elderly non-institutionalised South African subjects aged 65 years and older. Suggested reasons for dietary vitamin B_{12} deficiency include achlorhydria, chronic polypharmacy, physical inability to prepare food, and poverty (Charlton *et al* 1997). Vitamin B_{12} deficiency usually occurs through auto-immune destruction of the gastric parietal cells and loss of intrinsic factor. Deficiency of vitamin B_{12} leads to megaloblastic anaemia and if left untreated results in irreversible subacute combined degeneration of the spinal cord (Scott 1999). Folic acid can mask vitamin B_{12} deficiency through correction of the associated megaloblastic anaemia, therefore the potential masking of vitamin B_{12} deficiency is a major public health risk associated with fortification of foods with folic acid.

As described, certain events during the course of a woman's life, such as menstruation, pregnancy, lactation and the menopause, may impair haematinic nutrient status. The aim of the current study was to investigate iron, folate and vitamin B_{12} status and factors contributing to levels of these haematinic nutrients in a cross section of apparently healthy inner-city Dublin women. The purpose of this study was also to identify risk factors for poor haematinic nutrient status and characteristics, which are protective of iron, folate and vitamin B_{12} status in women in a primary care setting.

3.2 Subjects and methods.

3.2.1 Subjects.

Prior to the commencement of the study, ethical approval was sought and granted by the Irish College of General Practitioners ethics committee. Female volunteers were recruited from four general medical practices located in Dublin's south inner city. Two hundred consent forms inviting participation were circulated to each practice. Posters and leaflets describing the study were displayed in the waiting room of each medical

centre. Female volunteers were invited to complete the consent forms and were then contacted by telephone by the main investigator to arrange an appointment. All participating female volunteers were Irish, aged between 18 and 64 years and had no history of chronic disease or drug or alcohol abuse. Pregnant women were excluded as were women found to be anaemic during the twelve months prior to the study. Each participating female volunteer visited their relevant general practice once between June and December 1999. All female volunteers were reimbursed for travel expenses incurred.

3.2.2 Dietary Assessment.

During a one hour interview with the main investigator, the Food Intake Questionnaire was administered to each female volunteer. This standardised, interview assisted semi-quantitative food frequency questionnaire, derived from the Fat Intake Questionnaire (Cantwell *et al* 1997), was validated prior to use (see chapter 2) and was used in conjunction with a brand atlas of folic acid fortified foods on the Irish market specifically designed for the study (see chapter 2). A portion size atlas, derived from the Irish National Nutrition Survey (INDI 1990) and the Irish Universities Nutrition Alliance (IUNA 2001) atlases, was also used to enable volunteers to estimate portion sizes of foods consumed (see chapter 2). The dietary information was coded using McCance and Widdowson's food composition tables and their supplements (Holland *et al* 1988, Holland *et al* 1991, Holland *et al* 1995). Conversion from foodstuffs to energy and nutrients was performed using a computerised version of the Royal Society of Chemistry food and nutrient database, WISP version 1.26 for Windows. This computer package was modified to include information on foodstuffs on the Irish market fortified with folic acid, based on manufacturers' data (see chapter 2). The reported energy intakes were compared with the calculated energy requirements to identify and exclude under-reporters (Goldberg *et al* 1991). An EI:BMR cut-off point of 1.5 was used to determine under-reporting in the current sample of female volunteers (Goldberg *et al* 1991). The consumption of seventy-five commonly eaten food categories and the intake of these food categories among consumers were also assessed within the group of female volunteers.

3.2.3 Administration of the 'Interview Questionnaire'.

Demographic, lifestyle, medical and reproductive details were collected using a standardised, interview-assisted questionnaire, which was also validated prior to use

(see chapter 2). Information on age, time spent in formal education and occupation was collected using this 'Interview Questionnaire' (Appendix II). Data on menstrual history (age at menarche, duration of menses, length of cycle and subjective estimation of level of menstrual blood flow), reproductive history (number of pregnancies and miscarriages, age at first pregnancy, birth weight and state of health of offspring and birth interval) and contraceptive use (type and duration of use) were obtained using this questionnaire. The use of non-steroidal anti inflammatory drugs (frequency of both over-the-counter and prescription non-steroidal anti inflammatory drugs) and vitamin and mineral supplements was investigated using this questionnaire, as were body image concerns, slimming practices and nutritional knowledge relating to iron and folic acid. This 'Interview Questionnaire' was administered to each female volunteer by the main investigator to eliminate inter-observer bias.

3.2.4 Socio-economic Classification.

Data collected using the 'Interview Questionnaire' was used to calculate socio-economic class for each female volunteer. The socio-economic classification used for this analysis is based on the Irish social class scale for epidemiological health research devised by O'Hare (1982). For the purpose of meaningful statistical analysis, O'Hare's socio-economic classes one and two, three and four, five and six were combined to form socio-economic classes one two and three respectively. Socio-economic class one includes professionals, employers and managers. Socio-economic class two includes salaried employees and skilled manual workers. Socio-economic class three includes unskilled manual workers and the unemployed.

3.2.5 Anthropometry.

Height to the nearest mm and weight to the nearest 0.01kg were measured barefoot, in light indoor clothing and used to calculate body mass index (kg/m^2). Waist measurements were taken midway between the lowest rib and the iliac crest (Lean *et al* 1995). Hip measurements were taken at the level of the great trochanters (Lean *et al* 1995) using a metal tape. Waist to hip ratio was calculated. All measurements were taken by the principal investigator to eliminate inter-observer bias.

3.2.6 Blood sampling and analysis.

Venous blood specimens were taken during the interview. Nine millilitres of blood was obtained from each female volunteer using two vacutainers (Beckton Dickinson). One

vacutainer contained EDTA as an anticoagulant; the other contained no additives. Immediately after drawing the blood the vacutainers were refrigerated for transport to the Dublin Institute of Technology research laboratory within two hours of collection. Full blood count was measured in the anticoagulated sample using the Cobas Micros analyser and appropriate control samples (Boehringer Mannheim). For analysis of red cell folate 100µl of anticoagulated blood was aliquotted with 900µl of 1% ascorbic acid solution. This was stored in micronic tube strips at -20°C for transport to the Vitamin Research Laboratory, St. James's Hospital, Dublin 8. The remaining anticoagulated blood was centrifuged at 3500rpm for six minutes and the resulting plasma was aliquotted and stored in micronic tube strips at -20°C for transport to the Vitamin Research Laboratory, St. James's Hospital, Dublin 8 for analysis of vitamin B_{12} and homocysteine. The coagulated blood was centrifuged at 3500rpm for six minutes and one millilitre of the resulting serum was aliquotted and stored at -20°C for analysis of serum ferritin. A further one millilitre of serum was aliquotted and stored at -80°C for analysis of soluble transferrin receptor.

Analysis of serum ferritin was performed using the Enzymun-Test Ferritin kit (Boehringer Mannheim). This is an enzyme immunoassay for the quantitative determination of ferritin in vitro, the principle of which is an ELISA one step sandwich assay using streptavidin technology.

The soluble transferrin receptor was analysed using the Quantikine IVD Human sTFR Immunoassay kit (R & D Systems Ltd.). This immunosorbent ELISA assay is designed for the quantitative determination of the soluble transferrin receptor in human serum and plasma in the diagnosis of iron deficiency anaemia. Intra-assay precision for this kit was assessed by assaying three samples of known concentration ten times on one plate. Three samples of known concentration were assayed in eleven separate assays to assess inter-assay precision.

The appropriate samples were transported on ice to the Vitamin Research Laboratory, St. James's Hospital, Dublin 8 where red cell folate was analysed using a microbiological assay for serum, plasma and red cell folate using a cryopreserved microtitre plate method (Molloy & Scott 1997). Plasma vitamin B_{12} was determined using a microbiological assay for vitamin B_{12} performed in 96-well microtitre plates (Kelleher & O'Broin 1991). Plasma homocysteine was determined according to the method described by Nexo *et al* (2000).

3.2.7 Data management and statistical analysis.

All data obtained was entered into the Statistical Package for Social Sciences (SPSS) version 9.0 for Windows. Subjects were divided into quartiles of iron and folate status using the soluble transferrin receptor and red cell folate variables respectively. The distribution of data in each of these quartiles was checked for normality using the Kolmagorov-Smirnoff test. Data was then analysed using the Mann Whitney U test, One way ANOVA post hoc analysis-LSD test, Kruschall Wallis test or Chi square test as appropriate. Multiple linear regression analysis using stepwise selection was then performed to establish predictors of iron and folate status in the study population.

3.3 Results.

The response rate was 29.3% (n=176). Of these respondents 59% (n=104) participated fully in the study. Of those who were excluded 3% (n=5) were pregnant, 4% (n=7) under-reported dietary intake, 12% (n=21) were not Irish, and 22% (n=39) were unavailable at the time of data collection. Forty-two percent (n=44) of the participating female volunteers were recruited from Mercer's Medical Centre, Dublin 2, some 31% (n=32) were recruited from the City Medical Centre, Dublin 2, a further 24% (n=25) from the Rialto Medical Centre, Dublin 8 and 3% (n=3) from the Inchicore Medical Centre, Dublin 8. Mean age of the female volunteers was 32.8 years with a standard deviation of 11.2 years. Mean body mass index (BMI) was within the normal range at 24.7kg/m^2 (standard deviation 5.427kg/m^2). Mean value for waist to hip ratio (WHR) at 0.78 (standard deviation 0.58) was below the recommended cut off point for women (Lean *et al* 1995). Thirty five percent (n=36), 22% (n=23) and 43% (n=45) were in socio-economic classes one, two and three respectively. Of the total sample, 39% (n=41) were using vitamin and/or mineral supplements at the time of the study. Of the supplement users, 36% (n=15), 32% (n=13) and 32% (n=13) were in socio-economic classes one, two and three respectively. Of the total sample, 40% (n=42) were current cigarette smokers at the time of the study. Fourteen percent (n=15) of the female volunteers had ceased smoking more than six months prior to participating in the study and were therefore classified as ex-smokers, a further 45% (47) of the female volunteers had never smoked. Chi square tests revealed that significantly more of the current smokers were in socio-economic class three (7% (n=3) vs. 22% (n=9) and 71% (n=30) in socio-economic classes one, two and three respectively) ($p<0.001$). Significantly more of the female volunteers who had never smoked were in socio-economic class one (57% (n=27) vs. 17% (n=8) and 26% (n=12) in socio-economic classes one, two and

three respectively) ($p<0.001$). Of the ex-smokers 40% (n=6), 40% (n=6) and 20% (n=3) were in socio-economic classes one, two and three respectively.

The haematalogical analysis detected 2.8% (n=3) of the 104 female volunteers who were suffering from iron deficiency anaemia defined as haemoglobin levels <12g/dl (Hallberg & Rossander-Hulthen 1989). These anaemic women (2.8%, n=3) had soluble transferrin receptor levels above the recommended cut-off of 28.1nmol/l for adults (R & D Systems 1999). A further 14.4% (n=15) had low iron stores, defined as a serum ferritin level of less than 15μg/l (Hallberg & Rossander-Hulthen 1989). Of the 104 female volunteers 1.9% (n=2) were within the deficient range for red cell folate (<150μg/l). Almost half of the female volunteers, 48% (n=50), were found to have sub-optimal red cell folate levels for women of child-bearing age (< 400μg/l) (Daly *et al* 1995). Plasma vitamin B_{12} levels were found to be within the normal range (150-1000nmol/l) for all female volunteers. Plasma homocysteine levels were elevated (>15μmol/l) in 12.5% (n=13) of the subjects. The women suffering from clinical deficiencies were contacted through their GPs and treated.

Tables 1 and 3 summarise the observed macronutrient intakes among the female volunteers in each quartile of iron and folate status. Tables 2(a), 2(b), 4(a) and 4(b) present the micronutrient intakes recorded among the female volunteers in each quartile of iron and folate status.

Female volunteers in the quartile of lowest iron status as determined by soluble transferrin receptor (Q4) were found to have a significantly higher percentage of dietary energy from carbohydrate ($p=0.02$), higher Southgate fibre intake ($p=0.02$), and higher Englyst fibre intake ($p=0.01$) (Table 1). Female volunteers in the quartile of highest iron status (Q1) had a significantly higher percentage of dietary energy from monounsaturated fat ($p=0.01$) and polyunsaturated fat ($p=0.03$) (Table 1). Female volunteers in the quartile of highest iron status as determined by soluble transferrin receptor (Q1) had a significantly higher intake of dietary haem iron ($p=0.02$), iron from supplements ($p=0.04$) and folic acid from supplements ($p=0.04$) and a significantly lower calcium intake ($p=0.013$) than female volunteers in Q4 (Table 2(b)). Intake of total dietary folates was significantly higher among female volunteers in Q4 compared with Q1 of soluble transferrin receptor ($p=0.023$) (Table 2(a)). Dietary folic acid intake was significantly different between Q1 and Q2 ($p=0.04$) and Q1 and Q4 ($p=0.03$) of iron status as determined by soluble transferrin receptor (Table 2(a)).

Female volunteers in the quartile of highest folate status (Q4) as determined by red cell folate had a significantly higher percentage energy from carbohydrate compared to

those in Q1 (p=0.006), Q2 (p<0.001) and Q3 (p=0.04) (Table 3). Female volunteers in Q4 also had a higher mean carbohydrate intake compared to those in Q1 (p=0.04) and Q2 (p=0.03) (table 3). Percentage energy from fat was significantly lower in Q4 compared to Q1 (p=0.004), Q2 (p=0.007) and Q3 (p=0.04) (Table 3). Female volunteers in Q2 also had a significantly higher mean total fat intake (p=0.04) and saturated fat intake (p=0.04) than those in Q4. Percentage dietary energy from monounsaturated fat was significantly lower among female volunteers in Q4 of folate status compared to those in Q1 (p=0.006) and Q2 (p=0.004) (Table 3). Mean monounsaturated fat intake was significantly higher among female volunteers in Q2 of folate status compared to those in Q4 (p=0.04). Percentage energy from polyunsaturated fat was significantly lower in Q4 of folate status compared with Q1 (p=0.04), Q2 (p=0.01) and Q3 (p=0.03) (Table 3). Mean polyunsaturated fat intake was significantly lower among female volunteers in Q4 of folate status compared to those in Q2 (p=0.04) and Q3 (p=0.04). Female volunteers in the quartile of highest folate status (Q4) had a significantly higher intake of Englyst fibre (p=0.02) and Southgate fibre (p=0.006) compared to female volunteers in the quartile of lowest status (Q1) (Table 3).

Female volunteers in the quartile of lowest folate status (Q1) had significantly lower intakes of dietary non haem iron (p=0.04) and iron from supplements (p=0.03) compared to those in Q4 (Table 4(a)). Total dietary folate intake was significantly higher among women in Q4 compared to those in Q1 (p=0.03) and Q2 (p=0.03) (Table 4(a)). Female volunteers in Q3 also had a significantly higher intake of total dietary folate than those in Q1 (p=0.01) and Q2 (p=0.02) (Table 4(a)). Dietary folic acid intake from fortified foods was significantly higher in the quartile of highest folate status (Q4) compared with the lowest (Q1) (p<0.001) (Table 4(a)). Dietary folate intake was significantly lower in the quartile of lowest folate status (Q1) compared with Q3 (p=0.01) and Q4 (p=0.01) (Table 4(a)). Intake of folic acid from supplements was significantly higher in the quartile of highest folate status (Q4) compared with the lowest (Q1) (Table 4(a)).

Intake of vitamin B_1 was significantly lower in the quartile of lowest folate status (Q1) compared with Q3 (p=0.009) and Q4 (p=0.03) (Table 4(b)). Intake of vitamin B_2 was significantly lower in the quartile of lowest folate status compared with Q3 (p=0.011) and Q4 (p=0.015) (Table 4(b)). Intake of vitamin B_6 was significantly lower in Q1 of folate status compared to Q3 (p=0.002) and Q4 (p=0.007) (Table 4(b)).

Table 5 reports other variables found to be significantly different between the upper and lower quartiles of iron status. Some demographic and haematalogical observations are

also included here. Univariate analysis revealed a significant difference in age at first pregnancy (p=0.04) between women in the upper (Q1) and lower (Q4) quartiles of iron status. Female volunteers in Q1 had significantly more days between menstrual periods than those in Q3 (p=0.03) and Q4 (p=0.03) (Table 5). Analysis of food consumption showed that red meat intake was significantly lower among women in the quartile of lowest iron status (Q4) compared to Q1 (p=0.014), Q2 (p=0.024) and Q3 (p=0.024) (Table 5). Female volunteers in the quartile of lowest iron stores (Q4) consumed significantly more tea than those in Q1 (p<0.001), Q2 (p<0.001) and Q3 (p<0.001) (Table 5). Intake of wholemeal bread among women in the quartile of best iron status was significantly lower than in Q3 (p=0.02) and Q4 (p=0.03). Serum ferritin was significantly higher in female volunteers in the quartile of best iron status (Q1) compared to the quartile of lowest iron status (Q4) (p<0.001) (Table 5).

Table 6 reports other variables found to be significantly different between the quartiles of folate status as determined by red cell folate. Some demographic and haematalogical observations are also included here. Significantly more of the female volunteers in the highest quartile of folate status (Q4) were of higher socio-economic status compared to those in the lowest quartile of folate status (Q1) (p=0.04) (Table 6).

In terms of red cell folate status the most replete women (Q4) had spent significantly longer in formal education (p=0.01). Significantly more socio-economic class one women were in Q4 of red cell folate status (p=0.04). Significantly more women in Q4 chose to eat fortified whole-grain breakfast cereals (p=0.02) and fortified white bread (p=0.04) compared to Q1. Intake of salad vegetables was significantly higher among female volunteers in Q4 when compared to Q1 (p=0.045) (Table 6).

Multiple linear regression analysis produced multivariate models of the most potent predictors of iron and folate status. The multivariate model reported (Table 7) explained 7.9% of the variance in soluble transferrin receptor levels among the female volunteers. The multivariate model reported (Table 8) explained 46.5% of the variance in red cell folate levels among the female volunteers.

No significant differences in nutritional knowledge of sources and functions of iron and folic acid were found among female volunteers in the upper and lower quartiles of iron and folate status. However, significantly more female volunteers in socio-economic class one were aware of the functions of iron in the body (47% (*n*=17) vs. 22% (*n*=5) and 11% (*n*=5) in socio-economic classes one, two and three respectively) (p=0.001). Significantly more of the women in socio-economic class one correctly identified dietary sources of iron (97% (*n*=35) vs. 87% (*n*=20) and 77% (*n*=35) in socio-economic

classes one, two and three respectively) (p=0.04). Significantly more women in socio-economic class one were aware of the role of folic acid during pregnancy (97% (*n*=35) vs. 69% (*n*=16) and 77% (*n*=35) in socio-economic classes one, two and three respectively) (p=0.002). Significantly more women of higher socio-economic status were able to correctly identify dietary sources of folic acid (47% (*n*=17) vs. 22% (*n*=5) and 11% (*n*=5) in socio-economic classes one, two and three respectively (p=0.001).

Table 1: Macronutrient intakes among subjects in each quartile of iron status where Q1 denotes the best and Q4 the worst status as determined by soluble transferrin receptors.

Nutrient	Q1 (0-13.58 nmol/l)	Q2 (13.5801 -16.30 nmol/l)	Q3 (16.301 -21.15 nmol/l)	Q4 (>21.15 nmol/l)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
EI:BMR	1.7 ± 0.34	1.8 ± 0.36	1.7 ± 0.38	1.7 ± 0.38
Energy (MJ)	9.8 ± 2.2	10.3 ± 2.8	10.1 ± 1.9	10.4 ± 2.1
Energy (kcals)	2294 ± 532	2523 ± 553	2396 ± 445	2468 ± 503
Protein (g)	82.6 ± 10.9	87.7 ± 11.9	82.1 ± 10.8	87.6 ± 14.1
Protein (%)	14.4 ± 1.9	13.9 ± 1.9	13.7 ± 1.8	14.2 ± 2.3
CHO (g)	274 ± 48.9	281 ± 53.2	274 ± 48.5	283 ± 42.3
CHO (%)	43.0 ± 6.7[a]	44.6 ± 6.4	45.8 ± 8.1	47.5 ± 5.4
Total fat (g)	96.1 ± 16.8	107 ± 19.3	96.6 ± 18.4	96.8 ± 22.4
Total fat (%)	37.7 ± 6.6	38.3 ± 6.9	36.3 ± 6.9	35.3 ± 6.4
SFAs (g)	36.2 ± 10.5	42.8 ± 13.4	39.4 ± 10.9	40.0 ± 10.7
SFAs (%)	14.2 ± 4.1	15.3 ± 4.8	14.8 ± 4.1	14.6 ± 3.9
MUFAs (g)	31.4 ± 8.2	31.7 ± 9.5	26.9 ± 7.7	29.9 ± 9.0
MUFAs (%)	12.3 ± 3.2[a]	11.3 ± 3.4	10.1 ± 2.9	10.9 ± 3.3
PUFAs (g)	16.3 ± 3.8	17.1 ± 5.8	15.7 ± 4.8	15.4 ± 4.7
PUFAs (%)	6.4 ± 1.5[a]	6.1 ± 2.1	5.9 ± 1.8	5.3 ± 1.7
Englyst fibre (g)	14.9 ± 4.1[a]*	16.9 ± 6.0	18.9 ± 7.7	18.8 ± 6.1
Southgate fibre (g)	20.8 ± 4.7[a]*	25.3 ± 8.9	23.7 ± 6.9	25.5 ± 7.4
Alcohol (g)	14.0 ± 7.7	12.4 ± 7.2	12.8 ± 9.8	11.7 ± 8.4
Alcohol (%)	5.7 ± 3.2	4.4 ± 2.5	5.2 ± 4.5	4.7 ± 4.4

Q = quartile. SD = standard deviation. CHO = carbohydrate. SFAs = saturated fatty acids. MUFAs = monounsaturated fatty acids. PUFAs = polyunsaturated fatty acids.

[a] Signifieant difference between Q1 and Q4 according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 2(a): Haematinic micronutrient intakes among subjects in each quartile of iron status where Q1 denotes the best and Q4 the worst status as determined by soluble transferrin receptor.

Nutrient	Q1 (0-13.58 nmol/l)	Q2 (13.5801 -16.30 nmol/l)	Q3 (16.301 -21.15 nmol/l)	Q4 (>21.15 nmol/l)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Total iron (mg)	17.5 ± 5.9[a]	16.9 ± 5.3[b]	15.3 ± 4.1	14.7 ± 4.4
Total dietary iron (mg)	13.7 ± 3.9	14.2 ± 3.8	13.1 ± 3.2	12.6 ± 3.0
Dietary haem iron (mg)	3.3 ± 1.9[a]*	2.9 ± 1.9	2.9 ± 1.8	2.4 ± 2.5
Dietary non-haem iron (mg)	9.1 ± 3.2	11.3 ± 3.4	10.1 ± 2.9	10.2 ± 2.5
Supplementary iron (mg)	3.9 ± 4.3[a]*	2.8 ± 3.5	2.3 ± 3.4	2.4 ± 3.9
Total folates (μg)	392 ± 146[a]	402 ± 168[b]	396 ± 126[c]	448 ± 161
Total dietary folates (μg)	285 ± 54[a]	315 ± 126	308 ± 84	354 ± 137
Dietary folic acid (μg)	17 ± 29[a]*	44 ± 51	38 ± 42	48 ± 70
Dietary folate (μg)	268 ± 51	272 ± 103	265 ± 72	309 ± 103
Supplementary folic acid (μg)	135 ± 144[a]*	86 ± 121	88 ± 103	96 ± 137
Vitamin B_{12} (μg)	4.6 ± 2.8	4.3 ± 1.9	4.8 ± 2.2	4.0 ± 1.7

Q = quartile. SD = standard deviation. [a] Significant difference between Q1 and Q4, [b] significant difference between Q2 and Q4, [c] significant difference between Q3 and Q4 according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 2(b): Intakes of other micronutrients among subjects in each quartile of iron status where Q1 denotes the best and Q4 the worst status as determined by soluble transferrin receptor.

Nutrient	Q1 (0-13.58 nmol/l)	Q2 (13.5801 -16.30 nmol/l)	Q3 (16.301 -21.15 nmol/l)	Q4 (>21.15 nmol/l)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Calcium (mg)	945 ± 334[a]	1123 ± 281	1044 ± 314	1191 ± 452
Zinc (mg)	8.9 ± 2.3	9.9 ± 2.9	9.5 ± 3.0	9.7 ± 2.8
Vitamin B_1 (µg)	1.5 ± 0.34	1.7 ± 0.54	1.6 ± 0.51	1.8 ± 0.5
Vitamin B_2 (µg)	1.8 ± 0.5	2.1 ± 0.6	1.9 ± 0.6	2.2 ± 0.8
Vitamin B_3 (µg)	37.8 ± 7.7	41.9 ± 11.6	39.9 ± 9.8	40.9 ± 9.8
Vitamin B_6 (µg)	2.2 ± 0.43	2.5 ± 0.84	2.4 ± 0.65	2.6 ± 0.86
Vitamin C (mg)	121 ± 62	119 ± 71	93 ± 38	148 ± 71
Vitamin D (µg)	2.4 ± 1.7	3.1 ± 1.8	3.0 ± 1.8	2.8 ± 1.3
Vitamin E (µg)	7.4 ± 2.1	8.9 ± 3.8	7.5 ± 2.8	7.6 ± 2.3
REs	1332 ± 1188	1268 ± 1148	1351 ± 743	1433 ± 1262

Q = quartile SD = standard deviation REs = Retinol Equivalents [a] Significant difference between Q1 and Q4 according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 3: Macronutrient intakes among subjects in each quartile of folate status where Q1 denotes the worst and Q4 the best status as determined by red cell folate.

Nutrient	Q1 (0-311 µg/l)	Q2 (311.01 -409µg/l)	Q3 (409.01 -529.75µg/l)	Q4 (>529.75 µg/l)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
EI:BMR	1.63 ± 0.33	1.8 ± 0.41	1.89 ± 0.4	1.7 ± 0.3
Energy (MJ)	9.3 ± 2.7	10.8 ± 2.5	10.5 ± 2.0	9.9 ± 1.7
Energy (kcals)	2278 ± 537	2561 ± 603	2502 ± 474	2361 ± 393
Protein (g)	80.3 ± 9.1	91.6 ± 15.4	85.7 ± 12.5	83.2 ± 11.2
Protein (%)	14.1 ± 1.6	14.3 ± 2.4	13.7 ± 2.0	14.1 ± 1.9
CHO (g)	269± 38.7[a]	270 ± 46.1[b]	285 ± 48.3	295 ± 53.1
CHO (%)	44.1 ± 6.8[a]	42.2 ± 6.5[b]	45.5 ± 6.6[c]	49.0 ± 5.6
Total fat (g)	97.7 ± 15.9	106.7 ± 17.6[b]	100.1 ± 18.3	89.3 ± 14.7
Total fat (%)	38.6 ± 6.3[a]	38.9 ± 6.2[b]	36.0 ± 6.6[c]	33.9 ± 5.1
SFAs (g)	40.2 ± 12.4	43.3 ± 12.1	39.1 ± 10.8	36.2 ± 8.4
SFAs (%)	15.9 ± 4.9	15.2 ± 3.9	14.1 ± 3.9	13.8 ± 3.2
MUFAs (g)	30.4 ± 5.8	35.6 ± 6.5[b]	31.1 ± 6.4	27.9 ± 5.8
MUFAs (%)	12.0 ± 2.3[a]	12.5 ± 2.3[b]	11.2 ± 2.3	10.8 ± 2.2
PUFAs (g)	15.2 ± 5.3	17.9 ± 5.4[b]	17.2 ± 4.2[c]	14.3 ± 4.9
PUFAs (%)	6.0 ± 2.1[a]	6.3 ± 1.9[b]	6.2 ± 1.5[c]	5.3 ± 1.5
Englyst fibre (g)	14.3 ± 4.9[a]*	18.1 ± 5.8	18.6 ± 6.7	18.9 ± 6.7
Southgate fibre (g)	19.9 ± 5.8[a]*	24.6 ± 6.8	24.8 ± 7.9	26.1 ± 7.5
Alcohol (g)	9.3 ± 5.3	15.2 ± 8.4	15.3 ± 13.0	11.7 ± 8.9
Alcohol (%)	3.8 ± 2.4	5.7 ± 3.7	5.7 ± 4.3	4.7 ± 4.2

Q = quartile. SD = standard deviation. CHO = carbohydrate. SFAs = saturated fatty acids. MUFAs = monounsaturated fatty acids. PUFAs = polyunsaturated fatty acids. [a] Significant difference between Q1 and Q4, [b] significant difference between Q2 and Q4, [c] significant difference between Q3 and Q4 according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 4(a): Haematinic micronutrient intakes among subjects in each quartile of folate status where Q1 denotes the worst and Q4 the best status as determined by red cell folate.

Nutrient	Q1 (0-311 µg/l)	Q2 (311.01 -409µg/l)	Q3 (409.01 -529.75µg/l)	Q4 (>529.75 µg/l)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Total iron (mg)	14.1 ± 4.8[a]	16.1 ± 5.4	16.5 ± 4.6	18.1 ± 4.9
Total dietary iron (mg)	12.1 ± 3.2	13.6 ± 3.7	13.5 ± 3.3	14.4 ± 3.7
Dietary haem iron (mg)	3.3 ± 2.4	3.3 ± 2.2	2.5 ± 1.6	2.7 ± 2.0
Dietary non-haem iron (mg)	8.6 ± 3.0[a]	10.4 ± 2.9	11.0 ± 3.5[d]	11.6 ± 2.9
Supplementary iron (mg)	1.9 ± 3.5[a]	2.9 ± 4.2	3.0 ± 3.9	3.7 ± 3.6
Total folate (µg)	273 ± 86[a]	356 ± 102[b]	493 ± 129[c]	549 ± 122
Total dietary folates (µg)	247 ± 82[a]	296 ± 78[be]	364 ± 135[d]	357 ± 84
Dietary folic acid (µg)	10 ± 21[a]	25 ± 33	55 ± 50	57 ± 71
Dietary folate (µg)	237 ± 81[a]	271 ± 74	302 ± 87[d]	305 ± 89
Supplementary folic acid (µg)	26 ± 71[a]	60 ± 96	127 ± 140	192 ± 126
Vitamin B_{12} (µg)	4.1 ± 2.1	4.6 ± 2.6	3.9 ± 1.6	5.1 ± 3.9

Q = quartile. SD = standard deviation [a] Significant difference between Q1 and Q4, [b] significant difference between Q2 and Q4, [c] significant difference between Q3 and Q4 [d] significant difference between Q1 and Q3, [e] significant difference between Q2 and Q3, according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 4(b): Intakes of other micronutrients among subjects in each quartile of folate status where Q1 denotes the worst and Q4 the best status as determined by red cell folate.

Nutrient	Q1 (0-311 µg/l)	Q2 (311.01 -409µg/l)	Q3 (409.01 -529.75µg/l)	Q4 (>529.75 µg/l)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Calcium (mg)	965 ± 349	1136 ± 413	1091 ± 250	1123 ± 394
Vitamin B_1 (µg)	1.4 ±0.45[a]	1.7 ± 0.46[f]	1.8 ± 0.64[d]	1.7 ± 0.35
Vitamin B_2 (µg)	1.8 ± 0.65[a]	1.9 ± 0.51	2.1 ± 0.72	2.2 ± 0.59
Vitamin B_3 (µg)	36.5 ± 9.7	41.6 ± 10.5	42.6 ± 10.9	42.4 ± 7.5
Vitamin B_6 (µg)	2.1 ±0.72[a]	2.4 ± 0.51	2.7 ± 0.95[d]	2.6 ± 0.50
Vitamin C (mg)	105 ± 70	108 ± 51	127 ± 72	143 ± 58
Vitamin D (µg)	2.7 ± 1.4	3.3 ± 2.3	2.8 ± 1.5	2.6 ± 1.3
Vitamin E (µg)	6.8 ± 2.3	8.8 ± 2.9	8.3 ± 2.4	7.6 ± 3.5
REs	1342 ± 1084	1452 ± 774	1295 ± 520	1294 ± 895

Q = quartile SD = standard deviation REs = Retinol Equivalents [a] Significant difference between Q1 and Q4, [d] significant difference between Q1 and Q3, [f] significant difference between Q1 and Q2 according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 5: Age, socio-economic class, anthropometric, haematalogical and food consumption data observed in female volunteers in the upper (Q1) and lower (Q4) quartiles of iron status as determined by soluble transferrin receptor.

Nutrient		Q1 (0-13.58 nmol/l)	Q2 (13.5801 -16.30 nmol/l)	Q3 (16.301 -21.15 nmol/l)	Q4 (>21.15 nmol/l)
		% (*n*)	% (*n*)	% (*n*)	% (*n*)
SEC	1	26.9 (7)	28.6 (8)	45.8 (11)	38.5 (10)
	2	23.1 (6)	25.0 (7)	25.0 (6)	15.4 (4)
	3	50.0 (13)	46.4 (13)	29.2 (7)	46.2 (12)
		mean ± SD	mean ± SD	mean ± SD	mean ± SD
Age (years)		33.2 ± 10.8	33.7 ± 11.4	32.9 ± 9.5	31.1 ± 13.0
BMI (kg/m^2)		24.8 ± 5.3	23.8 ± 4.5	25.7 ± 7.4	24.8 ± 4.4
WHR		0.78 ± 0.46	0.78 ± 0.54	0.79 ± 0.57	0.79 ± 0.57
Age at first pregnancy (years)		25.2 ± 5.3[a]	23.9 ± 4.0	22.2 ± 6.2	21.9 ± 4.7
Menstrual frequency(days)		31.9 ± 5.1[a]	27.3 ± 3.9	26.2 ± 2.3[d]	26.4 ± 4.2
Hgb (g/dl)		13.0 ± 0.9	13.1 ± 0.9	12.9 ± 1.0	13.2 ± 1.2
MCV (fl)		89.7 ± 4.0	91.1 ± 4.0	89.2 ± 4.9	88.9 ± 3.6
MCHC (pg/ml)		32.8 ± 2.6	33.0 ± 2.9	33.5 ± 3.9	33.0 ± 3.3
Serum ferritin (µg/l)		69.5 ± 26.5[a]*	63.5 ± 50.2	51.5 ± 30.9	24.1 ± 23.1
RCF (µg/l)		509 ± 245	444 ± 227	426 ± 135	400 ± 177
Plasma vitamin B_{12} (ng/l)		519 ± 188	491 ± 215	461 ± 133	479 ± 157
Homocysteine (µg/l)		10.3 ± 2.6	11.2 ± 4.2	10.9 ± 4.0	10.4 ± 2.9
Red meat (g/day)		57.8 ± 22.4[a]	60.3 ± 24.9[b]	51.5 ± 30.9[c]	39.7 ± 24.2
Tea (g/day)		918 ± 348[a]	1008 ± 488[b]	1049 ± 591[c]	1789 ± 976
Wholemeal bread (g/day)*		86.3 ±70.7[a]	117.9 ± 79.6[b]	150.9 ± 91.4[c]	136.4 ± 94.8

Q = quartile. SD = standard deviation. RCF = red cell folate. MCHC = Mean cell haemoglobin content. MCV = Mean corpuscular volume. Hgb = Haemoglobin. SEC = socio-economic class. WHR = Waist to hip ratio. (g/day) = grams among consumers per day. [a] Significant difference between Q1 and Q4, [b] significant difference between Q2 and Q4, [c] significant difference between Q3 and Q4 [d] significant difference between Q1 and Q3 according to One Way ANOVA post hoc analysis LSD test, Chi square test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 6: Age, socio-economic class, haematalogical, anthropometric and food consumption data observed in female volunteers in the upper (Q4) and lower (Q1) quartiles of folate status as determined by red cell folate.

	Q1 (0-311 µg/l)	Q2 (311.01 -409µg/l)	Q3 (409.01 -529.75µg/l)	Q4 (>529.75 µg/l)
	% (*n*)	% (*n*)	% (*n*)	% (*n*)
SEC 1	13.9 (5)[a]	27.8 (10)	22.2 (8)	36.1 (13)
2	34.8 (8)	17.4 (4)	30.4 (7)	17.4 (4)
3	31.1 (14)	24.4 (11)	24.4 (11)	20.0 (9)
Consumers of:				
Fortified bran cereal	44.4 (12)[a]	48.0 (13)[b]	65 (17)[d]	85 (22)
Fortified white bread	85 (23)[a]	76 (19)[b]	81 (21)[c]	100 (26)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Salad vegetables(g/day)	47.7 ± 43.4[a]	57.9 ± 26.8	58.9 ± 43.0	69.3 ±35.3
Age (years)	31.7 ± 9.6	34.8 ± 11.0	30.7 ± 11.1	34.0 ± 12.9
BMI (kg/m^2)	25.3 ± 5.5	24.0 ± 5.1	24.1 ± 5.7	25.5 ± 5.5
WHR	0.78 ± 0.52	0.80 ± 0.56	0.77 ± 0.46	0.79 ± 0.56
Education (yrs)	13.5 ± 3.6[a]	14.3 ± 3.7	15.2 ± 4.0	17.5 ± 3.9
Hgb (g/dl)	13.1 ± 1.1	12.9 ± 0.98	13.1 ± 0.9	12.9 ± 1.1
MCV (fl)	90.9 ± 4.8	89.5 ± 4.1	90.1 ± 3.9	88.6 ± 3.6
MCHC (pg/ml)	32.9 ± 3.2	33.2 ± 2.9	33.7 ± 3.5	32.8 ± 3.1
Serum ferritin (µg/l)	40.8 ± 27.6	47.8 ± 31.2	62.5 ± 53.0	60.3 ± 35.8
sTFR (µg/l)	19.5 ± 5.1	17.4 ± 5.8	18.1 ± 5.5	16.5 ± 6.4
Plasma vitamin B_{12} (ng/l)	412 ± 141	474 ± 140	474 ± 184	595 ± 191
Homocysteine (µg/l)	12.5 ± 4.4	10.7 ± 3.4	10.1 ± 2.8	9.5 ± 2.5

Q = quartile. SD = standard deviation. RCF = red cell folate. MCHC = Mean cell haemoglobin content. MCV = Mean corpuscular volume. Hgb = Haemoglobin. STFR = soluble transferrin receptor. SEC = socio-economic class. WHR = Waist to hip ratio, (g/day) = grams among consumers per day [a] Significant difference between Q1 and Q4, [b] significant difference between Q2 and Q4, [c] significant difference between Q3 and Q4 [d] significant difference between Q1 and Q3 according to One Way ANOVA post hoc analysis LSD test, Chi square test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 7: Stepwise multivariate model explaining a proportion of the variance in serum soluble transferrin receptor levels among the female volunteers.

Step	Variable	β value	Adjusted r^2	Significance
1	Tea (g/day)	0.538	0.039	p=<0.001
2	Red Meat (g/day)	-0.377	0.021	p=<0.001
3	Menstrual Frequency (days)	0.178	0.019	p=<0.001

(g/day) = grams among consumers per day.

Table 8: Stepwise multivariate model explaining a proportion of the variance in red cell folate levels among the female volunteers.

Step	Variable	β value	Adjusted r^2	Significance
1	Supplementary folic acid	0.535	0.206	p=<0.001
2	Dietary folic acid	0.408	0.150	p=<0.001
3	Englyst fibre (g)	0.244	0.109	p=<0.001

(g) = grams.

3.4 Discussion:

The current study investigated haematinic nutrient status and found undiagnosed deficiencies of iron and folate and sub-optimal status in a sample of apparently healthy Irish adult women attending GPs in inner-city Dublin. The observed prevalence of frank clinical iron deficiency anaemia (2.8%, n=3) and depleted iron stores (14.4%, n=15) was comparable to that found in other studies of women of childbearing age in industrialised countries. A study of iron status among Danish adults found a prevalence rate of iron deficiency anaemia of 2.6% among pre-menopausal women aged between 30 and 50 years (Milman 1996). The prevalence of exhausted iron stores was found to be considerably higher among these Danish women at 18% (Milman 1996). A recent review of epidemiological studies examining iron deficiency and anaemia in Europe (Hercberg *et al* 2001) estimated the prevalence of iron deficiency anaemia to range between 1.5% and 14% of women of child-bearing age. Low iron stores have been detected in between 10% and 30% of European women (Hercberg *et al* 2001). A review of data on adolescent females revealed a high prevalence of both depleted iron stores and anaemia in European subjects (Hercberg *et al* 2001). Low iron stores and anaemia have been observed in 5% to 43% and 7% to 8% respectively of European adolescent females (Hercberg *et al* 2001). A study of the prevalence of iron deficiency among women of child-bearing age in the United States found that between 2% and 5% had iron deficiency anaemia (Looker *et al* 1997). The prevalence of iron deficiency anaemia was between 9% and 11% among American adolescent girls (Looker *et al* 1997). An investigation of haematinic status in a group of 99 post-menopausal South African women aged over 65 years found that iron status improved significantly after cessation of menses (Charlton *et al* 1997). No cases of iron deficiency anaemia were found and 5.2% were found to have depleted stores (Charlton *et al* 1997). This level of iron deficiency is similar to that observed in European adult men (0% to 3%) (Hercberg *et al* 2001). These findings appear to confirm that menstruating women and adolescents constitute a group at particularly high risk of iron deficiency and iron deficiency anaemia due to the demands of menstruation and pregnancy. Risk of this deficiency and the associated anaemia appears to decrease to a level similar to that observed in adult men after the menopause.

The most potentially serious complications of iron deficiency and iron deficiency anaemia may be their effect on pregnancy outcome. The female volunteers in the current study were of child-bearing age and potentially at risk of entering pregnancy in

sub-optimal iron status. This would greatly elevate their risk of developing iron deficiency anaemia of pregnancy and its associated complications. During pregnancy foetal demand increases maternal iron requirements from approximately 1.4mg/day to 2.5mg/day and 6.5mg/day in the third trimester (Steer 2000). This demand by the developing foetus may result in maternal anaemia if iron stores are depleted at the beginning of pregnancy (Steer 2000). Failure of the plasma volume to expand is associated with a three-fold increased risk of developing pre-eclampsia during pregnancy (Murphy *et al* 1986, Steer 2000). Sub-optimal expansion of the maternal blood volume during pregnancy is associated with poor placental blood flow, which affects the transfer of oxygen and nutrients to the foetus (Murphy *et al* 1986, Steer 2000). This can lead to restricted foetal growth, resulting in the infant being small for gestational age at birth (Steer 2000). Such infants are more vulnerable to the stress of labour (Steer 2000). Several studies attest to the higher incidence of premature labour and low birth weight in infants of iron deficient mothers (Allen 2000, Steer 2000). Infants who are small at birth, when corrected for gestational age, particularly those with a high ratio of placental to foetal weight are at increased risk of developing hypertension and non insulin dependant diabetes during adulthood (Barker 1993). Retrospective examination of birth weight data on 69,526 women involved in the Nurses' Health Study conclusively established that low birth weight as a marker of intrauterine under-nutrition is independently associated with increased risk of non-insulin dependent diabetes during adulthood (Rich-Edwards *et al* 1999). This association between low birth weight and insulin resistance has also been documented in British men and women (Barker 1993, Barker 1998, Godfrey & Barker 2001). A study of glucose and insulin metabolism in four year old children reported impaired glucose homeostasis among children who were light for gestational age (Yajnik *et al* 1995). Low birth weight and in particular a high placental weight to foetal weight ratio has been found to be an independent risk factor for the development of hypertension during adulthood (Barker *et al* 1990, Barker 1998, Godfrey & Barker 2001). Recent evidence suggests that the undernourished foetus is also at higher risk of developing disturbances in blood cholesterol metabolism and blood clotting mechanisms (Barker 1993, Godfrey & Barker 2001). This has negative implications for the cardiovascular risk factor profile of the offspring of iron deficient and anaemic women. Analysis of disease risk and its association with birth weight as a marker of foetal nutrition among adults who were ante-natally exposed to the Dutch famine found lower HDL cholesterol and higher LDL cholesterol concentrations and less favourable concentrations of

apolipoproteins among those who were exposed to famine in utero (Roseboom *et al* 2000). Maternal iron deficiency has also been associated with poor infant iron stores which may increase the risk of toddler anaemia and the negative developmental and immunological implications of this condition (Hurtado *et al* 1999, Allen 2000, Hall *et al* 2001).

Certain experts (Hallberg & Rossander-Hulthen 1991) argue that it is not possible to maintain iron balance during pregnancy through normal dietary practices and that iron prophylaxis is necessary. Maternal haemoglobin concentrations have a U shaped curve (Beard 2000). It is therefore unclear whether iron supplementation is necessary or advisable to ensure a favourable pregnancy outcome for all women. It would appear preferable to base the need for iron prophylaxis during pregnancy on subjective assessment of all women attending GPs for family planning advice or ante-natal care. Routine screening of women planning a pregnancy, and the consideration of risk factors for iron deficiency such as high menstrual losses, recent menarche or a vegetarian diet, by general practitioners would improve detection and facilitate more effective treatment of this deficiency prior to and during pregnancy. This would be likely to improve pregnancy outcomes and may also reduce the risk of chronic diseases during adulthood for infants born to high risk mothers. Also, assessing and treating women on an individual basis is likely to improve compliance with iron supplementation. This represents a more economical use of health care resources.

The current study highlighted certain risk factors for the development of iron deficiency anaemia. Menstrual blood loss is the greatest contributory factor to iron balance in women of childbearing age (Hallerg & Rossander-Hulthen 1991). This is supported by the results of the current study. Female volunteers in the two lowest quartiles of iron status had significantly shorter menstrual cycles and thus experienced menstrual bleeding more frequently than the iron replete women. The measure of actual menstrual blood loss in this study was a very subjective estimate of menstrual flow by each female volunteer. A study to directly quantify menstrual blood loss as conducted by Hallberg (1966) would be necessary for an accurate measurement of menstrual losses at an individual level. As the association between high menstrual losses and risk of iron deficiency and iron deficiency anaemia is well established awareness of menstrual and reproductive history as potential risk factors for iron deficiency anaemia should be raised among primary care personnel and the general public.

Pregnancy occurring at an early age was also a significant risk factor for poor iron status in the current study. A review of iron deficiency in European countries supports this

finding (Hercberg *et al* 2001). This suggests that particular attention should be paid to the haematinic status of women who become pregnant during adolescence or early adulthood, particularly where multiple pregnancies occur. Adolescents require substantial amounts of energy and nutrients for rapid growth and the development of body tissues including bone, brain and sexual organs (Herbold *et al* 2001). This period of rapid growth is second only to to the growth seen in infancy (Herbold *et al* 2001). During puberty adolescents gain 20% of their adult height and 50% of their adult weight and skeletal mass (Herbold *et al* 2001). Girls deposit twice as much body fat as boys and boys double their lean body mass (Herbold *et al* 2001). Evidence suggests that adolescents are not physically ready for pregnancy. Although fertile, adolescent girls have between 12% and 18% of their pelvic growth to complete (Herbold *et al* 2001). Nutritional deficiencies during adolescence have detrimental consequences for longterm health (Herbold *et al* 2001). The nutritional demands of a pregnancy coupled with the already elevated requirements of adolescence place pregnant teenage girls and their offspring at very high nutritional risk. Nutrients of particular concern among teenage girls include iron, calcium and energy due to unsafe slimming practices prompted by widespread body image concerns (Flynn 1997). Loss of adult height and osteoporosis are documented complications associated with restrictive dieting during adolescence (Herbold *et al* 2001). Maternal under-nutrition increases the risk of low birth weight and all its associated immediate and long-term complications. Pregnancies occurring close to menarche may therefore be associated with poorer outcomes for mother and foetus and pregnant adolescents should be considered as a subgroup of the population requiring very specialised ante and post-natal care. This would help to ensure a favourable pregnancy outcome initially but would also help to safeguard the long-term good health of mother and foetus. The need for specific ante-natal services for adolescents is becoming increasingly apparent as the incidence of adolescent pregnancy is increasing in Ireland (Central Statistics Office 2001). In 1980, 2312 babies were born to adolescent mothers, representing 3% of live births that year (Central Statistics Office 2001). In 1999 6.2% (n=3301) of live births were to adolescent mothers (Central Statistics Office 2001). Ensuring adequate care for this high risk group is likely to offset considerable morbidity and mortality and would have significant health and economic implications in developed countries including Ireland.

The current British reference nutrient intake for iron for women of childbearing age is 14.8mg/day (DHSS 1991). Mean total dietary iron intake was less than this value in all quartiles of iron status. The Irish RDA is 14mg/day (Food Safety Authority of Ireland

1999) and in the current study mean total dietary iron intake is greater than this level only in one quartile of iron status. Although mean dietary haem iron intake was significantly higher among the most iron replete women, overall mean iron intake excluding supplements would be insufficient to meet the requirements of a substantial minority of Irish menstruating women. The NSIFCS (2001) reported similar findings in relation to iron intake with 48% of women aged between 18 and 50 years having sub-optimal (Scientific Committee for Food 1993) intakes (IUNA 2001). Among women in this age group who used supplements the proportion with inadequate intakes was half that of women who did not take supplements (IUNA 2001). The results of the current study suggest that supplement use makes an important contribution to the dietary intake of iron among Irish menstruating women (IUNA 2001). Experts have suggested that recommendations should be increased to 20mg daily for menstruating women and 30mg/day during pregnancy (Hallerg & Rossander-Hulthen 1991). This is very unlikely to be met through diet alone by women in industrialised countries where less energy demanding lifestyles have led to lower intakes of energy and hence essential nutrients (Hallerg & Rossander-Hulthen 1991). This is compounded by dietary restraint and avoidance of staple foods due to fat misconceptions which appear to be rife among females in industrialised countries (Biener & Heaton 1995).

In industrialised countries consumption of insufficient bio-available dietary iron among vulnerable groups appears to be the principal cause of iron deficiency. The fortification of food products with iron is a suggested strategy for the prevention of iron deficiency (Hurrell 1997). As with any food fortification programme the vehicle for the nutrient must be chosen carefully in order to target groups at risk of deficiency while preventing toxicity in other sectors of the population. The fortification of infant formulae with iron has improved the iron status of infants and toddlers in developed countries (Hurrell 1997), however it is more difficult to accurately target women of childbearing age. For this group the fortification of a widely consumed foodstuff would also seem the best option but other groups, such as adult men and post-menopausal women, who do not require extra iron will also be likely to consume the fortified food. There is evidence to suggest that excess iron may increase the risk of atherosclerosis (Salonen *et al* 1992, Hurrell 1997) and cancer (Stevens *et al* 1988, Hurrell 1997) due to oxidative stress. In light of this evidence the safest option appears to be to increase the consumption of naturally iron rich foods among vulnerable sectors of the population coupled with the administration of supplemental iron, based on individual assessment by a health professional, to those who have higher than average requirements.

The NSIFCS (2001) revealed that Ireland is overall a well-nourished nation (IUNA 2001). In fact this study reported a high prevalence of overweight and obesity in Ireland (57% of the total population had a BMI >25kg/m^2) (IUNA 2001). Despite this iron, folate and calcium intakes were sub-optimal (Scientific Committee for Food 1993) in a significant proportion of Irish women (IUNA 2001). The increase in overweight and obesity currently being observed in developed countries has prompted much expenditure on public health campaigns to reduce this upward trend and the associated co-morbidities such as cardiovascular disease and non-insulin dependent diabetes. Public health recommendations to reduce chronic disease risk have advised populations to increase fibre and reduce total and saturated fat intake. In the current study female volunteers with the highest fibre intake had the poorest iron status. Overall nutritional status must be taken into account by public health campaigns for the prevention of chronic nutrition related diseases. Greater emphasis on the use of physical activity for its beneficial effects on weight, insulin resistance and lipid profiles combined with less dietary restraint may be more conducive to maintaining micronutrient status. Foods traditionally targeted on lipid lowering and weight reducing diets included red meat and dairy produce in the mistaken belief that they were fattening. The use of meat and dairy produce as recommended by the Irish Department of Health in the Food Pyramid together with *n*-3 polyunsaturated and monounsaturated fats and the avoidance of sources *trans* fatty acids may be the most effective strategy to maintain haematinic status, bone health and cardio-protective lipid profiles among women. The effect of inhibitors of iron absorption such as tea and fibre is evident in the current study. Practical advice to increase the use of promoters of divalent cation absorption should be provided in any in any public health strategy to increase fibre intake.

Approximately half of the female volunteers in the current study had sub-optimal folate status (Daly *et al* 1997) for women of child-bearing age and a small portion had levels in the deficient range. This has potentially serious implications for the health of the offspring of these inner city Dublin women. Several studies report the inverse relationship between maternal folate status and the risk of neural tube defects (Daly *et al* 1997). The periconceptual use of folic acid supplements reduces the first occurrence as well as the reoccurrence of neural tube defects (Scott 1999). An intake of folates (dietary folate, folic acid and supplementary folic acid) which elevates red cell folate level to 400μg/l or greater has been found to reduce the incidence of neural tube defects by up to 70% (Daly *et al* 1995). Expert groups recommend that all women capable of becoming pregnant consume an extra 400μg of folic acid per day. Although mean daily

intake of total folates was greater than the RNI and EAR (200μg) (DHSS 1991, Scientific Committee for Food 1993) in all quartiles of folate status, only female volunteers in the two highest quartiles were above the protective level of red cell folate for the prevention of neural tube defects (400μg/l) (Daly *et al* 1995). Intake of folates in the two highest quartiles of folate status was greater than 400μg per day. The use of folic acid containing supplements and folic acid fortified foods or a combination of these was significantly greater than among the most folate replete women. Without fortified foods and supplements or both, mean intake of folates in each quartile of folate status was lower than 400μg per day. Results of the Dietary and Nutrition survey of British adults (1994) revealed that of the women surveyed (n=1110) 47% in the 19 to 50 year age range had folate intakes below the reference nutrient intake of 200μg per day (DHSS 1991). Very few had intakes above 400μg per day. The NSIFCS (2001) reported similar findings. Only 2% and 5% of women aged from 18 to 35 years and 36 to 50 years respectively had optimal folate intakes (Scientific Committee for Food 1993) for women of childbearing age (IUNA 2001). All women who achieved the target consumed folic acid containing supplements (IUNA 2001). Therefore, dietary folate intakes alone appear to be insufficient to ensure protection against neural tube defect affected births.

Increasing the intake of folates to a level in line with current recommendations for preventing the first occurrence of NTDs is problematic for various reasons (McNulty *et al* 2000). Departments of Health in developed countries usually aim the recommendations at women planning to become pregnant. However an estimated 50% of pregnancies in the UK and USA are unplanned (Department of Health UK 1992, Grimes 1986, McNulty *et al* 2000) and NTDs occur during the fourth week of embryonic life, usually before a woman knows that she is pregnant. In order to be effective the to increase folic acid intake should be more actively targeted at all women in the population who could become pregnant (McNulty *et al* 2000). Also, the recommended amount of folates required to prevent NTDs represents a three-fold increase in current intakes (McNulty *et al* 2000, IUNA 2001) and this recommendation is unlikely to be achieved through habitual dietary intake in most women. Supplements are very effective in improving folate status however knowledge and use of folic acid supplements is apparently low (Sayers *et al* 1997, IUNA 2001). Food fortification is viewed by many as the most effective available option to improve folate status in the general population (McNulty *et al* 2000).

The fortification of all grain products is mandatory in the United States since 1998 (Wynn & Wynn 1998). This measure was implemented primarily to reduce the incidence of neural tube defects but also in light of research indicating that the risk of heart disease, cancer, stroke and nervous system disorders including Alzheimer's disease may be reduced by daily intakes of folates higher than is currently normal in the American population (Wynn & Wynn 1998). Since the introduction of mandatory fortification in the United States the incidence of neural tube defects has declined by 19% (Honein *et al* 2001). However, due to safety concerns the current American level of fortification is estimated to increase intake of folic acid by only approximately 100μg per person per day (Firth *et al* 1998). This level, although safe, may be ineffective to improve folate status significantly in the American population. The limitations on food folic acid fortification include the necessity to fortify at a level sufficient to be effective in increasing red cell folate status while avoiding complicating the diagnosis of vitamin B_{12} deficiency. In addition to possibly masking vitamin B_{12} deficiency diagnosis in certain sectors of the population, a broad spectrum fortification programme may complicate future research on folic acid. In the American population anybody who eats cereal products is receiving supplemental folic acid so the selection of a control group to investigate the effects of supplemental folic acid on various metabolic processes is hindered. It may be necessary to include 'wash-out' periods and to feed control groups specially manufactured 'folic acid free' cereal products in future investigations to accurately determine the effects of supplemental folic acid. This would add considerably to the cost of investigations and to the time commitments required of subjects. Food-nutrient conversion databases will require adaptation, as in the current study, to include foods fortified with folic acid to accurately assess intakes in populations. Even with these limitations food folic acid fortification was considered the most preferable method to improve red cell folate status in the American population. Representations have been made to the American Food and Drug Administration by American clinicians advocating the fortification of all grain products with vitamin B_{12} along with folic acid fortification (Wynn & Wynn 1998).

Another possible method of improving the safety profile of a fortification programme would be to target specific foodstuffs favoured by the population group most in need of additional folates. Although a broad-spectrum folic acid fortification programme is currently under consideration in Europe, the results of the current study reveal that the use of foodstuffs currently fortified with folic acid can make a significant difference to red cell folate levels. It may therefore be possible to educate shoppers to choose

sufficient amounts of these specific foods to improve folate status, thus eliminating the safety concerns associated with a broad-spectrum folic acid fortification programme. However, health education campaigns advising the use of folic acid supplements among women of childbearing age have thus far proved unsuccessful (Sayers *et al* 1997, IUNA 2001) and attempts to educate the public about folic acid fortified foods may also meet with limited success. Also, as a diet high in folates is generally more expensive this method may not be practical in low income households.

The significant inverse socio-economic and educational gradient observed in the use of vitamin supplements and folate status in the current study is supported by the literature. Higher socio-economic classes are more aware of current health recommendations and place more emphasis on diet for health (Roos *et al* 1996). A recent Irish study has shown that knowledge and use of folic acid is lowest in the lower socio-economic classes and among those who have spent less time in formal education (Sayers *et al* 1997). The incidence of neural tube defect affected births has been found to be higher among lower socio-economic classes (Sayers *et al* 1997). The provision of folic acid supplements free of charge to GMS patients attending general practitioners for family planning advice may increase awareness of and compliance with the recommendations for folic acid intake among this vulnerable population group.

Although nutritional knowledge was not found to be significantly greater among women with better iron and folate status a significant socio-economic gradient was apparent in the level of nutritional knowledge. Knowledge of folic acid in particular was generally poor among the female volunteers. This is supported by the results of a survey of nutritional knowledge and use of folic acid among women of child-bearing age in Dublin (Sayers *et al* 1997). This author found that knowledge was significantly associated with higher social status and higher education (Sayers *et al* 1997). It is important to note that knowledge is just one aspect of nutritional behaviour and that several other factors impact on food choice and supplement use (Vaandrager *et al* 1997). Knowledge alone may not be sufficient to promote healthy practices (Vaandrager *et al* 1997).

This study of haematinic nutrient status in apparently healthy women revealed a substantial minority who were functioning in sub-optimal iron and folate status. Certain dietary, reproductive and socio-economic characteristics were established as risk factors for these deficiencies. Increasing awareness of these risk factors among primary care personnel and the general public would ensure that fewer cases remain undetected. In addition, a more holistic approach to the treatment of disorders of nutritional excess in

developed countries is warranted to simultaneously maintain the micronutrient intakes of vulnerable groups. Healthy eating advice designed to promote moderate energy intakes through the inclusion of sufficient amounts of nutrient dense staple foods, such as meat, dairy products and fortified cereals, together with daily physical activity will be most effective to maintain healthy body mass indices and adequate iron, folate and calcium intakes among Irish women.

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Chapter 4

Lifestyle characteristics and attitudes to nutrition of Irish adult women.

4.1 Introduction:

The link between diet, the maintenance of health and the development of chronic disease has become increasingly evident in recent years (Martinez-Gonzalez *et al* 2000). It is gradually being accepted that understanding personal and socio-economic predictors of nutrition behaviour and dietary change is necessary for the development of effective health promotion campaigns (Kearney *et al* 2000). Food intake is a complex process affected by social, cultural, economic and political factors (Vaandrager & Koelen 1997). Eating habits are often developed early in life and are often stable and long-lasting (Vaandrager & Koelen 1997). Health promotion programmes designed to promote better nutrition are generally based on the assumption that poorly balanced diets are due to a lack of nutritional knowledge (Vaandrager & Koelen 1997). However, knowledge is just one aspect of nutrition behaviour; personal tastes, cultural traditions, economic factors, the variety of foodstuffs available and time constraints all have important effects on food choice (Vaandrager & Koelen 1997).

Various studies in developed countries have examined determinants of food choice among consumers. Factors such as the quality or freshness of food, taste and nutritional value have been cited as determinants of food choice by Irish adults (Kearney *et al* 2000). Studies in other developed countries have reported taste (Glanz *et al* 1998, Kearney & McElhone 1999, Johansson *et al* 1999), time constraints (Kearney & McElhone 1999) and cost (Glanz *et al* 1998, Kearney & McElhone 1999) as the most important determinants of food choice. The nutritional value of the food, although cited as a determinant of food choice (Glanz *et al* 1998, Kearney & McElhone 1999, Johansson *et al* 1999) appears to be less important than personal preference. Female gender, higher socio-economic status, length of formal education and increasing age appear to be independent sociodemographic factors affecting the selection of nutritional value as an important determinant of food choice (Glanz *et al* 1998, Kearney & McElhone 1999, Johansson *et al* 1999, Kearney *et al* 2000).

Awareness of the need to alter one's diet is an important stage in changing dietary behaviour (IUNA 2001, Kearney *et al* 2001). A high level of perception of personal dietary habits as already sufficiently healthy has been reported among Irish and European consumers (Kearney & McElhone 1999, Kearney *et al* 2000, IUNA 2001, Kearney *et al* 2001). Fifty two percent of participants in the North/South Ireland Food Consumption Survey (NSIFCS) considered that they did not need to make changes to their dietary habits for health reasons (IUNA 2001, Kearney *et al* 2001). This self-

reported perception of diet is not necessarily reflected in nutritional adequacy and healthy BMI. The NSIFCS found inadequate calcium intakes in 23% of women surveyed and inadequate iron intakes (Scientific Committee for Food 1993) in 48% of pre-menopausal women (18-50 years) (IUNA 2001, Hannon *et al* 2001). Intake of folates was below the recommendation for women of childbearing age (Scientific Committee for Food 1993) in 98% of women aged between eighteen and thirty five years and in 95% of women aged between thirty six and fifty years (IUNA 2001, O'Brien *et al* 2001). Mean percentage energy from dietary fat was above the level recommended for cardiovascular disease prevention in the population and fibre intakes were inadequate in 67% of men and 87% of women (DHSS 1991, IUNA 2001). Subjects in the older age group (51-64 years) were most likely to view their diets as sufficiently healthy, however the greatest prevalence of obesity was recorded in this group with 24.4% and 29.6% of men and women respectively having a BMI in the obese range (IUNA 2001).

A rising trend in overweight and obesity has been observed in many developed countries (Flegal *et al* 1998, McCrory *et al* 2000, IUNA 2001, McCarthy *et al* 2001). Obesity prevalence has reportedly increased by 67% in Ireland since 1990 (IUNA 2001, McCarthy *et al* 2001). Various expert groups have cited the increasingly sedentary lifestyles in industrialised countries as the major contributory factor to the upsurge in obesity (Prentice & Jebb 1995, IUNA 2001, McCarthy *et al* 2001, Livingstone *et al* 2001). European and American research has reported that sedentary leisure pursuits such as television watching are correlated with BMI (Martinez-Almendros *et al* 2000, Crespo *et al* 2001, IUNA 2001, Livingstone *et al* 2001). The NSIFCS found that, on average, overweight and obese individuals spent between two and three more hours per week in television viewing than their normal weight counterparts (IUNA 2001, Livingstone *et al* 2001). Overweight and obese subjects also spent significantly less time in vigorous recreational activity (IUNA 2001, Livingstone *et al* 2001).

Increasing physical activity during leisure time is vital to reduce BMIs within the population. Regular physical activity affords a variety of other well documented health benefits including a reduced risk of cardiovascular disease, cerebrovascular disease, hypertension, non-insulin dependent diabetes and osteoporosis (Vuori 2001). A study examining perceived benefits and barriers to physical activity in a representative sample of adults in the EU found a high level of awareness of the health benefits of regular exercise (Zunft *et al* 1999). The most important reported barriers to increasing physical

activity related to work or study commitments and the subjects' belief that they are not 'the sporty type' (Zunft *et al* 1999).

A holistic approach combining dietary and lifestyle factors is required to reverse the upward trend in overweight and obesity. Health promotion campaigns in the new millennium must aim to provide nutritional knowledge but also practical solutions to enable individuals to recognise areas in their diet and lifestyle, which require attention and ways in which to implement the necessary changes.

The present study examined demographic characteristics, attitudes to nutrition and self reported perception of diet in relation to food choice, nutritional adequacy and haematinic status in a sample of Irish adult women attending general practitioners in inner city Dublin. Self reported occupational and leisure time activity levels were also examined in relation to BMI in the group.

4.2 Subjects and methods.

4.2.1 Subjects

Prior to the commencement of the study, ethical approval was sought and granted by the Irish College of General Practitioners ethics committee. Female volunteers were recruited from four general medical practices located in Dublin's south inner city. Two hundred consent forms inviting participation were circulated to each practice. Posters and leaflets describing the study were displayed in the waiting room of each medical centre. Female volunteers were invited to complete the consent forms and were then contacted by telephone by the main investigator to arrange an appointment. All participating female volunteers were Irish, aged between 18 and 64 years and had no history of chronic disease or drug or alcohol abuse. Pregnant women were excluded as were women found to be anaemic during the twelve months prior to the study. Each participating female volunteer visited their relevant general practice once between June and December 1999. All female volunteers were reimbursed for travel expenses incurred.

4.2.2 Lifestyle characteristics and attitudes to nutrition.

Data on lifestyle characteristics and attitudes to nutrition were collected using section one (questions h, i, j and k) and section two of the standardised 'Interview Questionnaire' (Appendix II) which was validated prior to use (see chapter 2). The

'Interview Questionnaire' was administered to each female volunteer by the main investigator. The relevant information was obtained using six multiple-choice questions, each including a 'don't know' option. The female volunteers were initially asked to report the extent to which they are involved in food purchasing and cooking in their households. Information on determinants of food choice was obtained in the next multiple-choice question where female volunteers were asked to identify which factors (taste preferences, cost, nutritional value, convenience, local availability, storage and/or cooking facilities) they would consider when purchasing food for their households. Female volunteers were informed that more than one of the factors could be cited if appropriate, they were also invited to volunteer any other information deemed relevant. Self-reported perception of current dietary habits ('mainly healthy', 'both healthy and unhealthy' or 'mainly unhealthy') was then recorded for each female volunteer.

The remaining questions related to physical activity. Self-reported perceptions of occupational and leisure time physical activity levels ('mainly active', both active and sedentary' or 'mainly sedentary') were recorded for each participant. Finally the female volunteers were asked to estimate the amount of time spent in television viewing each day. Weekly television viewing was calculated from this information.

4.2.3 Dietary Assessment.

Refer to chapter 3.

4.2.4 Socio-economic Classification.

Refer to chapter 3.

4.2.5 Anthropometry.

Refer to chapter 3.

4.2.6 Blood sampling and analysis.

Refer to chapter 3.

4.2.7 Data management and statistical analysis.

All data obtained was entered into the Statistical Package for Social Sciences (SPSS) version 9.0 for Windows. Data was then analysed using the Mann Whitney U test, One

way ANOVA post hoc analysis-LSD test, Kruschall Wallis test or Chi square test as appropriate.

4.3 Results

The response rate was 29.3% (n=176). Of these respondents 59% (n=104) participated fully in the study. Of those who were excluded 3% (n=5) were pregnant, 4% (n=7) under-reported dietary intake, 12% (n=21) were not Irish, and 22% (n=39) were unavailable at the time of data collection. Forty-two percent (n=44) of the participating female volunteers were recruited from Mercer's Medical Centre, Dublin 2, some 31% (n=32) were recruited from the City Medical Centre, Dublin 2, a further 24% (n=25) from the Rialto Medical Centre, Dublin 8 and 3% (n=3) from the Inchicore Medical Centre, Dublin 8. Of the one hundred and four female volunteers who completed the study 84.5% (n=88) did the majority of food purchasing for their households, 9.5% (n=10) shared this task with other members of the household and the remaining 5% (n=6) did not habitually purchase food for their households. Forty four percent (n=45) of the female volunteers did the majority of food preparation and cooking for their households, a further 55% (n=57) of the female volunteers shared these tasks with other members of the household and 1% (n=2) did not habitually prepare and cook food for their households.

Taste preference was reported as a determinant of food choice by 94% (n=98) of the female volunteers. Cost, convenience, storage and/or cooking facilities and local availability were reported as determinants of food choice by 44% (n=45), 37% (n=38), 23% (n=24) and 19% (n=20) of female volunteers respectively. Seventy percent (n=73) of the sample reported nutritional value as a determinant of food choice. Significantly more female volunteers who cited nutritional value as a consideration when purchasing food were in socio-economic class one (47% (n=34) vs. 22% (n=16) and 31% (n=23) in socio-economic classes two and three respectively) ($p<0.001$). Female volunteers who cited nutritional value as a determinant of food choice had spent significantly longer in formal education (15.5 ± 3.8 years) than those who did not consider nutritional value to be important (13.4 ± 3.5 years) ($p=0.01$).

Table 1 summarises the observed macronutrient intakes among the female volunteers in each dietary perception category (i.e. those who reported their diet to be 'mainly healthy', 'both healthy and unhealthy' or 'mainly unhealthy'). Tables 2(a) and 2(b)

present the micronutrient intakes recorded among the female volunteers in each dietary perception category. Female volunteers who perceived their diets to be 'mainly healthy', as assessed by the Interview Questionnaire, had a significantly lower fat intake (p=0.04) and percentage energy from fat (p=0.04) compared to those who perceived their diets to be 'mainly unhealthy' (Table 1). The female volunteers in the 'mainly healthy' dietary perception category also had significantly higher intakes of Englyst fibre and compared with those in the 'both healthy and unhealthy' (p=0.001) and 'mainly unhealthy' (p=0.001) dietary perception categories (Table 1). Intake of Southgate fibre was also significantly higher among those in the 'mainly healthy' dietary perception category compared with the 'both healthy and unhealthy' (p=0.03) and 'mainly unhealthy' (p=0.01) categories (Table 1). Female volunteers who perceived their diets to be 'both healthy and unhealthy' had significantly higher intakes of Englyst fibre (p=0.004) and Southgate fibre (p=0.03) compared with those who perceived their diets to be 'mainly unhealthy' (Table 1).

Analysis also revealed some significant differences in haematinic micronutrient intakes (Table 2[a]). Female volunteers who perceived their diets to be 'mainly healthy' (p=0.02) and 'both healthy and unhealthy' (p=0.006) as assessed by the Interview Questionnaire, had a significantly higher total iron intakes (diet and supplements) compared with those who perceived their diets to be 'mainly unhealthy'. Dietary haem iron intakes were significantly lower among the female volunteers in the 'mainly healthy' dietary perception category compared with those in the 'both healthy and unhealthy' (p=0.01) and 'mainly unhealthy' (p=0.01) dietary perception categories. Female volunteers who perceived their diets to be 'mainly healthy' had significantly higher intakes of dietary non-haem iron compared to those who perceived their diets to be 'both healthy and unhealthy' (p=0.04) and 'mainly unhealthy' (p=0.001). Dietary non-haem iron intake was significantly higher among the female volunteers in the 'both healthy and unhealthy' dietary perception category compared with those in the 'mainly unhealthy' (p=0.02) dietary perception category. Dietary folic acid intakes were significantly lower among female volunteers in the 'mainly unhealthy' dietary perception category compared to those in the 'mainly healthy' (p=0.001) and 'both healthy and unhealthy' (p=0.001) dietary perception categories.

Table 2(b) illustrates other significant differences in micrcronutrient intakes between the dietary perception categories. Female volunteers who perceived their diets to be 'mainly healthy' (p=0.04) and 'both healthy and unhealthy' (p=0.04) had significantly

higher intakes of vitamin B_1 compared with those who perceived their diets to be 'mainly unhealthy'. Female volunteers who perceived their diets to be 'mainly healthy' (p=0.01) and 'both healthy and unhealthy' (p=0.01) also had significantly higher intakes of vitamin B_6 compared with those in the 'mainly unhealthy' dietary perception category. Significantly higher intakes of vitamin E were also observed among female volunteers in the 'mainly healthy' (p=0.03) and 'both healthy and unhealthy' (p=0.02) dietary perception categories compared to those who perceived their diets to be 'mainly unhealthy'.

In terms of nutritional adequacy in each dietary perception category; Chi-square tests revealed some significant differences in the proportions meeting requirements (DHSS 1991, Scientific Committee for Food 1993, Food Safety Authority of Ireland 1999) for certain nutrients between the dietary perception categories (data not shown). A significantly higher proportion of the female volunteers in the 'mainly healthy' (94%, *n*=34) dietary perception category achieved the RDA for calcium compared to the 'both healthy and unhealthy' (74%, *n*=38) and 'mainly unhealthy' (65%, *n*=11) categories (p=0.02) (Food Safety Authority of Ireland 1999). A significantly higher proportion of the female volunteers who perceived their diets to be 'mainly healthy' (69%, *n*=25) achieved the recommendation for Englyst fibre compared to the female volunteers who perceived their diets to be 'both healthy and unhealthy' (40%, *n*=21) or 'mainly unhealthy' (7%, *n*=1) (p=0.003) (DHSS 1991). None of the female volunteers in the 'mainly unhealthy' dietary perception category achieved the recommended intake of folates for the prevention of neural tube defects compared with 25% (*n*=9) and 25% (*n*=13) of those in the 'mainly healthy' and 'both healthy and unhealthy' categories respectively (p=0.05) (Scientific committee for Food 1993).

Table 3 reports the results of the analysis of food consumption in each dietary perception category. Significantly fewer female volunteers in the 'mainly healthy' category consumed full fat polyunsaturated spreads compared to the 'both healthy and unhealthy' and 'mainly unhealthy' categories (p=0.04). A significantly higher proportion of the female volunteers who perceived their diets to be 'both healthy and unhealthy' consumed low fat milk compared with those who perceived their diets to be 'mainly healthy' or 'mainly unhealthy' (p=0.02). Significantly fewer women in the 'mainly healthy' dietary perception category consumed red meat compared with those in the 'both healthy and unhealthy' and 'mainly unhealthy' categories (p=0.04). Consumption of beer, stout and cider was significantly higher among female volunteers

who perceived their diets to be 'both healthy and unhealthy' compared to those who perceived their diets to be 'mainly healthy' (p=0.04) and 'mainly unhealthy' (p=0.05). Consumption of confectionery was also significantly higher among female volunteers who perceived their diets to be 'both healthy and unhealthy' compared to those who perceived their diets to be 'mainly healthy' (p=0.01) and 'mainly unhealthy' (p=0.05). Consumption of fortified white cereals was significantly higher among female volunteers who perceived their diets to be 'mainly healthy' compared to those who perceived their diets to be 'both healthy and unhealthy' (p=0.01) and 'mainly unhealthy' (p=0.05). Consumption of low fat cheese was also significantly higher among female volunteers in the 'mainly healthy' dietary perception category compared to those in the 'both healthy and unhealthy' (p=0.008) and 'mainly unhealthy' (p=0.03) categories. Consumption of offal was significantly higher among female volunteers who perceived their diets to be 'mainly unhealthy' compared to those who perceived their diets to be 'mainly healthy' (p=0.01) and 'both healthy and unhealthy' (p=0.04).

Table 4 summarises haematological and anthropometric parameters, socio-economic class, smoking habits, education and current supplement use observed among female volunteers in each dietary perception category. Mean red cell folate levels were significantly higher among female volunteers who perceived their diets to be 'mainly healthy' compared to those who perceived their diets to be 'mainly unhealthy' (p=0.02). Mean plasma vitamin B_{12} levels were also significantly higher among female volunteers who perceived their diets to be 'mainly healthy' compared to those who perceived their diets to be 'both healthy and unhealthy' (p=0.02) and 'mainly unhealthy' (p=0.02). Mean BMI was significantly greater among women in the 'mainly unhealthy' dietary perception category compared with those in the 'mainly healthy' (p=0.003) and 'both healthy and unhealthy (p=0.03) categories. Female volunteers who perceived their diets to be 'mainly healthy' had spent significantly longer in formal education compared with those who perceived their diets to be 'both healthy and unhealthy' (p=0.001) and 'mainly unhealthy' (p=0.001). Significantly fewer of the female volunteers in the 'mainly healthy' dietary perception category were current smokers compared to those in the 'both healthy and unhealthy' and 'mainly unhealthy' categories (p=0.007). A significantly higher proportion of the female volunteers who perceived their diets to be 'mainly healthy' never smoked compared with those who perceived their diets to be 'both healthy and unhealthy' and mainly unhealthy' (p=0.007). Significantly more of the female volunteers in the 'mainly healthy' dietary perception category were in socio-

economic class 1 compared to those in the 'both healthy and unhealthy' and 'mainly unhealthy' categories (p=0.001). Significantly more of the female volunteers in the 'mainly unhealthy' dietary perception category were in socio-economic class 3 compared to those in the 'mainly healthy' and 'both healthy and unhealthy' categories (p=0.001). One aspect of nutritional knowledge was found to be significantly different among the female volunteers in the dietary perception categories (data not shown). Significantly more of the female volunteers in the 'mainly healthy' dietary perception category (97%, *n*=35) correctly identified the biological function of iron compared to those in the 'both healthy and unhealthy' (68%, *n*=35) and 'mainly unhealthy' (65%, *n*=11) categories (p=0.005).

Table 5 presents self-reported perception of occupational and leisure-time physical activity level, dietary perception and weekly television viewing in relation to BMI among the female volunteers. Significantly fewer of the obese individuals perceived themselves as 'mainly active' during leisure time (p=0.04). A significantly higher proportion of the female volunteers in the obese BMI range perceived themselves as 'mainly sedentary' during leisure time (p=0.04). Significantly more of the normal weight female volunteers perceived their diets as 'mainly healthy' (p=0.04) and significantly more of the obese female volunteers perceived their diets to be 'mainly unhealthy' (p=0.04). Female volunteers in the obese BMI range spent significantly longer watching television each week than those in the underweight (p=0.001), normal weight (p<0.001) and overweight (p=0.007) BMI ranges.

Table 1: Macronutrient intakes among female volunteers in each dietary perception category (i.e. 'mainly healthy', 'both healthy and unhealthy' or 'mainly unhealthy').

Nutrient	Mainly healthy (n=36)	Both healthy & unhealthy (n=51)	Mainly unhealthy (n=17)
	mean ± SD	mean ± SD	mean ± SD
EI:BMR	1.73 ± 0.36	1.80 ± 0.39	1.63 ± 0.29
Energy (MJ)	9.8 ± 2.5	10.3 ± 2.3	10.1 ± 1.8
Energy (kcals)	2382 ± 497	2459 ± 550	2400 ± 441
Protein (g)	83.9 ± 10.6	86.7 ± 11.0	82.2 ± 7.9
Protein (%)	14.1 ± 2.1	14.1 ± 2.0	13.7 ± 1.8
CHO (g)	275.1 ± 31.8	274.8 ± 39.1	267.7 ± 30.9
CHO (%)	46.2 ± 6.4	44.7 ± 7.1	44.6 ± 7.0
Total fat (g)	92.6 ± 15.9[a]	102.2 ± 18.7	104.8 ± 23.6
Total fat (%)	35.0 ± 6.4[a]	37.4 ± 6.1	39.3 ± 5.9
SFAs (g)	37.1 ± 9.7	40.4 ± 11.3	43.2 ± 16.6
SFAs (%)	14.0 ± 3.9	14.8 ± 4.1	16.2 ± 4.0
MUFAs (g)	28.6 ± 6.5	32.2 ± 7.8	32.6 ± 10.3
MUFAs (%)	10.8 ± 2.6	11.8 ± 2.0	12.2 ± 3.0
PUFAs (g)	15.9 ± 3.1	16.2 ± 4.4	15.8 ± 4.9
PUFAs (%)	6.0 ± 1.9	5.9 ± 1.6	5.9 ± 2.2
Englyst fibre (g)	19.5 ± 6.7[ab]	17.6 ± 5.6[c]	12.7 ± 4.3
Southgate fibre (g)	25.9 ± 7.7[ab]	23.9 ± 7.0[c]	19.3 ± 5.6
Alcohol (g)	15.1 ± 10.7	12.4 ± 9.3	8.9 ± 5.2
Alcohol (%)	5.8 ± 4.1	4.8 ± 3.7	3.4 ± 1.9

SD = standard deviation. CHO = carbohydrate. SFAs = saturated fatty acids. MUFAs = monounsaturated fatty acids. PUFAs = polyunsaturated fatty acids. [a] Significant difference between 'mainly healthy' and 'mainly unhealthy', [b] significant difference between 'mainly healthy' and 'both healthy & unhealthy', [c] significant difference between 'both healthy & unhealthy' and 'mainly unhealthy' according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 2(a): Haematinic micronutrient intakes among female volunteers in each dietary perception category (i.e. 'mainly healthy', 'both healthy and unhealthy' or 'mainly unhealthy').

Nutrient	Mainly healthy (n=36)	Both healthy & unhealthy (n=51)	Mainly unhealthy (n=17)
	mean ± SD	mean ± SD	mean ± SD
Total iron (mg)	16.2 ± 4.9[a]	16.8 ± 5.2[c]	14.5 ± 4.8
Total dietary iron (mg)	13.6 ± 3.3	14.0 ± 3.7	11.3 ± 2.3
Dietary haem iron (mg)	2.1 ± 1.3[ab]*	3.6 ± 2.5	3.0 ± 1.3
Dietary non-haem iron (mg)	11.3 ± 3.3[ab]	10.4 ± 3.3[c]	8.3 ± 2.4
Supplementary iron (mg)	2.5 ± 3.4	3.0 ± 4.0	3.2 ± 4.1
Total folates (μg)	412 ± 146	427 ± 158	397 ± 172
Total dietary folates (μg)	322 ± 97	327 ± 121	268 ± 75
Dietary folic acid (μg)	46 ± 49[a]*	38 ± 57[c]*	15 ± 21
Dietary folate (μg)	278 ± 84	288 ± 91	253 ± 75
Supplementary folic acid (μg)	89 ± 117	100 ± 131	129 ±141
Vitamin B_{12} (μg)	4.1 ± 2.4	4.8 ± 3.3	4.0 ± 1.7

SD = standard deviation. [a] Significant difference between 'mainly healthy' and 'mainly unhealthy', [b] significant difference between 'mainly healthy' and 'both healthy & unhealthy', [c] significant difference between 'both healthy & unhealthy' and 'mainly unhealthy' according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 2(b): Intakes of other micronutrients among female volunteers in each dietary perception category (i.e. 'mainly healthy', 'both healthy and unhealthy' or 'mainly unhealthy').

Nutrient	Mainly healthy (n=36)	Both healthy & unhealthy (n=51)	Mainly unhealthy (n=17)
	mean ± SD	mean ± SD	mean ± SD
Calcium (mg)	1110 ± 283	1045 ± 352	1107 ± 504
Zinc (mg)	9.4 ± 2.5	9.8 ± 3.0	8.6 ± 2.2
Vitamin B_1 (μg)	1.7 ± 0.5[a]	1.7 ± 0.5[c]	1.4 ± 0.4
Vitamin B_2 (μg)	2.1 ± .05	2.0 ± 0.7	1.9 ± 0.8
Vitamin B_3 (μg)	41.8 ± 9.5	40.3 ± 10.2	36.6 ± 9.1
Vitamin B_5 (μg)	4.9 ± 1.2	5.0 ± 1.5	4.7 ± 1.5
Vitamin B_6 (μg)	2.5 ± 0.6[a]	2.5 ±0.8[c]	2.0 ± 0.6
Vitamin C (μg)	119 ± 60	130 ± 71	98 ± 45
Vitamin D (μg)	3.0 ± 2.1	2.9 ± 1.5	2.4 ± 1.2
Vitamin E (μg)	8.2 ± 3.2[a]	8.1 ± 2.8[c]	6.3 ± 1.7
REs	1316 ± 714	1358 ± 787	1366 ± 705

SD = standard deviation REs = Retinol Equivalents. [a] Significant difference between 'mainly healthy' and 'mainly unhealthy', [b] significant difference between 'mainly healthy' and 'both healthy & unhealthy', [c] significant difference between 'both healthy & unhealthy' and 'mainly unhealthy' according to One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 3: Food consumption among female volunteers in each dietary perception category (i.e. 'mainly healthy', 'both healthy and unhealthy' or 'mainly unhealthy').

Nutrient	Mainly healthy (n=36)	Both healthy & unhealthy (n=51)	Mainly unhealthy (n=17)
	% (n)	% (n)	% (n)
Consumers of:			
Full fat polyunsaturated spreads	3 (1)[ab]	22 (11)	18 (3)
Low fat milk	6 (2)	31 (16)[bc]	12 (2)
Red meat	77 (28)[ab]	90 (46)	100 (17)
	mean ± SD	mean ± SD	mean ± SD
Beer/stout/cider (g/day)	346 ± 277	520 ± 355[bc]	305 ± 321
Confectionery (g/day)	23.1 ± 15.9	15.1 ± 10.3[bc]	22.6 ± 12.1
Fortified white cereal (g/day)	24.8 ± 17.3[ab]	16.3 ±7.2	19.3 ± 11.8
Low fat cheese (g/day)	13.5 ± 10.6[ab]	6.0 ± 5.3	6.2 ± 6.0
Offal (g/day)	4.5 ± 1.0[a]	6.8 ± 4.5[c]	13.3 ± 5.5

SD = standard deviation, (g/day) = grams among consumers per day [a] Significant difference between 'mainly healthy' and 'mainly unhealthy', [b] significant difference between 'mainly healthy' and 'both healthy & unhealthy', [c] significant difference between 'both healthy & unhealthy' and 'mainly unhealthy' according to Chi square test, One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 4: Haematological and anthropometric parameters, smoking habits, supplement use, education and socio-economic class of female volunteers in each dietary perception category (i.e. 'mainly healthy', 'both healthy and unhealthy' or 'mainly unhealthy').

Nutrient	Mainly healthy (n=36)	Both healthy & unhealthy (n=51)	Mainly unhealthy (n=17)
	mean ± SD	mean ± SD	mean ± SD
Hgb (g/dl)	12.8 ± 1.0	13.0 ± 1.0	13.4 ± 0.9
MCV (fl)	88.6 ± 3.9	90.5 ± 4.3	90.0 ± 4.1
MCHC (pg/ml)	33.6 ± 3.4	33.0 ±3.0	33.1 ± 3.2
Serum ferritin (µg/l)	48.5 ± 29.2	53.4 ± 45.8	57.5 ± 32.8
sTFR (µg/l)	17.4 ± 4.6	18.4 ± 6.5	17.2 ± 5.6
RCF (µg/l)	500 ± 262[a]	435 ± 161	358 ± 143
Plasma vitamin B_{12} (ng/l)	547 ± 161[ab]	456 ± 194	461 ± 120
Homocysteine (µg/l)	10.5 ± 3.8	10.6 ± 3.2	11.5 ± 3.7
BMI (kg/m^2)	23.2 ± 3.3[a]	24.7 ± 5.3[c]	27.9 ± 7.8
WHR	0.78 ± 0.52	0.79 ± 0.53	0.80 ± 0.55
Education (years)	16.3 ± 4.6[ab]	14.8 ± 3.1	12.1 ± 2.1
	% (n)	% (n)	% (n)
Smoking status:			
Current	19 (7)[ab]	45 (23)	71 (12)
Ex-smoker	17 (6)	14 (7)	12 (2)
Never smoked	64 (23)[ab]	41 (21)	17 (3)
Supplement use	36 (13)	45 (23)	33 (5)
SEC 1	58 (21)[a]	27 (14)[c]	6 (1)
2	14 (5)	29 (15)	18 (3)
3	28 (10)[a]	44 (22)[c]	76 (13)

SD = standard deviation, SEC = socio-economic class, RCF = red cell folate, MCHC = Mean cell haemoglobin content, MCV = Mean corpuscular volume, Hgb = Haemoglobin, sTFR = soluble transferrin receptor, WHR = Waist to hip ratio, [a] significant difference between 'mainly healthy' and 'mainly unhealthy', [b] significant difference between 'mainly healthy' and 'both healthy & unhealthy', [c] significant difference between 'both healthy & unhealthy' and 'mainly unhealthy' according to Chi square test, One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

Table 5: Self-reported perception of occupational and leisure-time physical activity level, dietary perception and weekly television viewing among female volunteers in each BMI category.

BMI (kg/m^2)	Under-weight [<20]	Normal weight [20-24.9]	Over-weight [25-29.9]	Obese [>30]
	(*n*=8)	(*n*=58)	(*n*=24)	(*n*=14)
	% (*n*)	% (*n*)	% (*n*)	% (*n*)
Perception of occupational activity level:				
Mainly active	25 (2)	28 (16)	33 (8)	21 (3)
Active & sedentary	37.5 (3)	41 (24)	29 (7)	21 (3)
Mainly sedentary	37.5 (3)	31 (18)	38 (9)	58 (8)
Perception of leisure-time activity level:				
Mainly active	25 (2)[a]	31 (18)[b]	33 (8)[c]	7 (1)
Active & sedentary	50 (4)	55 (32)	55 (13)	43 (6)
Mainly sedentary	25 (2)[a]	14 (8)[b]	12 (3)[c]	50 (7)
Perception of diet:				
Mainly healthy	13 (1)[d]	48 (28)	25 (6)[e]	7 (1)[b]
Both healthy & unhealthy	62 (5)	43 (25)	58 (14)	50 (7)
Mainly unhealthy	25 (2)[a]	9 (5)[b]	17 (4)[c]	43 (6)
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Television viewing (hours/week)	12.7 ± 10.1[a]	12.2 ± 8.9[b]	15.3 ± 9.9[c]	25.8 ± 19.8

SD = standard deviation, [a] significant difference between underweight and obese, [b] significant difference between normal weight and obese, [c] significant difference between overweight and obese, [d] significant difference between underweight and normal weight, [e] significant difference between overweight and normal weight according to Chi square test, One Way ANOVA post hoc analysis LSD test, Kruschall-Wallis test* or Mann Whitney U test* as appropriate.

4.4 Discussion.

The current study investigated lifestyle characteristics and attitudes to nutrition in relation to nutritional adequacy and anthropometry in a sample of apparently healthy Irish adult women attending GPs in inner-city Dublin. The association between dietary and lifestyle practices and chronic conditions such as obesity and its co-morbidities including non-insulin dependant diabetes, hypertension, dyslipidaemia, coronary heart disease and certain types of cancer is well established (Solomon & Manson 1997). Public health interventions to stem the rise in obesity levels are currently high on government agendas in many developed countries (Solomon & Manson 1997, Buttriss 1997). Similarly increasing the periconceptual use of folic acid to reduce the incidence of neural tube defects is of priority (Murphy *et al* 2000, Honein *et al* 2001). Health promotion campaigns designed to improve nutritional status in population are often based on the assumption that unhealthy diet and lifestyle choices are due to a dearth of information (Vaandrager & Koelen 1997). However although nutritional knowledge is important, it is not always sufficient to promote behavioural change (Vaandrager & Koelen 1997). Awareness of the need to change dietary and lifestyle practices is an important first stage in behavioural change (IUNA 2001, Kearney *et al* 2001). The results of the current study reveal a high level of perception of current dietary habits as already sufficiently healthy among the female volunteers. This finding is similar to observations made by the Irish Universities Nutrition Alliance (2001) regarding dietary perception among subjects in the North/South Ireland Food Consumption Survey. These investigators reported that 52% of subjects considered that they did not need to make changes to their diet for health reasons (IUNA 2001, Kearney *et al* 2001). In a pan-EU consumer attitudinal survey Kearney and McElhone (1999) reported that a major obstacle to nutrition education is the fact that 70% of EU subjects believe that their diets are already healthy. This phenomenon is known as optimistic bias and indicates an unrealistic optimism in self-perception of dietary quality (Kearney & McElhone 1999).

Although female volunteers in the current study did exhibit a degree of optimistic bias, those who perceived their diets to be 'mainly healthy' did have intakes of dietary total, saturated, monounsaturated and polyunsaturated fat more in line with current cardiovascular disease prevention guidelines (Krauss *et al* 2000). Total plasma and LDL cholesterol levels can be reduced by replacing dietary saturated fatty acids (SFAs) and *trans* fatty acids isoenergetically with either monounsaturated (MUFAs) or

polyunsaturated (PUFAs) fatty acids (Gibney 1999). The triacylglycerol raising effect of a high carbohydrate diet may be attenuated through the addition of *n*-3 PUFAs (Gibney 1999). However although a reduction in fat intake is being achieved in many countries, the reduction appears to be uniform across all fatty acid categories (Gibney 1999) similar to that observed in the current study.

Although significantly more of the female volunteers in the 'mainly healthy' dietary perception category had adequate fibre and calcium intakes, mean dietary iron intakes were lower than the RNI (DHSS 1991) in each dietary perception category and lower than the RDA (Food Safety Authority of Ireland 1999) among those who perceived their diets to be 'mainly healthy' and 'mainly unhealthy'. Haem iron intake in particular was significantly lower among female volunteers who perceived their diets to be 'mainly healthy'. Although mean dietary folic acid intake was significantly higher among female volunteers who perceived their diets to be 'mainly healthy', mean dietary intake of folates was below the recommended level for the prevention of neural tube defects in each dietary perception category. Although not significant, folic acid intake from supplements was lowest in the 'mainly healthy' dietary perception category suggesting poor compliance with the recommendation that women of child-bearing age consume folic acid supplements to help prevent neural tube affected births. Similarly, despite a high level of optimistic bias, iron intakes were found to be sub-optimal in 48% of menstruating females and intake of folates was inadequate (Scientific committee for Food 1993) in a large majority of women of child-bearing age in the NSIFCS (IUNA 2001, O'Brien *et al* 2001, Hannon *et al* 2001).

Public health interventions designed to reduce BMIs and cardiovascular disease in populations have largely focused on dietary fat intake. In addition to being ineffective, percentage energy from dietary fat has remained static in Ireland since 1990 but obesity has increased by 67% in the population (IUNA 2001), this approach appears to have led to misconceptions about sources of fat in the diet (Buttriss 1997). Consumers attempting to reduce fat intake are reducing consumption of or omitting staple foods such as meat and milk (Buttriss 1997). This has potentially negative implications for nutritional status as the results of the current study suggest. Significantly fewer of the female volunteers who perceived their diets to be 'mainly healthy' consumed red meat. Mean daily consumption of offal meats was also significantly lower here and, although not significant, serum ferritin levels were lowest among this group. Positive food choices were also detected in the 'mainly healthy' dietary perception category where

significantly higher consumption of fortified cereals was recorded. This is likely to contribute to their significantly higher intakes of vitamin B_1, vitamin B_6 and fibre and to the fact that female volunteers in this group were significantly more folate and vitamin B_{12} replete. This positive association between the consumption of fortified food and improved folate status has been extensively recorded (Murphy *et al* 2000, Honein *et al* 2001) and suggests that food fortification represents the most effective option available to reduce the incidence of neural tube defects on a population basis. Public health campaigns to promote better cardiovascular health and lower BMIs in the population should also focus on the preservation of micronutrient intakes especially among vulnerable groups such as women of reproductive age. To this end, misconceptions about the fat content of nutrient dense staple foods such as meat need to be corrected.

Regular physical activity gives rise to substantial dose-dependant health enhancing effects including reduced risk of cardiovascular disease, stroke, hypertension, non-insulin dependant diabetes, overweight, obesity and osteoporosis (Vuori 2001). However, the accurate measurement of physical activity for research purposes is often considered problematic. Methods for assessing energy expenditure include behavioural observation, questionnaires, mechanical and electronic motion detectors and heart rate monitoring (Philippaerts *et al* 1999). In the last decade the doubly labelled water technique has become the 'gold standard' in the assessment of physical activity in field studies (Philippaerts *et al* 1999). Unfortunately this technique is very costly and time consuming and requires considerable commitment from subjects making it unsuitable for use in all research studies where an estimation of physical activity level is required. Various questionnaires have been developed and validated against the doubly labelled water technique (Philippaerts *et al* 1999) and are considered acceptable for use in research studies. The estimation of physical activity used in the current study although tested for reproducibility (see chapter 2) must be considered very crude. Although it is acknowledged that these self-reported physical activity levels are very subjective they can be considered at least reasonable markers of physical activity level due to their observed correlation with BMI in the study population.

It is well documented that lifestyles in industrialised countries today are considerably less energy demanding than in previous decades (Prentice & Jebb 1995, Vuori 2001). Experts have cited this increasingly sedentary lifestyle as the major contributory factor to the spiralling obesity levels that have been observed since the 1980s (Prentice & Jebb 1995, Vuori 2001). With increasing mechanisation occupational physical activity has

decreased (Prentice & Jebb 1995), this appears to be the case for the majority of individuals in developed countries. Therefore, leisure-time physical activity level is potentially the most potent predictor of BMI. In the current study female volunteers who perceived themselves to be 'mainly active' during leisure-time were significantly less likely to be obese. In contrast, those who perceived themselves as 'mainly sedentary' during leisure-time were significantly more likely to have a BMI in the obese range. The obese female volunteers in the current study also spent significantly longer watching television each week. The Irish Universities Nutrition Alliance (2001) also observed this inverse correlation between leisure-time physical activity level and obesity in Ireland. Obese respondents in the NSIFCS also spent a significantly greater portion of leisure time in television viewing and significantly less time in vigorous recreational activity than their normal weight counterparts (IUNA 2001). A Spanish study examining energy expenditure during leisure-time and BMI among one thousand adults found a significantly positive association between BMI and the number of hours spent sitting down during leisure-time each week (Martin-Almendros *et al* 2000). A recent American study examining television viewing, energy intakes and obesity in children found that television watching was positively associated with obesity independently of age, ethnicity, family income and energy intake (Crespo *et al* 2001). The importance of increasing leisure-time physical activity levels in the population for obesity prevention and improved general health cannot be over-emphasised. This is particularly important in light of recent evidence that low cardiorespiratory fitness is an independent predictor of all-cause and cardiovascular disease mortality in men in all BMI ranges (Lee *et al* 1999). Increasing physical activity is also likely to indirectly improve micronutrient status in the population by allowing the maintenance of acceptable BMIs without excessive dietary restriction. This advantage of regular physical activity should be communicated to women in the population as this group appears to be more likely to engage in dietary restraint rather than exercise in an attempt to achieve and maintain an acceptable body weight (Gilbody *et al* 1998).

Overall nutritional value was reported to be a priority when purchasing food among the majority of female volunteers in the current study. Higher socio-economic class women who had spent longer in formal education were significantly more likely to consider nutritional value when purchasing food for their households. Also female volunteers in the current study who perceived their diets to be 'mainly healthy' were significantly less likely to smoke, significantly more likely to have a normal BMI and had significantly

better nutritional knowledge. A substantial majority of the female volunteers in the current study did most of the food purchasing and preparation for their households. It is therefore possible that their positive nutritional and lifestyle behaviours will impact not only on their own health but also on that of their families. It is also possible that these women's dietary misconceptions together with their potentially negative health implications will be passed on to their families. For this reason women of reproductive age appear to be a particularly important population group to target in health education campaigns. The promotion of regular physical activity combined with moderate energy intakes would effectively maintain BMIs within the normal range while simultaneously preserving micronutrient intakes in this vulnerable group. The correction of dietary misconceptions, which appear to be widespread among women in developed countries should form an integral part of population based nutrition education campaigns.

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Chapter 5

Body image concerns and slimming practices of inner-city Dublin women.

5.1 Introduction

Despite the global increase in overweight and obesity prevalence (Prentice & Jebb 1995, Flegal *et al* 1998, IUNA 2001, McCarthy *et al* 2001) preoccupation with body image has never been greater, particularly among women. Currently in the United States it is estimated that 33-40% of adult women and 20-24% of adult men are attempting to lose weight (Serdula *et al* 1993, Lindeman 1998). A further 28% of the American population are thought to be trying to maintain weight (Serdula *et al* 1993, Lindeman 1998). Approximately two thirds of the adult population of the UK appear to be concerned about weight control (Wardle *et al* 2000). As would be expected more women (36%) than men (28%) exhibit body weight concern (Wardle *et al* 2000). Dissatisfaction with body weight or body shape or both is not confined to the overweight and obese individuals as studies report that normal weight subjects regularly attempt to lose weight (Willaimson *et al* 1992, Biener & Heaton 1995, Allaz *et al* 1999). It is reported that women may have unrealistic expectations of attainable body weight and shape associated with the Western emphasis on 'thinness' (Lindeman 1998). This weight and shape dissatisfaction and the associated preoccupation with slimming are well documented in adolescents and young women (Lindeman 1998). Recent research suggests that this phenomenon also affects middle-aged and older women in developed societies (Allaz *et al* 1999). In addition it is suggested that body image concerns commencing during adolescence may persist into adulthood (Ressler 1998, Allaz *et al* 1999).

Obesity greatly increases the risk of non insulin dependant diabetes, hypertension, stroke, coronary heart disease and some cancers (Solomon & Manson 1997). An intra-abdominal body fat distribution appears to be predictive of cardiovascular risk at any given level of adiposity (Lean *et al* 1995) and recent recommendations advise that these 'apple shaped' individuals be preferentially targeted for weight reduction advice. The scientific community does advise overweight and obese individuals to attempt to reduce their weight. However, depending on the characteristics of the person engaging in weight loss behaviour it can be potentially beneficial or harmful. Unnecessary dietary restriction may expose certain individuals, for example underweight or normal weight persons or adolescents, to health risks (Biener & Heaton 1995, Neumark-Sztainer *et al* 2000).

Lower intakes of macronutrients and micronutrients have been reported in weight reduction diets (Biener & Heaton 1995, Gendall *et al* 1997, Neumark-Sztainer *et al* 2000). The avoidance of staple foods, such as meat and dairy produce, is a common weight loss strategy among young females (Flynn 1997), and calcium and iron intakes have been found to be lower among adolescent girls trying to control weight (Neumark-Sztainer *et al* 2000). The avoidance of meat and dairy produce eliminates important sources of haem iron and calcium from the diet and therefore is potentially detrimental to haematinic nutrient status (Hallberg & Rossander Hulthen 1991) and bone health (Anderson 1999). Vegetarianism is increasingly being used as a possible means of weight control (Gilbody *et al* 1999, Martins *et al* 1999). High levels of eating restraint have been observed among women and adolescent girls following a vegetarian diet or avoiding red meat (Martins *et al* 1999). It has been suggested that these behaviours are markers for weight dissatisfaction and a tendency to diet (Martins *et al* 1999). Nutrient deficiencies and concomitant health complications are well documented in clinical eating disorders (Gendall *et al* 1997). However, complications and nutrient deficiencies may occur in those suffering from sub-clinical eating disorders not just in cases of anorexia and bulimia nervosa, which fulfil traditional diagnostic criteria (Whitehouse *et al* 1992, Biener & Heaton 1995).

Unhealthy weight control behaviours have been recorded in adolescent and adult females (Biener & Heaton 1995, Neumark-Sztainer *et al* 2000). It has been suggested that adolescents are more likely to employ extreme dieting strategies (Neumark-Sztainer *et al* 2000). Drugs, such as amphetamines, laxatives and diuretics, have been used by individuals attempting to lose weight (Forman *et al* 1989, Biener & Heaton 1995). Negative health effects associated with these drugs include electrolyte imbalances and cardiac arrythmias (Forman *et al* 1989, Biener & Heaton 1995, Neumark-Sztainer *et al* 2000). Another complication, which has been documented in normal weight and, to a lesser extent, overweight slimmers is impaired cognitive performance (Rogers & Green 1993, Bryan & Tiggemann 2001). Studies have suggested that attention span, reaction time and recall performance may be affected in women who are dieting (Rogers & Green 1993, Bryan & Tiggemann 2001). In addition frequent dieting and weight cycling may be associated with low self-esteem (Garner & Wooley 1991, Bryan & Tiggemann 2001), some loss of the ability to control

food intake (Bryan & Tiggemann 2001) and an increased risk of developing an eating disorder (Wilson 1993, Bryan & Tiggemann 2001).

Studies have suggested that weight concerns and dieting behaviours are positively related to adolescent smoking particularly among females (Tomeo *et al* 1999, King *et al* 2000). A study of reported slimming practices among Irish adolescent females found that 13% had commenced or continued to smoke in an attempt to control weight (Ryan *et al* 1998). The recent North/South Ireland Food Consumption Survey (NSIFCS) reported that 32% of Irish adult women are currently smoking and, although it is unclear whether it is connected with weight concern, 42% of women in the 18 to 35 year age category are smoking (IUNA 2001). Adolescent and adult women are susceptible to society's emphasis on slenderness and many believe that smoking is an effective weight control method (Tomeo *et al* 1999, King *et al* 2000). Cigarette smoking is associated with a lower body weight and smoking cessation is associated with weight gain (Flegal *et al* 1998, King *et al* 2000). Upon cessation of smoking women gain more weight on average than men (5kg versus 4.4kg) (Flegal *et al* 1998) and concern about weight appears to be important in why women start smoking and a key reason why so many do not even try to stop (Califano 1995, King *et al* 2000). Women who smoke are at a significantly higher risk of cardiovascular disease, stroke, cancer and osteoporosis (King *et al* 2000). Smoking has been described as the most important modifiable cause of poor pregnancy outcome (Pomerleau *et al* 2000). Although smoking cessation rates are higher among pregnant women than for the general population (Pomerleau *et al* 2000), female smokers with high cognitive eating restraint scores are more likely to continue to smoke during pregnancy or to relapse immediately post-partum (Pomerleau *et al* 2000). The upsurge in female smoking is potentially disastrous for women's health and the health of their offspring in future decades (Califano 1995, King *et al* 2000, Pomerleau *et al* 2000). It is imperative that public health campaigns promote healthy methods of weight maintenance to dispel the notion of tobacco use as a method of weight control (Tomeo *et al* 1999, Pomerleau *et al* 2000).

It is hypothesised that the media, family members and peers can intensify the cultural preoccupation with female thinness that is pervasive in developed societies (Byely *et al* 1999). An American study of 6770 girls and 5287 boys aged between nine and fourteen years found, over a one year follow-up period, that children who perceived their mother to be frequently trying to lose weight were more likely to become highly

concerned with weight (boys) or constant dieters (girls) (Field *et al* 2001). This suggests that mothers play an important role in the transmission of body image concerns. The phenomenon of maternal dieting predicting dieting behaviour among children has also been documented in a group of 197 five year old girls (Abramovitz & Birch 2000). Compared to girls whose mothers did not diet, girls whose mothers reported current or recent dieting were more than twice as likely to have ideas about dieting (Abramovitz & Birch 2000). These findings further underline the need to encourage women to use health promoting weight control behaviours, not only for their own well being but to reduce the likelihood of their offspring adopting unsafe slimming practices.

The emergence of girls' body image concerns and dieting behaviour is thought to often coincide with pubertal development (Byely *et al* 2000). The normative increases in body fat associated with puberty may be the impetus for concerns about body weight (Byely *et al* 2000). Dieting at this age may have an important effect on the long-term health of individuals. Physical growth is not yet complete and unsafe slimming practices at this time may be particularly hazardous (Grunewald 1985, Byely *et al* 2000). Reliable sources of information on weight loss may not be readily available to this age group (Grunewald 1985, Byely *et al* 2000). An American study of female college students found that the average age of dieters when they went on their first reducing diet was 16.2 years (Grunewald 1985). The 166 young women surveyed reported that their principal sources of health information during adolescence were magazines, newspapers, friends and relatives (Grunewald 1985). Ninety-one percent of this group who had commenced dieting during adolescence continued into adulthood, suggesting that body weight concerns and unsupervised attempts to lose weight are not confined to adolescents but are a feature throughout the female life-span (Grunewald 1985, Ressler 1998). Several authors have commented on the intractable nature of eating disorders, particularly anorexia nervosa (Ressler 1998).

Strict dieting and excessive exercising in an attempt to control weight has been found to be more common in higher socio-economic adolescents and adults (Wardle & Griffith 2001). Eating disorder prevalence has been shown to have a similar socio-economic gradient (Wardle & Griffith 2001). The prevalence of anorexia nervosa and bulimia nervosa among British women is thought to be between one and two percent (Whitehouse *et al* 1992). Hidden cases of eating disorders or partial syndromes are relatively common in general practice (Whitehouse *et al* 1992, Cooley & Toray 2001).

The prevalence of partial syndromes has consistently been found to be considerably greater than that of clinical eating disorders (Whitehouse *et al* 1992, Cooley & Toray 2001). Given the relatively high prevalence of sub-clinical eating disorders in community samples, and the aforementioned physical and psychological complications, there is likely to be considerable hidden morbidity among women consulting their general practitioner (Whitehouse *et al* 1992, Cooley & Toray 2001).

This study examined the self-perception of and satisfaction with body weight and shape among a group of women attending general practitioners in inner-city Dublin. Age of onset of body weight dissatisfaction was also investigated. Slimming practices employed by the women were also assessed. The effect of socio-economic class on all outcomes measured was also examined.

5.2 Subjects and Methods.

5.2.1 Subjects.

Twenty-four percent (n=32) of the female volunteers for this study were recruited from within the group of mothers of primary school children (n=35) who participated in the pilot study (see chapter 2). The remaining 76% (n=102) were those recruited from the group (n=104) who participated in the main investigation of haematinic status of adult women attending general practitioners in inner-city Dublin (see chapter 3). Ninety-six percent (n=134) of the total group of female volunteers (n=139) provided information on body image concerns and slimming practices. The female volunteers (n=134) were aged between 18 and 64 years and had no history of chronic disease, alcohol or drug abuse. Pregnant women were excluded as were women found to be anaemic over the previous twelve months. Two of the female volunteers excluded from the pilot test group gave different information at each interview and were therefore considered unreliable, one other woman was unwilling to answer questions on body image concerns and slimming practices. Two women were unable to participate in this section of the study due to time constraints. All participating female volunteers attended general practitioners, as private or public patients, in inner-city Dublin.

5.2.2 Body image concerns and slimming practices.

Data on body image concerns and slimming practices were collected using section seven of the standardised 'Interview Questionnaire' (Appendix II) which was validated

prior to use (see chapter 2). This section of the questionnaire consisted of nine multiple-choice questions, each question including a 'don't know' option, and was administered to each female volunteer by the main investigator.

Female volunteers were first asked to identify their self-perceived body shape as: apple shaped, pear shaped or other. A standardised drawing, illustrating the different body shapes, was used to enable the female volunteers to identify their self-perceived body shape. Satisfaction with self-perceived body shape as assessed in Irish teenage girls (Ryan *et al* 1998) was then ascertained.

The next multiple-choice questions asked female volunteers to identify their self-perceived body weight as: very underweight, slightly underweight, normal weight, slightly overweight or very overweight. Current and lifelong satisfaction with body weight was then determined. Among female volunteers who reported weight dissatisfaction, the age of onset of this dissatisfaction was recorded.

Female volunteers were then asked if they had ever tried to lose weight and the remaining multiple-choice questions examined slimming practices used by those who had previously tried to lose weight. From a list of commonly used slimming practices (Ryan *et al* 1998) female volunteers were asked to identify those which they had used over the previous two years in an attempt to lose weight. They were also asked to identify slimming practices, which they had not used recently (over the previous two years) but had used in the past to lose weight. Female volunteers were also invited to describe slimming practices that they had used which were not listed on the questionnaire. The final question asked female volunteers to choose food groups (bread, breakfast cereal and potatoes; red meat; crisps and confectionery; chicken and fish: alcohol), which they would cut down on or avoid if on a weight reduction diet.

5.2.3 Anthropometry.

Refer to chapter 3.

5.2.4 Socio-economic class.

Refer to chapter 3.

5.2.5 Data management and statistical analysis.

All information was coded for entry into the Statistical Package for Social Sciences (SPSS) version 9 for Windows. Data were analysed using the Chi-square test and significance was read at $p< 0.05$.

5.3 Results

The female volunteers in each socio-economic class were comparable in terms of age, body mass index and waist/hip ratio (Table 1). Of the 134 female volunteers surveyed 22.4% (n=30) were found to be overweight and 13.4% (n=18) were in the obese body mass index range (Table 2). Although not significant, a trend towards a higher rate of overweight and obesity was observed in the lower socio-economic classes (Table 2).

A highly significant disparity existed between actual and self-perceived body weight with female volunteers in each socio-economic class perceiving themselves as heavier than they actually were (Table 3). Of those who had an inaccurate self-perception of body weight 86% (n=18), 83% (n=10) and 76% (n=19) in socio-economic classes one, two and three respectively overestimated their body weight.

Overall a high level of dissatisfaction with body weight was found among the group of women surveyed. Of the 134 female volunteers 82% (n=110) expressed dissatisfaction with their self-perceived body weight. From actual body weight measurements it was found that a desire to be lighter was not exclusive to the overweight and obese individuals. Of the dissatisfied underweight female volunteers over one-third (n=3) wanted to be lighter. Of the dissatisfied normal weight female volunteers 91% (n=51) expressed a desire to be lighter.

Table 4 reports the age of onset of dissatisfaction with body weight among women in each socio-economic class. The majority of female volunteers in each socio-economic class reported becoming dissatisfied with their body weight during adolescence. Significantly more subjects in socio-economic class one reported never experiencing body weight dissatisfaction.

Over four-fifths (n=110) of the female volunteers had previously tried to lose weight and their reported slimming practices are summarised in Table 5. When asked about specific food groups 99% (n=132) of the total sample said that they would avoid confectionery and savoury snacks if they were trying to lose weight, 57% (n=76) would avoid cereal foods, 52% (n=69) would avoid alcohol, 43% (n=58) would avoid dairy products and 37% (n=49) would avoid red meat.

The self-perception of body shape was more accurate than that of body weight among the female volunteers in each socio-economic class with 98% (n=52) of those with an abdominal body fat distribution or 'apple' shape correctly identifying this. Ninety-six percent (n=78) of the female volunteers with a gluteal-femoral body fat distribution or 'pear' shape recognised this. Almost two thirds (n=78) of the female volunteers were satisfied with their self-perceived body shape.

Table 1: Age and anthropometric measurements of female volunteers in each socio-economic class (SEC).

	Total	SEC 1	SEC2	SEC3	Significance
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	
% (*n*)	100 (134)	42.5 (57)	20.1 (27)	37.4 (50)	ns
Age (years)	34.7 ± 10.7	34.5 ± 10.5	32.3 ± 10.5	36.3 ± 11.0	ns
BMI (kg/m^2)	24.7 ± 5.2	23.8 ± 4.8	24.9 ± 5.6	25.7 ± 5.2	ns
WHR	0.80 ± 0.06	0.80 ± 0.07	0.79 ± 0.06	0.80 ± 0.06	ns

SD = standard deviation. BMI = body mass index. WHR = waist/hip ratio. ns = not significant

Table 2: Body weight status of Irish adult women (n=134) attending general practitioners in inner city Dublin.

	Total	SEC 1	SEC 2	SEC 3	Significance
	% (n)	% (n)	% (n)	% (n)	
BMI<19 kg/m^2					
Underweight	6.7 (9)	7.0 (4)	7.4 (2)	6.0 (3)	ns
BMI 19-24.9kg/m^2					
Normal weight	57.5 (77)	68.4 (39)	51.9 (14)	48.0 (24)	ns
BMI 25-29.9kg/m^2					
Overweight	22.4 (30)	15.8 (9)	25.9 (7)	28.0 (14)	ns
BMI ≥ 30 kg/m^2					
Obese	13.4 (18)	8.8 (5)	14.8 (4)	18.0 (9)	ns

ns = not significant

Table 3: Actual versus self-perceived body weight of the female volunteers (n = 134).

	Actual weight	Self-perceived weight	Significance
	% (n)	% (n)	
Underweight	6.7 (9)	9.7 (13)	ns
Normal weight	57.5 (77)	29.9 (40)	**
Overweight	22.4 (30)	44.0 (59)	**
Obese	13.4 (18)	16.4 (22)	ns

** = statistically highly significant difference ($p<0.001$). ns = not significant

Table 4: Age of onset of dissatisfaction with body weight among female volunteers in each socio-economic class (SEC).

	Total	SEC 1	SEC 2	SEC 3	Significance
Age Range	% (*n*)	% (*n*)	% (*n*)	% (*n*)	
0-10 yrs	4.5 (6)	1.7 (1)	3.7 (1)	8.0 (4)	ns
10-20 yrs	38.8 (52)	35.1 (20)	55.6 (15)	34.0 (17)	ns
20-30 yrs	14.2 (19)	12.3 (7)	14.8 (4)	16.0 (8)	ns
30-40 yrs	15.7 (21)	15.8 (9)	7.4 (2)	20.0 (10)	ns
40-50 yrs	7.5 (10)	7.0 (4)	7.4 (2)	8.0 (4)	ns
> 50 yrs	1.5 (2)	-	-	4.0 (2)	ns
Never	17.9 (24)	28.1 (16)	11.1 (3)	10.0 (5)	*

* = statistically significant difference (p=0.04). ns = not significant. yrs = years

Table 5: Reported slimming practices of the female volunteers in each socio-economic class (SEC) who had previously attempted to lose weight (n = 110).

	Total	SEC 1	SEC 2	SEC 3	Significance
Slimming Practice	% (n)	% (n)	% (n)	% (n)	
Dieting	75 (83)	80 (33)	77 (21)	64 (29)	ns
Skipping meals	32 (35)	37 (15)	21 (5)	33 (15)	ns
Diet pills	15 (16)	9.8 (4)	25 (6)	13.3 (6)	ns
Laxatives	2.7 (3)	-	8.3 (2)	2.2 (1)	ns
Vomiting	12 (13)	7.3 (3)	20.8 (5)	11.1 (5)	ns
Smoking	30 (33)	19.5 (8)	33 (8)	38 (17)	ns
Exercise	76 (84)	85 (35)	75 (18)	69 (31)	ns

ns = not significant.

5.4 Discussion.

The significant discrepancy found between actual and self-perceived body weight may partly explain the high level of dissatisfaction with body weight and attempts to slim reported by the underweight and normal weight female volunteers. This inaccuracy of self-reported weight has been documented in female patients with eating disorders (McCabe *et al* 2000). Patients with anorexia nervosa have been found to overestimate weight and bulimic patients have a tendency to under-report weight (McCabe *et al* 2000). Subjects exhibiting disordered eating patterns which do not fulfil the diagnostic criteria for clinical eating disorders have also been shown to inaccurately self-report their body weight (McCabe *et al* 2000). Different investigators have shown a greater tendency to under-report (Cash *et al* 1992) or over-report (Klesges *et al* 1991) weight with increasing levels of dietary restraint.

The high level of dissatisfaction with body weight observed in normal weight and even underweight female volunteers in the current study has been documented in other investigations involving women in developed countries. A recently published study of 225 American, female, first-year college students found an average BMI of 22.9kg/m^2 in the group (Cooley & Toray 2001). However, when asked what they would ideally like to weigh 94% of the women chose a weight less than their current weight (Cooley & Toray 2001). The perception of being too heavy, not the actual weight level, was found to be the greatest predictor of disordered eating symptoms (Cooley & Toray 2001). Therefore, the need to diet or achieve a smaller body size is apparently motivated by factors other than actual obesity. In developed societies girls and women are saturated with images of thinness (Califano 1995, Lindeman 1998). The desire for an unrealistically slim appearance has been promoted widely by the media and weight loss and fashion industries (Biener & Heaton 1995, Lindeman 1998). Detrimental effects of the pressure to conform to this ideal of female beauty on women's physical and psychological health have been documented (Biener & Heaton 1995, Lindeman 1998). It is noteworthy that many normal weight dieters cite health as their primary motivation (Biener & Heaton 1995). Health reasons and a desire to improve physical fitness are reported motives for attempting weight loss among higher socio-economic groups in particular (Wardle *et al* 2000, Wardle & Griffith 2001). The widespread body weight dissatisfaction observed in the current study and many others would suggest that the definition of normal weight appears to have become distorted. What is

considered normal weight for good health is not necessarily what is considered aesthetically pleasing.

Onset of dissatisfaction with body weight in females commonly occurs during adolescence. This was found to be the case among the majority of female volunteers surveyed. Studies indicate that up to 70% of adolescent girls have attempted to lose weight (Flynn 1997). Unsupervised attempts to lose weight are common among adolescent girls (Flynn 1997, Neumark-Sztainer *et al* 2000, Wardle & Griffith 2001). The use of unsafe slimming practices is pervasive in this group (Flynn 1997, Neumark-Sztainer *et al* 2000, Wardle & Griffith 2001). Almost one third (30.4%) of a group of 130 American adolescent females, aged between twelve and eighteen years, had used unsafe practices, such as taking diet pills, laxatives or diuretics, self-induced vomiting, skipping meals or fasting, in an attempt to lose weight (Neumark-Sztainer *et al* 2000). Among these adolescent girls the use of extreme weight control behaviours was found to increase with household income (Neumark-Sztainer *et al* 2000). The tendency for higher socio-economic class individuals, particularly females, to monitor weight closely appears to continue into adulthood (Wardle & Griffith 2001). However, the use of unsafe slimming practices may reverse its socio-economic gradient with increasing age (Wardle & Griffith 2001). Higher socio-economic class British men and women are more likely to use healthy eating and regular vigorous physical activity to control weight compared with those from lower socio-economic classes (Wardle & Griffith 2001). The smaller proportion of the higher socio-economic class women in the current study who reported using unsafe slimming practices supports findings that higher socio-economic class individuals may be more aware of and benefit more from public health recommendations (Roos *et al* 1996). Although use of extreme weight control behaviours appears to be less pervasive among higher socio-economic class adults these individuals do appear to be more concerned with weight and have a lower threshold for defining themselves as overweight compared with their lower socio-economic class counterparts (Wardle & Griffith 2001). Greater body weight concern and more deliberate attempts to control weight have been suggested as a possible explanation of the lower incidence of overweight and obesity among higher socio-economic classes observed in the current study and several others (Wardle *et al* 2000, Wardle & Griffith 2001).

It is thought that body weight dissatisfaction arising in adolescence may persist into adulthood (Grunewald 1985, Biener & Heaton 1995, Neumark-Sztainer *et al* 2000,

Cooley & Toray 2001). The current study supports this hypothesis, as all the female volunteers who were dissatisfied with their body weight as adolescents remained so as adults. Although there is little information available on body weight dissatisfaction among middle-aged or elderly women a recently published review suggests that body image concerns among women are independent of age (Allaz *et al* 1999). A study of the desired weights and dieting behaviour in a randomised sample of 1053 Swiss women aged 30 to 70 years found that 71% of the sample expressed a desire to be lighter and that 42% were dicting even though 73% were of normal weight (Allaz *et al* 1998, Allaz *et al* 1999). A smaller study in the United Kingdom compared 50 normal weight young women with 50 normal weight women over sixty years age. The average desired level of weight loss was comparable in both groups and approximately half of the elderly women were chronic dieters (Hetherington & Burnett 1994, Allaz *et al* 1999). Body image concern and a tendency to diet persisting into adulthood and old age may have deleterious consequences. Poor nutrition represents a significant health hazard in the elderly and can negatively impact on quality of life and life expectancy (Allaz *et al* 1999). The risk of malnutrition in the elderly is likely to be increased by voluntary dietary restraint (Allaz *et al* 1999). Longitudinal studies are warranted to examine the phenomenon of dieting across the female life-span. The current study highlights the need for reliable education on weight loss during adolescence because many women begin lifelong dieting behaviour at this time.

The relatively large number of the women surveyed who reported using smoking as a slimming strategy is of concern. The upsurge in female smoking has potentially serious implications for the future health of women and their offspring (Califano 1995, Pomerleau et al 2000). Smoking among women as a means of weight control is associated with an increased likelihood of smoking during pregnancy and immediately post-partum (Pomerleau *et al* 2000). Death rates from lung cancer among female smokers increased 500 percent from the early 1960s to the mid 1980s in the U.S. (Califano 1995). Between the ages of 30 and 49 years the risk of myocardial infarction in smokers is five times that in non-smokers (Parish et al 1995). The prevalence of smoking among American high-school students increased from 27.5% in 1991 to 36.4% in 1997 (Tomeo *et al* 1999). It has been reported that at least 75% of adult smokers are addicted to nicotine before the age of twenty-one and that around 90% try, most unsuccessfully, to stop smoking (Califano 1995). Given the intractable nature of the addiction and the rising prevalence of obesity among adolescents, strategies to

educate adolescents on body image and healthy weight loss practices must be developed to counteract both obesity and tobacco use (Tomeo et al 1999). Careful monitoring of weight during pregnancy and the promotion of safe, gradual weight loss after delivery may reduce the risk of smoking among women who are concerned about excessive weight gain associated with pregnancy.

The appreciable use of slimming practices such as skipping meals, inducing vomiting and taking laxatives or diet pills highlights the need to assess the prevalence of sub-clinical eating disorders in Ireland. Several studies comment on the high rate of sub-clinical eating disorders in general practice (Whitehouse *et al* 1992). These conditions do not fulfil the normal diagnostic criteria, but patients engage in extreme weight loss practices and present a chaotic eating pattern (Whitehouse *et al* 1992). The number of the female volunteers who reported that they would avoid staple foods, such as meat and dairy produce, if on a weight reducing diet is of concern due to the high rate of dieting observed in this group. Meat avoidance places individuals at risk of iron deficiency (Hallberg & Rossander Hulthen 1991). This is compounded in women due to the demands of menstruation, pregnancy, lactation and the menopause (Hallberg & Rossander Hulthen 1991). Hip fracture incidence rates secondary to osteoporosis are predicted to increase dramatically world-wide in the first half of the 21st century (Anderson 1999). The major risk factors are a genetic predisposition, inadequate calcium intake, limited physical activity and low lifelong oestrogen exposure (Anderson 1999). Body weight concerns leading to the avoidance of dairy produce, the major source of bio-available calcium in the western diet, may act to exacerbate the increasing prevalence of osteoporosis in women.

The relatively high level of body weight dissatisfaction and use of unsafe slimming practices observed in the current group of Irish women of child-bearing age may have implications for their offspring. There is considerable evidence to suggest that mothers may transmit their own weight concerns to their children, particularly their daughters. Researchers have reported higher levels of body weight dissatisfaction, body image distortion and dietary restraint among children whose mothers exhibit these characteristics compared to children whose mothers are less concerned with weight and dieting (Byely *et al* 2000, Abramovitz & Birch 2000, Field *et al* 2001). Recognising this maternal influence on the development of unhealthy weights and weight control practices is important and has implications for public health policy. Targeting all

women of child-bearing age with safe weight loss and maintenance advice is likely to have an impact on the weight loss practices of future generations. In addition, educating women about normal healthy weight ranges may help to reduce the desire to achieve an unrealistically thin body size and may prevent the perpetuation of this desire among their daughters.

The rate of overweight among female volunteers in the current study is lower than that observed in the Irish adult female population (IUNA 2001, McCarthy *et al* 2001) however the rate of obesity is comparable to that seen in adult females in Ireland (IUNA 2001, McCarthy *et al* 2001). The trend towards a higher rate of overweight and obesity in the lower socio-economic classes observed in this study has been described by several authors (Ford *et al* 1994), (Roos *et al* 1996). Studies describe a higher prevalence of mortality from all causes including cardiovascular disease in the lower socio-economic classes (Ford *et al* 1994). Disability and chronic ill health appear to be associated with poor school achievement (Ford *et al* 1994). Higher socio-economic classes have been found to consume more of the foods recommended for good health such as fruit and vegetables and whole-grain cereals (Roos *et al* 1996). A higher socio-economic status has been associated with a lower dietary fat intake (Roos *et al* 1996). Greater knowledge of current dietary recommendations and greater emphasis on diet for health may partially explain the greater rate of normal weight observed among the women of higher socio-economic class in this study.

This study confirms that body image concern and dieting are not confined to adolescent females in developed countries but may persist into adulthood and even old age. In fact women of all ages appear to experience a degree of weight dissatisfaction and are likely to try to slim. Effective and safe weight control strategies, based on healthy eating and regular exercise, are required to keep BMIs in the population within the normal range and to reduce the use of unsafe slimming practices. Future health promotion campaigns to prevent obesity should target groups at risk of receiving misinformation, such as adolescents, and should aim to dispel the notion that smoking and the avoidance of staple foods are effective weight loss strategies.

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APPENDIX I

Food Intake Questionnaire

Food Intake Questionnaire.

Volunteer identification:_____ **Date:**_____

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/mooth	Rarely /Never	Indicate the following:	Foodbase Code	Weight
1	Fruit Juice -Breakfast	1 small glass		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Sweetened/ unsweetened		
	-Light meal	1 small glass		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Sweetened/ unsweetened		
	- Main meal	1 small glass		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Sweetened/ unsweetened		
	- snacks	1 small glass		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Sweetened/ unsweetened		
2	Cornflakes, Special K, Rice Krispies,Puffed Wheat, -Breakfast	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Brands		
	- snacks	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Brands		
3	Weetabix, Shredded wheat,All Bran,Bran Buds, Sultana Bran, -Breakfast	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Brands		
	-snacks	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Brands		
4	Muesli -Breakfast	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	+/- Sugar +/ Fruit/ Nuts Brands		
	-snacks	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	+/- Sugar +/ Fruit/ Nuts Brands		
5	Porridge - Breakfast	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/mooth	Rarely /Never	Made on water / milk/ cream.		
	-snacks	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/mooth	Rarely /Never	Made on water / milk/ cream.		
6	Corn Pops, Coco Pops, Sugar Puffs, Frosties . -Breakfast	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Brands		
	-snacks	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Brands		
7	Sugar on cereal	1 dessertsp.														
8	Milk on cereal	1 cup												Do you drink all of the milk? Yes / No		
9	What type of milk do you take on your cereal ? (e.g. low fat, full fat , Super milk, soya milk, goats milk etc.)															

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase Code	Weight
10	Rasher -Breakfast	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Streaky/ Back Grilled/ Fried		
	-Light meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Streaky/ Back Grilled/ Fried		
	- Main meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Streaky/ Back Grilled/ Fried		
	-snacks	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Streaky/ Back Grilled/ Fried		
11	Sausage - Breakfast	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Grilled/ Fried		
	-Light meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Grilled/ Fried		
	-Main meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Grilled/ Fried		
	-Snacks	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Grilled/ Fried		
12	Black Pudding/ White Pudding	1 slice (30g)		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Grilled/ Fried		
13	Egg - Breakfast	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Boiled / Poached / Fried / Scrambled / Omelette		
	-Light meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Boiled / Poached / Fried / Scrambled / Omelette		
	-Main meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Boiled / Poached / Fried / Scrambled / Omelette		
	-Snacks	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Boiled / Poached / Fried / Scrambled / Omelette		
14	Bread Breakfast	1 slice		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Wholemeal / White Brand Home Sliced/Pre-sliced + / - Butter/ Spread, + / - preserve		
	-Light meal	1 slice		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Wholemeal / White Brand Home Sliced/Pre-sliced + / - Butter/ Spread, + / - preserve		
	-Main meal	1 slice		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Wholemeal / White Brand Home Sliced/Pre-sliced + / - Butter/ Spread, + / - preserve		
	-Snacks	1 slice		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Wholemeal / White Brand Home Sliced/Pre-sliced		

														+ / - Butter/ Spread, + / - preserve		
16	**Bread Rolls/ French Stick others..... e.g. Pitta bread, chapatis etc.**	**S, M, L Roll**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**Wholemeal / White Brand Home Sliced/Pre-sliced + / - Butter/ Spread, + / - preserve Type of roll Is this instead of , or as well as bread already mentioned?**		

17 **Do you spread your butter / Spread thickly_____? or thioly_____?**

What type of butter/ spread do you use on the following?

	Brand	Foodbase Code	Weight
Bread			
Toast			
Sandwiches			

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase Code	Weight
18	**Yoghurt**	**1 carton**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**Brand LF / FF / Diet**		
19	**Tea Infusion** -Breakfast	**1 Mug**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**+/- Milk +/- Sugar**		
	-Light meal	**1 Mug**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**+/- Milk +/- Sugar**		
	-Main meal	**1 Mug**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**+/- Milk +/- Sugar**		
	-Snacks Morning Afternoon Bedtime	**1 Mug**		7 7 7	6 6 6	5 5 5	4 4 4	3 3 3	2 2 2	1 1 1	1/14 1/14 1/14	1/month 1/month 1/month	Rarely /Never	**+/- Milk +/- Sugar**		
20	**Coffee Infusion** -Breakfast	**1 Mug**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**+/- Milk +/- Sugar**		
	-Light meal	**1 Mug**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**+/- Milk +/- Sugar**		
	-Main meal	**1 Mug**		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	**+/- Milk +/- Sugar**		

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase Code	Weight
	-Snacks -Morning -Afternoon -Bedtime	1 Mug		7 7 7	6 6 6	5 5 5	4 4 4	3 3 3	2 2 2	1 1 1	1/14 1/14 1/14	1/month 1/month 1/month	Rarely /Never	+/- Milk +/- Sugar		
21	Milk	1 glass		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
22	Hot choc, Cappuccino etc.	1 Mug		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
23	Milk on tea/coffee	1 Ultra Pack														
24	Sugar on tea/ coffee	1 teasp.														

25 What type of milk do you usually use for the following?

	Type
a) Tea	
b) Coffee	
c) Milk on its own or milky drinks	

If you eat these foods more than once per day, increase your portion size as necessary.

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase codes	Weight
26	Soup	S,M,L bowl		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade/retail/Type		
27	Shellfish , Type	Indicate no.		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Cooking Method /Sauce added		
28	Carrots,Parsnips, swedes, turnips, Beetroot other root veg.	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	+ / - Butter Cooking method		
29	Cabbage, broccoli, spinach, brussel sprouts.	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	+ / - Butter Cooking method		
30	Peas, butter beans, kidney beans, lentils	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	+ / - Butter Cooking method		
31	Baked Beans	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			

32 How much butter / Spread do you put on your vegetables? Number of pats? _____

Which type of butter/ spread do you use on your vegetables?

Brand	Foodbase Code	Weight
_____	_____	_____

Which type of fat do you use in the preparation of vegetables (e.g for stir fries, or for frying vegetables)?

Brand	Foodbase Code	Weight
_____	_____	_____

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase codes	Weight
33	Salad e.g. lettuce , scallions, cucumber, tomato. peppers.			7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
34	Salad Dressings, Type.	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Type of sauce Low fat? / Full Fat?		
35	Mayonnaise	1 tablespoons		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Type of sauce Low fat? / Full Fat?		
36	Coleslaw, Potato salad etc.	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
37	Potato chipped/ Fried -Light meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade / Retail /		
	-Main meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade / Retail /		
	-Snacks	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade / Retail /		
38	Potatoes Roast -Light meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade / Retail /		
	-Main meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade / Retail /		
39	Potatoes boiled/baked/mashed -Light meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	+ / - Fat added		
	-Main meal	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	+ / - Fat added		
	Others.....			7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			

40 How much butter / Spread do you put on potatnes? Number of pats? _____
Which type of butter/ spread do you use on your potatoes?

Brand	Foodbase Code	Weight

Which type of fat do you use in the preparation of chips, roast potatnes or fried potatoes?

Brand	Foodbase Code	Weight

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase codes	Weight
41	Pasta -Light meal	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	White/wholegrain		
	-Main meal	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	White/wholegrain		
42	Rice -Light meal	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	White/wholegrain		
	-Main meal	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	White/wholegrain		
43	Beef, fried/roast /grilled	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Stewed / Baked / Grilled/ Fried / Roast		
44	Bacon/Ham	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Stewed / Baked / Grilled/ Fried / Roast		
45	Pork	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Stewed / Baked / Grilled/ Fried / Roast		
46	Lamb	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Stewed / Baked / Grilled/ Fried / Roast		
47	Chicken/turkey, Breast ? Leg?	S, M L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Stewed / Baked / Grilled/ Fried / Roast		
48	Liver/Kidney/Heart	S, M, L portion		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Stewed / Baked / Grilled/ Fried / Roast		
49	Gravy	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Granules? + / - Juices		

50 Which type of fat do you use for frying meat , chicken , fish?

Brand	Foodbase Code	Weight

Are you in the habit of eating or not eating the visible fat on meat? Yes / No ?
Ham? Yes / No ?
Chicken? Yes / No ?

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase codes	Weight
51	White fish (cod, haddock, plaice, hake)	1 S,M,L Fillet		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	breadcrumbed/ Battered Fresh		
52	Kippers, herring, pilchards, salmon, mackerel	1 S,M,L Fillet		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	breadcrumbed/ Battered Fresh		
53	Tinned fish	Small tin		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Tinned in Oil, Brine or Sauce		
54	Fish Fingers	Indicate No.		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Fried/ Grilled?		
55	White/ Cheese/ Parsley Sauce	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade/ Retail? + / - milk + / - Fat		
56	Stuffing, Type	1 tablespoon		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Homemade/ Retail? Type of fat used?		
57	Beef burgers	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Fried/ Grilled Homemade/ Retail?		
58	Meat pies/ Pasties/Tinned meats	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
59	Sausage Rolls	Small or large?		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
60	Pizza, Type			7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Thin base/ Deep pan?		

61
How many mixed dishes do you eat per week?_____
(e.g. Bolognaise, Lasagne, Stews, Curries, Chinese dishes etc.)

Type	Foodbase Code	Weight
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Note: Meat, vegetables and pasta / Rice already accounted for. Only include those sauces which are eaten e.g. Dolmio, Sweet 'n Sour sauce, curry paste/ sauce/ powder?

62
How many take-away foods do you eat per week ? e.g burgers, Battered sausages , Big mac, Quarter Pounders , Spice burgers etc ? _____

Type	Foodbase Code	Weight
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

	How often do you usually eat the following foods at each meal mentioned.	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase codes	Weight
63	**Full fat cheeses** (cheddar, Leicester, stilton, Brie and soft cheeses)	Matchbox size		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
64	Easi-Singles, Galtee, Calvita	1 Slice or 1 Triangle		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
65	**Low fat cheeses** (Reduced fat cheddar, reduced fat soft cheeses, Edam Blarney)	Matchbox size		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			

66
How often do you have a dessert per week?_____
(e.g. Apple Tart, Cheesecake, Jelly, Milk Puddings, Gateaux etc)

Type	Foodbase Code	Weight
_____ **(+/- Cream, Icecream, Custard, Topping)**	_____	_____
_____ **(+/- Cream, Icecream, Custard, Topping)**	_____	_____
_____ **(+/- Cream, Icecream, Custard, Topping)**	_____	_____
_____ **(+/- Cream, Icecream, Custard, Topping)**	_____	_____
_____ **(+/- Cream, Icecream, Custard, Topping)**	_____	_____
_____ **(+/- Cream, Icecream, Custard, Topping)**	_____	_____
_____ **(+/- Cream, Icecream, Custard, Topping)**	_____	_____

	How often do you eat the following foods?	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase codes	Weight
67	**Icecream, on its own (e.g choc ice,magnum etc)**	1 average ice cream		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			

Cut Cake (e.g Madeira, Fruit, Sponge, Ginger etc.):

68 How many slices of cake do you eat per day?_____ or per week?_____

Type	Brand	Foodbase Code	Weight

Individual Cakes & Pastries:

69 How many individual cakes do you eat per week?_____
(e.g. Scones , doughnuts, cream cakes, apple pies, jam tarts etc)

Type Scones (+ / - Butter, = / - Jam)_	Brand	Foodbase Code	Weight

Sweet Biscuits:

70 How many biscuits do you eat per day?_____ or per week?_____

What types do you eat e.g. Chocolate , Sandwich, Plain, Hob Nob Type etc.

Type	Brand	Foodbase Code	Weight
_____(+/- Butter)			
_____(+/- Butter)			
_____(+/- Butter)			
_____(+/- Butter)			
_____(+/- Butter)			

Crackers & Savoury Biscuits:

71 How many crackers do you eat per day?_____

Type	Brand	Foodbase Code	Weight
_____(+/- Butter)			
_____(+/- Butter)			
_____(+/- Butter)			
_____(+/- Butter)			
_____(+/- Butter)			

	How often do you consume the following foods?	Standard portion	Your Portion	7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	Indicate the following:	Foodbase codes	Weight
72	Beer	1 pint		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never	type		
73	Wine	1 glass		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
74	Spirits	1 Measure		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
75	Crisps:	1 average size pkt.(28g)		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
76	Peanuts Salted/Dry Roasted	1 Pkt.		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
77	apple	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
78	Orange/ Grapefruit/Mandarins/ Clementines	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
79	Banana	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
80	Pears, peaches , plums	1 average		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
81	Tinned Fruit, Stewed fruit	1 tlbsp.		7	6	5	4	3	2	1	1/14	1/mooth	Rarely /Never	+/- Cream, Icecream, Custard, Topping		
Sweets & Confectionery																
82	Club Milk/ Snack Bar/ Kitkat	1		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
83	Mars Bar/ Twix/ Snickers	1		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
84	Dairy Milk Chocolate/ Wispa/ Aero/ Buttons/ Choc Orange	1		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
85	Fruit Gums / Pastilles/ mints/Wine Gums/ Boiled Sweets/marsh mallows/ snowballs	1 Pkt.		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
86	Toffees/ Caramels	1 Pkt.		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
87	Minerals:	1 can		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			
88	Fruit Drinks:	1carton		7	6	5	4	3	2	1	1/14	1/month	Rarely /Never			

APPENDIX II

'Interview Questionnaire'

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Anaemia in Irish Women

⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘⌘

⌘⌘⌘ Questionnaire ⌘⌘⌘

Date of interview:

Study ID: [ ]

Section 1: Demographic and Household Details.

These first questions are about who lives with you in your home, your job and your opinion on your diet. This information is very important for the study. Thank you for agreeing to answer these quite personal questions and before we begin I would like to tell you again that all the information you give me is strictly confidential and it will not be seen by anybody outside the research group. Ethical approval was given for this study by the Irish College of General Practitioners and one of the conditions that they set out is complete confidentiality so you can be sure that all the information that I collect will only be used for this study of anaemia in Irish women.

(a) Do you have a medical card? 1. Yes 2. No 3. Don't know

Patient type:

Do you have health insurance such as VHI or Bupa?
1. Yes 2. No 3. Don't know

GMS=1
Private=2
Neither=3

(b) What age were you on your last birthday? [ ]

(C) What is your date of birth?

Age in years: [ ]

Next I need to find out who lives with you in your home.

(d) How many people are in your household?

Household no. [ ]

(e) Can you describe the people in your household; for example yourself and your husband or you may live with your parents and brothers and sisters or you may live alone.

This information is important because the people in your house may affect the foods you buy and the way that you cook them.

(f) What is your occupation?

(g) What is your husband's/partner's/parent's occupation?

(If unemployed state previous occupation)
(If never employed state occupation of supporting family member)
(O'Hare 1983)

S.E.C. ☐
1=1+2
2=2+3
3=4+5

(h) Do you do the the shopping for the household?

1. Yes
2. No
3. Share

Subject shopping? ☐

(I) Do you do the cooking for the household?

1. Yes
2. No
3. Share

Subject cooking? ☐

(j) Which of the following things do you think about when buying food for your household? (You can chose more than one)

Yes=1, 2=No, 3=Don't know

1. The likes and dislikes of your family — **Likes + dislikes** ☐
2. The cost of the food — **Cost of food** ☐
3. Whether the food is healthy — **Healthy food** ☐
4. Whether the food is quick and easy to prepare — **Quick + easy** ☐
5. Whether the food is available in your local shop — **Available locally** ☐
6. The equipment, for example a freezer or a grill that you have at home — **Equipment** ☐
7. Other (please state) — **Other** ☐

(k) Here I am going to ask you what you think of your own diet. Do you think that it is:

1. Mainly healthy
2. Both healthy and unhealthy
3. Mainly unhealthy
4. Don't know

Perception of diet ☐

Section 2: Physical Activity.

The next section covers exercise.

(a) In your job would you describe yourself as:

1. Mainly active
2. Both active and sedentary
3. Mainly sedentary
4. Don't know

Perception of Occupational activity ☐

(b) In your free time would you describe yourself as:

1. Mainly active
2. Both active and sedentary
3. Mainly sedentary
4. Don't know

Perception of Leisure activity ☐

(C) How many hours do you spend watching television each day?

Hrs. T.V. per week ☐

Section 3: Education

Next I need to find out some details about your education.

Can you tell me:

(a) What age were you when you started school?

(b) What age were you when you left school?

(C) When you left school did you do any other full-time or part-time course, for example a FAS course, a secretarial course, a cert, a diploma, a degree or a postgraduate qualification?

1. Yes
2. No
3. Don't know

*** If 'no' go to 4(a)**

(d) How long did you spend doing the course after school?

Years in education: ☐

Section 4: Smoking, Supplement use and Medication.

Here I am going to ask you about cigarette smoking and any vitamin and mineral tablets that you may take. I also need to know about the medicines that you take because they may affect the iron in your blood.

*(a) Are you: 1. A current smoker
2. An ex-smoker (we can only call you an ex-smoker if you gave up cigarettes > 6 months ago
or 3. Have you never smoked

Smoking status: ☐

*** If the subject has never smoked go to 4(e)**

(b) What age were you when you started smoking?

(C) If you stopped smoking when did you stop? (Please tell me here if you stopped smoking at any time in your life and then started again)

Yrs stopped smoking ☐

Total yrs. spent smoking: ☐

(d) How many cigarettes do you/did you smoke per day?

Average no. smoked per day: ☐

* (e) Have you taken a tonic or vitamin or mineral tablets over the last year?

1. Yes
2. No
3. Don't know

Supplement use over last year ☐

*** If 'no' go to 4(k)**

(f) Can you tell me the name of it?

(g) When did you start taking it?

(h) Are you still taking it?

1. Yes
2. No
3. Don't know

Current supplement use: ☐

(l) If you have stopped taking it, when did you stop?

Duration of use: ____
Time since stopping supplement: ____

(j) How often do you/did you take your vitamin tablets?
Do you/did you take them:

1. Every day
2. Between 3 and 5 days of the week
3. One or two days of the week
4. Once per fortnight
5. Once per month
6. Less often than once per month
7. Don't know

Supplement freq. ____

ug folic acid/day: ____
mg iron/day: ____
ug B12/day: ____

---

* (k) The next few questions are about the medicines that you may take. Some medicines can affect the iron in your blood.

Do you ever take any over-the-counter medicines for pain, colds or flu? (By over-the-counter I mean medicines that you can buy without a prescription)

1. Yes
2. No
3. Don't know

*** If 'no' go to 4(n)**

(l) If you do is it any of the following?

Advil	Disprol
Anadin	Ibuprofen
Asprin	Nurofen
Disprin	Nurofen Cold and Flu
Disprin Extra	Nurofen Plus

O.T.C. NSAID use: ____

1=Yes
2=No

(m) If you do take one of the medicines on the list how often do you need to take it?

Do you need to take it:

1. Every day
2. Between 3 and 5 days of the week
3. One or two days of the week
4. Once per fortnight
5. Once per month
6. Less often than once per month
7. Don't know

O.T.C. NSAID freq. ____

Section 5: Menstrual and Reproductive Details.

In this section I will ask you about your periods, pregnancies and about methods that you may use or have used to prevent a pregnancy.

(a) Can you tell me what age you were when you had your first period?

Menarche: ☐

(b) How would you describe your periods? Do you think they are:

1. Mainly regular
2. Both regular and irregular
3. Mainly irregular

Menstrual frequency ☐

(C) Thinking about your periods over the last 6 months, can you tell me how many days there are usually between your periods, counting from the first day of one period to the first day of the next one?
(If your periods are irregular, you can give me a range of days, for example 16 to 25 days)

Average days between periods: ☐

(d) How long do your periods usually last?

Duration of periods: ☐

(e) Next I am going to ask you about the type of sanitary protection (by that I mean towels or tampons) that you use during your period.

What do you use at the start of your period?

Do you use:
1. Towels
2. Tampons
3. Both

Are they:
1. Light
2. Normal
3. Super
4. Super plus

Early stage:

Type: ☐

Level of protection: ☐

What do you use at the end of your period?

Do you use:
1. Towels
2. Tampons
3. Both

Are they:
1. light
2. Normal
3. Super
4. Super plus

Late stage:

Type: ☐

Level of protection: ☐

(f) Are you still having periods?

1. Yes
2. No
3. Don't know

*** If 'yes' go to 5(l)**

(g) What age were you when you had your menopause?

Menopause: ☐

(h) How long since your last period?

Time since last period ☐

* (l) Have you ever been pregnant?

1. Yes
2. No
3. Don't know

*** If 'no' go to 6(a)**

(j) How many times have you been pregnant?

No. of pregnancies:

(k) How many children do you have?

Total: ☐

Last 10 yrs. ☐

(l) Could you tell me the date of birth of each of your children?

Last 5 yrs. ☐

Last 2 yrs. ☐

	D.O.B.	Age	Birthwt.	Gestation	N.T.D.	Prem.	Stillbirth
C1							
C2							
C3							
C4							
C5							
C6							
C7							
C8							
C10							

(m) If you can remember, can you tell me the birthweight of each of your children?

(n) Again, if you can remember, can you tell me how many weeks pregnant you were when each of your children was born?

(o) Were there any problems with any of your pregnancies? For example, were any of your children:

1. Born prematurely **(ask for D.O.B., Birthwt., Gestation)**
2. Stillborn **(ask for D.O.B., Gestation)**

Birth interval: ☐

(p) Did any of your children have health problems after they were born? (for example: spinabifida, cystic fibrosis, cerebral palsy, cot death etc.)

(q) Did you ever have a miscarriage?

1. Yes
2. No
3. Don't know

*** If 'no' go to 6(a)**

Age at 1st pregnancy

(R) How many did you have?

	Date	Gestation	Time since
Mis1			
Mis2			
Mis3			
Mis4			
Mis5			
Mis6			
Mis7			
Mis8			
Mis9			
Mis10			

No. of miscarriages:
Total:
Last 10 yrs.
Last 5 yrs.
Last 2 yrs.

(s) Can you tell me when it/they happened?

(t) Can you tell me how many weeks pregnant you were when it/they happened?

Section 6: Contraceptive Details.

The next questions are about the methods that you may use or have used in the past to prevent a pregnancy.

(a) Have you ever taken the Pill?

1. Yes
2. No
3. Don't know

O.C.P. use:

*** If 'no' go to 6(e)**

(b) Are you still taking it?

1. Yes
2. No
3. Don't know

Current O.C.P. use:

(C) When did you start taking it?

(d) When did you stop taking it?

Duration of OCP use: ☐

* (e) Have you ever had any devices put into your womb in order to prevent a pregnancy, for example a coil?
(Don't get confused with devices used to support the womb.)

1. Yes
2. No
3. Don't know

I.U.D. use: ☐

*** If 'no' go to 7(a)**

(f) Is it still in place?

1. Yes
2. No
3. Don't know

Current I.U.D. use: ☐

(g) When did you have it put in?

(h) When was it removed?

Duration of IUD use: ☐

Section 7: Body Image Concerns.

Here I will ask you about you some questions about how you feel about your body to find out how it affects the way you eat.

(a) Thinking about your body shape, would you describe yourself as:

1. Apple Shaped
2. Pear Shaped
3. Other (please describe)

Body shape: ☐

(Show picture to illustrate different body shapes)

(b) Are you satisfied with your body shape?

1. Yes
2. No
3. Don't know

Shape satisfied: ☐

(C) Thinking about your weight, would you describe yourself as:

1. Very underwieght
2. Slightly underweight
3. Of normal weight
4. Slightly overweight
5. Very overweight
6. Don't know

Weight perception: [ ]

(d) Are you satisfied with your body weight?

1. Yes
2. No
3. Don't know

Current wt. satisfied: [ ]

(e) Were you ever dissatisfied with your body weight?

1. Yes
2. No
3. Don't know

Ever not wt. satisfied: [ ]

*** If 'no' go to 7(j)**

(f) At what age did you become dissatisfied with your weight?

Age dissatisfied with weight: [ ]

(g) If you are/were dissatisfied with your weight do/did you want to be:

1. Lighter than you are/were
2. Heavier than you are/were
3. Don't know

Desired change: [ ]

(h) Have you ever tried to lose weight?

1. Yes
2. No
3. Don't know

*** If 'no' go to 7(j)**

(l) Next I am going to ask you about some of the things people do to lose weight. I want to find out if you have ever used these methods, or if you have done so recently.

Have you ever:	Have you over the last two years:	Yes=1, No=2 Ever	Last 2 yrs
1. Gone on a diet to lose weight			
2. Skipped meals to lose weight			
3. Taken diet pills to lose weight			
4. Taken laxatives to lose weight			
5. Made yourself vomit to lose weight			
6. Began or continued smoking to lose weight			
7. Exercised to lose weight			
8. Drank lots of water to lose weight			
9. Other (please describe)			

(j) If you were trying to lose weight which of the following foods would you cut down on?

	Yes=1, No=2
1. Bread, breakfast cereals, potatoes	
2. Red meat	
3. Milk, cheese, yogurt	
4. Biscuits, cakes, chocolate, crisps	
5. Chicken, fish	
6. Alcohol	

Section 8: Nutritional Knowledge.

The vitamins that we are looking at in this study are iron, folic acid, and vitamin B_{12}. In the next section I will ask you why you think we need these vitamins and where they can be found in our diets.

1= Correct
2= Incorrect/Don't know

(a) Why do you think we need iron?

1. For healthy bones and teeth
2. For healthy blood
3. To help us to see
4. Don't know

(b) Can you tell me if this statement is true or false: 'Men need more iron than women and teenage girls.'

1. True
2. False
3. Don't know

(C) Which of the following foods are high in iron?

1. Liver
2. Potatoes
3. Milk
4. Red meat
5. Oranges
6. Black pudding
7. Don't know

(d) Can you complete this sentence: Folic acid is important for women in order to have:

1. Healthy hair
2. Healthy kidneys
3. Healthy babies
4. Don't know

(e) Which of the following foods are high in folic acid?

1. Fruit and vegetables
2. Chicken
3. Some breakfast cereals
4. Yogurt
5. Super milk
6. Don't know

Anaemia in Irish Women.

Study ID: _____ Date: _____

Height:_____m.

Weight:_____kg.
(Without shoes in indoor clothing) B.M.I._____kg/m^2

Waist:_____cm.
(Minimum girth between the lower rib margin and the iliac crest)

Hip:_____cm.
(Maximum girth over the great trochanters)

Waist/Hip:_____