Dietary fat intake and obesity:

An empirical study in Greek adults

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ABSTRACT

The empirical evidence concerning the associations between diet, particularly fat intake, and obesity is inconclusive.

The aim of the present study was to investigate cross-sectional associations between general and central adiposity, and dietary and other socio-demographic and behavioural factors influencing energy balance. Study subjects were 961 women and 596 men aged 30-75 years who participated in the Greek segment of the European Prospective Investigation into Cancer and Nutrition (EPIC) Study.

General obesity was evaluated by Body Mass Index (BMI) and central obesity by Waist to Hip ratio (WHR) and Waist Circumference (WC). Dietary intake was estimated through a validated semi-quantitative Food Frequency Questionnaire (FFQ) obtained at baseline. Time weighted occupational and leisure activities, as well as socio-demographic and behavioural data were assessed through a life-style questionnaire. The methodological issues related to under-reporting and adjustment for energy intake have been considered in depth.

Obesity indices (BMI, WHR, WC) were initially regressed, separately for men and women, on energy intake and energy expenditure, after adjusting for the confounding effects of age, socio-economic status and smoking habits and controlling for dietary under-reporting. Results indicated that increasing physical activity is less effective than decreasing energy intake in reducing BMI. WHR and WC were not affected by energy intake, whilst energy expenditure reduced WHR and WC independently of BMI.

Obesity indices (BMI, WHR, WC) were subsequently regressed on nutrient intake after controlling for the confounding effects of energy intake, energy expenditure, age, socio-economic status and smoking habits, including and excluding under-reporters of energy intake. Among women, but not men, the nutrient more strongly positively associated to BMI was protein and to a lesser extent mono-unsaturated and total fat intake. WHR and WC do not seem to be differentially affected by energy equivalent amounts of energy generating nutrients.

To my parents

To my mentor, Antonia Trichopoulou

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LIST OF ABBREVIATIONS

The following list of abbreviations has been used within the thesis:

BMI	Body Mass Index
BMR	Basal Metabolic Rate
CI	Confidence Intervals
DLW	Doubly Labelled Water
SD	Standard Deviation
SE	Standard Error
HDL	High Density Lipoprotein
EE	Energy Expenditure
EI	Energy Intake
EPIC	European Prospective Investigation into Cancer and nutrition
FFQ	Food Frequency Questionnaire
METs	Metabolic equivalents (The ratio of the metabolic rate associated with a given activity to the resting metabolic rate)
MUF	Mono-unsaturated fat intake
NIDDM	Non Insulin Dependent Diabetes Mellitus
PAL	Physical Activity Level
PUF	Poly-unsaturated fat intake
RMR	Resting Metabolic Rate
SAT	Saturated fat intake
TEE	Total Energy Expenditure
WHO	World Health Organisation
WC	Waist circumference
WHR	Waist to Hip ratio

SECTION I. BACKGROUND AND LITERATURE REVIEW

1. SIZE AND PUBLIC HEALTH SIGNIFICANCE OF THE PROBLEM

Chapter 1.1 considers the true costs of obesity both in terms of physical and mental ill health, as well as the human and financial resources being diverted to deal with it. *Chapter 1.2* examines the definition and classification of obesity. *Chapter 1.3* presents up-to-date regional data on the prevalence of obesity and finally *chapter 1.4* examines the etiological factors implicated in weight gain and obesity.

1.1 Health risks of obesity

The life insurance industry has known, since the beginning of this century, that people who are above a certain 'desirable' weight for height are liable to die young, and hence are less profitable to insure. Although obesity has not yet achieved the full disease status of other bodily malfunctions, it constitutes a major medical concern, since it is a powerful risk factor for numerous life-threatening and debilitating disorders.

This chapter examines the health risks associated with overweight and obesity in adults; these are many ranging from an increased risk of premature death to several non-fatal but debilitating conditions that impact immediate quality of life. Also, the significance of weight loss is tackled along with the issue of weight stability and weight cycling.

1.1.1 Obesity related mortality

The relationship between obesity and mortality has been a controversial issue since different studies have provided conflicting results (WHO Report on Obesity, 1997, Garrow, 1999). There has been a number of studies demonstrating a U- or J- shaped association between obesity and mortality with higher mortality rates accumulating at both upper and lower parts of the weight range (Seidell et al, 1996, Seidell et al, 1999). Some others have shown a linear and gradual increase in mortality with increasing weight (Lee et al, 1993, Manson et al, 1995). Finally, there have been some others which have reported no association at all (WHO Report on Obesity, 1997, Lee & Manson, 1998, Garrow, 1999). Potential reasons for the discrepancies observed may be different type of biases that may have been involved in the design of many of the studies relating obesity and mortality. These biases comprise the failure to control for cigarette smoking, (which would lead to an artificially high mortality in leaner subjects), the inappropriate control for conditions such as hypertension or hyperglycemia which although taken into consideration as confounding factors, to a large degree consist of secondary disorders of obesity (which would distort the true association between obesity and mortality), the failure to control for weight loss associated with illness (which would produce an underestimation of the impact of obesity in mortality) and finally failure to standardize for age. All these may have led to a systematic under-estimation of the impact of obesity in premature mortality (WHO Report on Obesity, 1997, Seidell et al, 1999, Garrow, 1999). In fact, the latter argument has been well documented in the context of the Nurses' Health Study. When such biases were removed from the analysis the increased relative mortality risk at low weights disappeared and there was almost a linear relationship between BMI and mortality with no particular lower threshold (Manson et al, 1995).

Nevertheless, whatever the shape of the curve, there seems to be a scientific consensus that the lowest mortality appears to be associated with a BMI between 18 and 25 kg/m^2. This was actually the conclusion reached by the American Institute of Nutrition after analyzing numerous studies relating obesity to mortality risk (Garrow, 1993, James, 1996, WHO Report on Obesity, 1997, Garrow, 1999, Willett et al, 1999).

1.1.2 Obesity related morbidity

There has been strong scientific evidence suggesting that those who suffer from excessive body weight and fat are at elevated risk for several chronic health problems, including cardiovascular disease, hypertension, stroke, certain types of cancer (particularly those which are hormone-dependent or gastrointestinal), diabetes mellitus, and gallbladder disease. Furthermore, obesity has been associated with several endocrine disturbances, such as insulin resistance, menstrual problems and obstetric complications as well as metabolic disorders such as dyslipidaemia, and several aspects of the metabolic syndrome; these involve insulin resistance, glucose intolerance, hypertension, hypertriglyceridaemia, and low HDL-cholesterol, and visceral adipose tissue accumulation (central adiposity). Furthermore, obesity has been associated with several debilitating health problems such as osteoarthritis and gout, and certain pulmonary impairments such as hypoxaemia and sleep apnea (Garrow, 1988, National Research Council, 1989, Pi-Sunyer, 1993, Berger, 1995, Bray, 1996, Sjostrom, 1997, WHO Report on Obesity, 1997, James, 1998, Garrow, 1999, Khan & Bowman, 1999).

The independent and differential effect of obesity on the health of men and women is still under debate. This has been complicated by findings from empirical studies which suggest that it is the distribution of body fat, rather than gross adiposity per se,

which is more important as a risk factor for ill-health. Thus, central adiposity -excess fat in the intra-abdominal cavity- is of particular concern, since it is associated with elevated risks to health in comparison to a more peripheral fat distribution (e.g. subcutaneous fat on the limbs) (Lapidus et al, 1984, Rossner, 1984). A report from a well-known study in Gothenburg, Sweden suggested that in both men and women a high ratio of waist to hip circumference was more important than BMI as a risk factor for cardiovascular disease and death during 12 years of follow-up (Lapidus, 1984, Larson et al, 1984). However, further analysis suggested that the health risks associated with a high waist to hip ratio in the Gothenburg studies might have been confounded by factors such as cigarette smoking and high alcohol intake which are both associated with central adiposity. The latter factors may have contributed to some of the excess observed mortality (Larson, 1987). In conclusion, although the association between abdominal obesity and health risk has been clearly established in many studies, it is not yet known to what extent the health risk consists of a primary effect of visceral fat or if disease risk and the visceral fat are both secondary indicators of other risk factors (Van Loan, 1996, Molarius & Seidell, 1998).

In addition to the elevated risk of morbidity and mortality, obesity is associated with decreased quality of life although the latter may not be bound to any specific diagnosis. In this context, social prejudice and discrimination, psychological effects, body shape dissatisfaction and eating disorders have been shown to be strongly associated with obesity. All the above conditions are particularly evident in women and children. Most of them are improved with modest weight loss (Stunkard & Sobal, 1995, Seidell, 1998).

Finally, the economic costs of obesity have been assessed in several developed countries. These can be broken down into: a) Direct costs referring to the diversion of resources to the diagnosis and treatment of diseases related to obesity, as well as the treatment of obesity itself. These costs have been estimated to range from 1-10% of

total health care costs; b) Indirect costs related to the loss of productivity caused by absenteeism, disability pensions and premature death. Data on aspects such as societal costs and personal costs are rather too loose to allow calculation of the expenses involved (Seidell, 1995, Colditz, 1999). However, an appropriate analysis of all costs associated with obesity might help to persuade responsible bodies to develop strategies towards the prevention and long-term management of obesity.

1.1.3 The health benefits of weight loss

While the effects of obesity on health and quality of life have been studied extensively, the impact of weight loss on the same conditions is rather less well documented. There have been some short-term studies which have demonstrated that a modest weight loss results in clear benefits in most of the associated disorders of obesity. However, there have been only a few studies designed to examine the benefits of long-term (two years or longer) weight loss. The reasons for that involve inherent difficulties implicated in such study design. These comprise the difficulty of maintaining long-term weight loss as well as the need to separate intentional from unintentional weight loss (Goldstein, 1992, Williamson & Ramuk, 1993, De Leiva, 1998, Gaesser, 1999).

However, there is sufficient scientific evidence to support the fact that intentional weight loss results in marked improvements in NIDDM (glucose tolerance improves, insulin secretion decreases, and insulin resistance is reduced), dyslipidaemia, hypertension, cardiovascular risk factors, associated hormonal abnormalities and menstrual function. It also improves measures of general health including breathlessness, sleep quality, sleep apnea, back and joint pain and osteoathritis. As far as hazards of weight loss are concerned (when weight loss is rapid) these are limited to an increased risk of gallstones (most probably due to the fact that during

weight loss the cholesterol in adipose tissue is mobilized and the bile may become even more liable to form cholesterol stones) and possibly a reduction in bone density (WHO Report on Obesity, 1997, Shetty & Tedstone, 1997, Garrow, 1999).

1.1.4 Weight cycling and stable weights

According to the Report of a WHO Consultation on Obesity (1997), weight cycling refers to the repeated weight loss and regain which can occur as a result of recurrent dieting. Although there is no implicit definition of weight cycling and comparison between different studies is difficult, it has been suggested that weight cycling is associated with inverse health outcomes, since it makes future weight loss more difficult and results in a decrease in lean-to-fat tissue ratio. Although, supporting evidence has been conflicting, it has not been sufficiently compelling so as to override the potential benefits of moderate weight loss in obese patients (Lissner et al, 1999, Blair et al, 1993, Report of National Task Force for the Prevention and Treatment of Obesity, 1994, Jeffery, 1996).

1.2 Definition and measurement of obesity

It is necessary to have a working definition of 'overweight' and 'obesity'. Overweight is an increase of body weight above a standard, defined in relation to height. Obesity, on the other hand, is a condition of accumulation of excess body fat, which may be generalized or localized, associated with elevated health risks. The latter statement begs two questions: a) how does one know how much fat an individual has stored and b) how much is excessive?

In order to determine whether an individual is obese or simply overweight because of increased muscle mass or large frame, one needs techniques and standards for quantifying body weight, body fat, and distribution of body fat. Several approaches to determine this are listed in Table 1 (adapted from Bray, 1996), which also includes an estimate of the cost, ease of use, and accuracy of these methods.

Some of the methods, although useful for measuring body fat in certain clinical situations, are too complex for use in epidemiological studies, so less direct anthropometric assessment methods are used. These are described below:

Table 1. Methods of estimating body fat and its distribution (adapted from Bray, 1996)

Method	Cost	Ease of use	Accuracy	Regional fat
Height and weight	£	Easy	High	No
Skinfold thicknesses	£	Easy	Low	Yes
Circumferences	£	Easy	Moderate	Yes
Ultrasound	££	Moderate	Moderate	Yes
Density				
Immersion	£	Moderate	High	No
Plethysmograph	£££	Difficult	High	No
Heavy water				
Tritiated	££	Moderate	High	No
Deuterium oxide or heavy oxygen	£££	Moderate	High	No
Potassium isotope (^{40}K)	££££	Difficult	High	No
Total body electrical conductivity	£££	Moderate	High	No
Bioelectric impedance	££	Easy	High	No
Fat-soluble gas	££	Difficult	High	No
Absorptiometry (dual energy x-ray)	£££	Easy	High	No
Absorptiometry (dual photon absorptiometry)				
Computer tomography (CT)	££££	Difficult	High	Yes
Magnetic resonance imaging (MRI)	££££	Difficult	High	Yes
Neutron activation	££££	Difficult	High	No

£: low cost, ££: moderate cost, £££ : high cost, ££££: very high cost

General adiposity can be expressed as relative weight or ratios of weight to height. Relative weight is the ratio of actual to standard weight as determined from a table of reference body weights for men and women expressed relative to height (100 * body weight/standard weight). Weight to height ratios can be expressed in several ways.

The most widely used index for the classification of overweight and obesity (general adiposity) in adults is the Body Mass Index (BMI) or Quetelet index (QI) which is body weight (in kilograms) divided by the square of the height (in meters), weight/height^2. Table 2 presents a comprehensive classification for determining obesity according to BMI. This has been proposed by Garrow and is based on epidemiological evidence for the association between obesity and excess morbidity and mortality. A BMI of 20-24.9 kg/m^2 is a desirable range, a BMI of 25-29.9 kg/m^2 is overweight, a BMI over 30 kg/m^2 is obese, and a BMI over 40 kg/m^2 very obese (Garrow, 1988). This classification is in agreement with that recommended by WHO except from the fact that desirable weight has been defined within a broader range, starting from a BMI of 18.5 kg/m^2 (WHO Report on Physical Status, 1995).

Table 2. Classification of obesity according to BMI*

Grade	BMI (kg/m^2)	Classification
Grade 0	20-24.9	'desirable weight'
Grade I	25-29.9	'overweight'
Grade II	30-40	'obese'
Grade III	>40	'very obese'

* adapted from WHO Report on Obesity, 1997

Additionally, a recent Report of a WHO Consultation on Obesity Committee (1997) has slightly modified the classification presented above by including an additional subdivision at BMI 35-39.9 kg/m^2 in recognition of the fact that management options for dealing with obesity differ above a BMI of 35 kg/m^2, and by reducing the lower limit of 'normal' to 18.5 kg/m^2 (Table 3).

Table 3. Classification of obesity according to BMI (associated with health risk)

Classification	BMI	Risk of co-morbidities
Underweight	<18.5	Low (risk of other clinical problems increased)
Normal range	18.5-24.9	Average
Overweight	≥25	
Pre-obese	25-29.9	Increased
Obese class I	30-34.9	Moderate
Obese class II	35-39.9	Severe
Obese class III	≥40	Very severe

From all evidence reviewed above, it seems that there has been an 'international consensus' that a BMI of 30 kg/m^2 or more is widely accepted as denoting 'obesity'. Some studies, however, have used alternative BMI cut-off points, both above and below 30 kg/m^2. For instance in the USA, obesity has been routinely classified on the basis of the distribution of BMI in the National Health and Examination Survey (NHANES), a representative study of the US population: 27.8 kg/m^2 in men and 27.3 kg/m^2 in women are taken as the 85th percentile (Kuczmarski et al, 1994). Such differences in cut-off points used have a great impact on estimates of the prevalence of obesity, in given populations and may complicate comparisons between different studies.

Concerning the validity of BMI as an indicator of the accumulation of excess energy as fat, it has been reported that BMI can efficiently be used as such in adult white men and women living in Europe and North America; the correlation of BMI with body fat as measured from body density is between 0.7 and 0.8 (Bray, 1996). It is probably less appropriate in other populations who differ in body build and body proportions. Using BMI to classify individuals, according to fatness, may result in some misclassification because of the varying contribution of bone mass, muscle mass, and fluid to body weight. Furthermore, since there is no allowance for age, sex, and frame size in this classification, differences in the percentage of body fat through

different ages or sexes may not be revealed (Garrow, 1988, Ross et al, 1994, Garrow, 1993, Bray, 1992, Rolland-Cachera, 1991). Deurenberg reported that equations including BMI, sex and age for Dutch adults were shown to predict body fat percentage relatively accurately (r^2 about 0.8, SE about 4%) (Deurenberg et al, 1991). This prediction error is similar to values obtained with other more elaborated methods such as measurement of skinfold thickness or bioelectrical impedance (WHO Report on Physical Status, 1995).

Since it has been well documented that obese individuals with excess fat in the intra-abdominal depots are of particular risk of the negative health consequences of obesity, and abdominal fat mass may vary widely within a narrow range of total body fat as well as BMI (Lemieux et al, 1993), a variety of indicators for abdominal adiposity has been proposed in the literature (Molarious & Seidell, 1998). The most popular are WHR and WC.

The ratio of waist or abdominal circumference to the hip or gluteal circumference (WHR) provides an index of the regional fat distribution and has proven valuable as a guide to health risks in epidemiologic studies (WHO Report on Obesity, 1997, Bray, 1996, WHO Report on Physical Status, 1995). The waist circumference measures predominantly visceral organs and abdominal (both subcutaneous and intra-abdominal) fat. The hip circumference standardizes waist circumference for body build and may reflect different aspects of body composition that is muscle mass, and skeletal frame. Concerning the validity of this measure for classifying abdominal obesity, problems of misclassification may occur as different contributions of muscle mass and bone structure as well as stature and abdominal muscle tone may lead to different associations between WHR and visceral fat accumulation. This issue combined with the observations that a high WHR may reflect either small hips or a large waist and often implies different health risks for different racial groups and at different ages has complicated the development of

universal cut-off points for WHR (Dowling & Pi Sunyer, 1993, WHO Report on Physical Status, 1995, Bray, 1996, WHO Report on Obesity, 1997).

Several attempts have been made to derive appropriate WHR cut-off points. Bjorntorp first recommended that 'the risk of complicating disorders to obesity increases sharply at a WHR exceeding 1.00 in middle aged men and 0.80 in middle aged women (Bjorntorp, 1985). These cut-off points were derived from the analysis of a relatively small number (792) of middle aged men all born in the same year and a larger sample (1,462) of middle aged women, in the context of the prospective Gothenburg studies of men and women. Bray subsequently recommended the cut-off points 1.00 for men and 0.90 for women (Bray, 1987). Other cut-off points for WHR have also been suggested from the US Department of Agriculture, 0.95 for men and 0.80 for women (US Dept of Agriculture, 1990). Recently, Lemieux proposed possible cut-off points for the WHR, 0.94 in men and 0.88 in women (Lemieux et al, 1996). These corresponded to the critical amount of 130 cm^2 of visceral fat, as this had been measured using computed tomography. Their study population was relatively small (213 men, 190 women).

Recent evidence suggests that waist circumference (WC) alone - measured at the midpoint between the lower border of the rib cage and the iliac crest - may have certain advantages as an indicator of adiposity in predicting risk of obesity-related diseases, particularly to the extent that visceral fat is the predominant risk factor for abdominal obesity related diseases (WHO Report on Physical Status, 1995, James, 1996, Bray, 1996, WHO Report on Obesity, 1997, Molarious & Seidell, 1998, Van Itallie, 1998). WC is a practical research tool, which has been found through computed tomography to be closely correlated with visceral fat accumulation (Despres et al, 1991, Pouliot et al, 1994). Evidence has emerged that men and women in the top quintile for waist circumference, i.e. the top 20%, have been found to be in a substantially increased risk of heart disease, diabetes and other forms of chronic

disease, even though the risks seem to vary in different populations (WHO Report on Obesity, 1997, Bray, 1996, Han et al, 1995, Pouliot et al, 1994). This is one of the reasons why globally applicable cut-off points cannot and have not been developed.

Several attempts however, have been undertaken. Lean has suggested two 'action levels' for waist circumference. According to the 'action level 1' men with waist circumference ≥ 94cm and women with WC ≥ 80cm should not gain any further weight. According to 'action level 2' men with WC ≥ 102cm and women with WC ≥88 cm should reduce their weight (Lean et al, 1995). These cut-off points are presented in Table 4 and are actually based on cut-off points from BMI 25kg/m^2 at 'action level 1' and 30 kg/m^2 at 'action level 2' and WHR 0.95 for men and 0.80 for women at both action levels (Lean et al, 1995). These cut-off points derived from the analysis of a relatively large sample (990 men, 1,216 women) representative of the population aged 25-74 years in Glasgow. Later, the same team verified the cut-off points in a random sample of 2,183 and 2,698 women aged 20-59 years from the Netherlands (Han et al, 1995).

Table 4. Sex specific waist circumference that denote 'increased risk' and 'substantially increased risk' of metabolic complications associated with obesity in Caucasians

Sex	Risk of obesity-associated metabolic complications	
	Increased	*Substantially increased*
Men	≥ 94 cm	≥ 102 cm
Women	≥ 80 cm	≥ 88 cm

Lemieux used another approach for calculating cut-off points. They used regression models to find the cut-off points for WC that best predicted the absolute amount of 130cm^2 visceral fat which has been measured using computed tomography scan (Lemieux et al, 1996). They concluded that a WC cut-off point of 100 cm should be

used for subjects ≤40 years and 90 cm for subjects ≥ 40 years of age, for both men and women. Their study population was relatively small (213 men, 190 women).

1.3 Epidemiology of obesity

Studies investigating obesity have generally reported an increasing prevalence of obesity worldwide; both developed and developing countries are affected. Thus, in many developing countries, where undernutrition is the predominant public health nutrition problem, obesity has been rare, although it is rising, particularly in countries experiencing the epidemiological transition from poverty or agrarian lifestyles to more affluence (Popkin, 1994, Popkin & Doak, 1998). In these countries, obesity often co-exists with under-nutrition (BMI<18.5 kg/m^2); it is more prevalent in urban than in rural populations, whilst in economically advanced regions, prevalence may be as high as in industrialized countries (WHO Report on Obesity, 1997). In contrast, obesity is considered one of the major public health problems with a rapidly increasing prevalence in industrialized countries. Northern America and several European countries -especially Southern and Eastern European countries- are characterized by high prevalence rates of obesity (Garrow, 1993). Available data from European countries participating in the WHO-MONICA study between 1983 and 1986 as well as some monitoring projects and repeated surveys suggest that since 1980 the prevalence of obesity has risen (Seidell, 1995, Seidell & Flegal, 1997). It has actually been estimated that more than half of adults aged 35-65 years living in Europe are either overweight or obese. The average prevalence of obesity is about 15% for men and 22% for women. The prevalence varies considerably between countries and between regions within countries (Seidell, 1995, James, 1996, Seidell & Flegal, 1997). A recent review of the prevalence of obesity (BMI>30 kg/m^2), among men and women aged 40-60 years, in different regions of Europe, shows that

in every geographical region, in the age group 40-60 years, the prevalence of obesity is higher among women than among men, although Grade I (BMI 25-29.9 kg/m^2) tends to be more common among men than among women of the same age (Seidell, 1995, James, 1996, Seidell & Flegal, 1997). This indicates that obesity is more common among women and the percentage of women relative to men, increases with severity of obesity. Men tend to reach the maximum prevalence of obesity at about age of 45 years, but in women the prevalence increases to age of 65 years and then starts to decline (Seidell, 1995, James, 1996, Seidell & Flegal, 1997).

In developed countries the prevalence of obesity is usually inversely related to social class with the highest prevalence being in the lowest social class groups, particularly among women (Seidell, 1995, James, 1996, Seidell & Flegal, 1997). A study in Finland examining determinants of weight gain and overweight in adult Finns showed that the risk of rapid weight gain (>5 kg/5 years) in adulthood was related to a low educational level, morbidity, low physical activity during leisure hours, high alcohol consumption, cessation of cigarette smoking and high parity in women (Rissanen et al, 1991).

1.3.1 Prevalence of obesity in Greece

There are indications of a high prevalence of obesity in the Mediterranean region (WHO MONICA Project, 1988, Crepaldi, 1991). Greece once characterized by low mortality rates due to cardiovascular disease (WHO Statistics, 1963), now has one of the most rapidly growing death rates due to cardiovascular disease (Trichopoulou et al, 1989, Trichopoulou et al, 1994). In addition, there has been well documented evidence of a decrease in physical activity and a deterioration in dietary patterns (a rapid increase of meat consumption and gradual decrease in pulses intake) and serum lipid profiles in Greece (Trichopoulou et al, 1993a, Trichopoulou et al, 1993b,

Trichopoulou et al, 1995, Petridou et al, 1995). These observations suggest that the prevalence of obesity may be rising in Greece. However, national data are not available to assess either the prevalence of obesity or trends over time. Several small-scale studies have been undertaken but interpretation of trends from these is complicated by the small number of studies, the heterogeneity of design, difficulties of extrapolation of findings due to lack of representativeness of the national population, as well as small sample sizes.

In 1988, a study of an urban Cretan area was conducted by the Preventive Medicine and Nutrition Clinic of the Department of Social Medicine, University of Crete and the Faculty of Medicine of the University of Stellenbosch in South Africa (Kafatos et al, 1991). The subjects were 387 adult bank employees (256 males, 131 females), aged 20-60 years. Most of the sample (86%) was overweight (BMI>25 kg/m^2). With the exception of the 20-30 year age group, all other age groups were overweight (mean BMI>25 kg/m^2). Similar observations were made with respect to the mean WHR with the senior age groups obtaining the highest mean scores. Finally, the male age groups had a high abdominal fat distribution (mean WHR>0.90), while the female groups were characterized by an intermediate distribution of fat (0.80<WHR<0.90).

In 1988-1989, fieldwork for the EURONUT, SENECA study was conducted in 19 areas from 12 European countries. The study involved samples of men and women, all aged 70-75 years. There were two Greek rural samples. One was from mainland Greece, while the other was from Crete. Greek males were characterized by central obesity (mean WHR>0.90), while Greek women showed an intermediate body fat distribution (0.80<WHR<0.90). The percentage overweight and mean BMI (kg/m^2) of both males and females of the Cretan group were among the lowest in Europe, while the reverse held true for the mainland group (de Groot et al, 1991). Data from the mainland group, 33 men and 27 women, aged 74-75 years, randomly selected

from the electoral registries of two traditional Greek towns near Athens was subsequently used by the Department of Nutrition, National School of Public Health, in an effort to assess relationships between BMI, energy intake and walking. The subjects were found to be overweight, especially the women (48% of the women had BMI>30). The mean value for BMI was 28.6 kg/m^2, 27.5 kg/m^2 for men and 29.7 kg/m^2 for women (Vassilakou & Lagiou, 1991).

In 1990, a study of all the junior students of Medicine of the University of Crete was conducted. The sample consisted of 43 males and females, aged 21-22 years. Half the females had total body fat over 30%. Only a minority of students (25%) exercised regularly (Vassilakis et al, 1991).

In 1990, 837 male conscripts were examined and anthropometric measurements were taken. Although the mean BMI of the sample is within the optimal range, 22.8% of the sample was overweight (25<BMI<29.9 kg/m^2) and 4.4% was found to be obese (BMI>30kg/m^2) (Georgiadis et al, 1993).

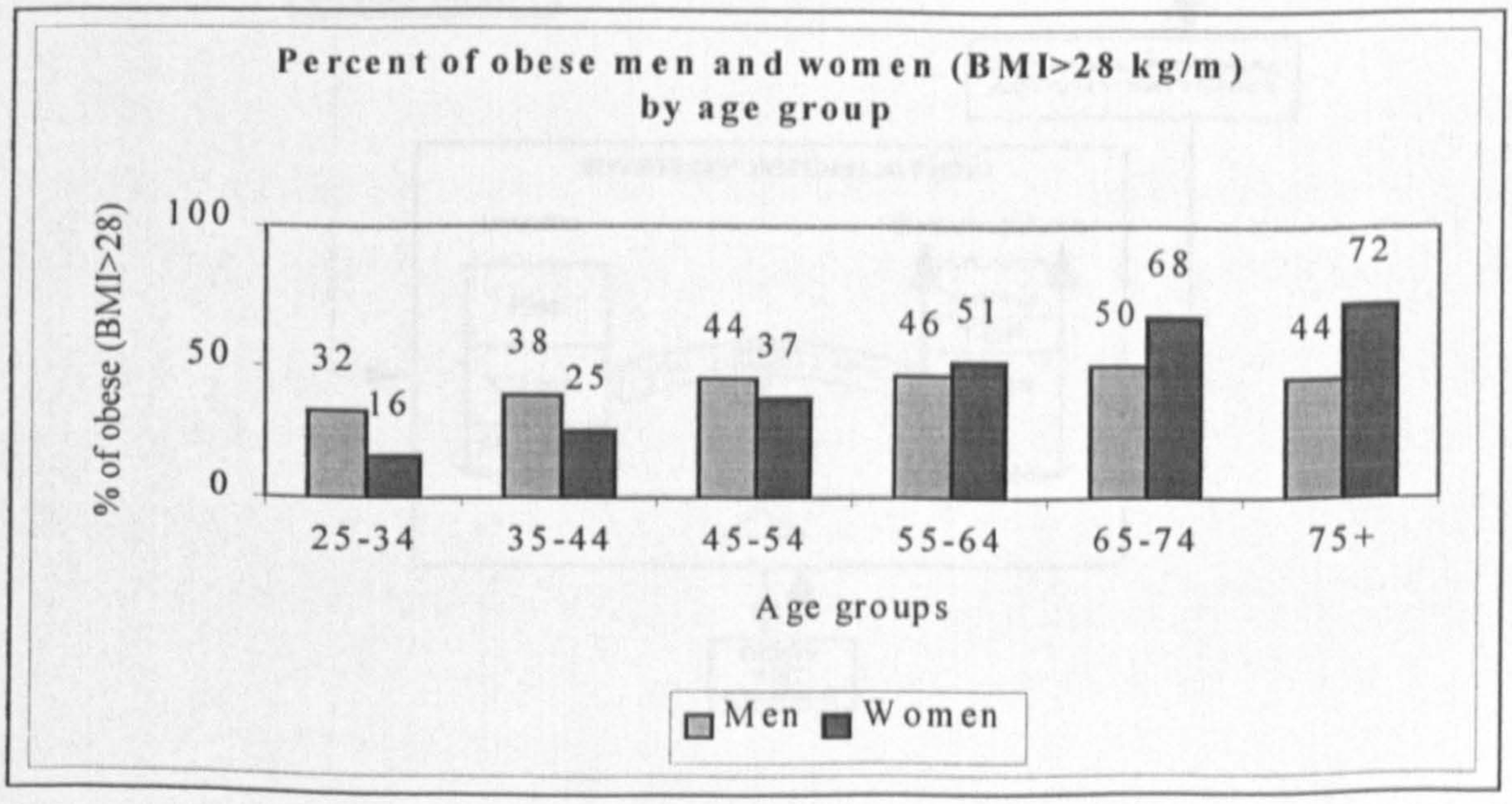
Follow-up of the rural Cretan samples that had participated in the Seven-Countries study in 1960, indicate an increase in obesity over the years studied (Kafatos & Mamalakis, 1993). Examination of the mean BMI of the different age groups of the surviving men of the Seven Countries study in 1991, indicated that the 54-69 and 70-79 years age groups were overweight (BMI>25 kg/m^2). In addition, central obesity (WHR>0.90) was evident among all age groups examined (Mamalakis & Kafatos, 1996).

In conclusion, lack of national data precludes an accurate description of the prevalence of obesity and trends over time within Greece. Available data indicate that obesity is present. There is some indication that it is rising as in other southern European countries. In contrast to females, Greek males appear to be characterized

by central obesity. The lack of national data highlights the need for national surveillance data to assess the size of the problem and trends over time.

The Greek segment of the EPIC Study is expected to provide valuable data on the prevalence of overweight and obesity in the Greek population. It should be noted that although ethical reasons limited the study sample to volunteers and one could not claim that study subjects consist of an 'exemplary' representative sample of the Greek population, there has been a conscious effort to cover all regions of Greece and all social classes in the study design. The most recent data published in 1996, have derived from a preliminary descriptive analysis of a subsample of 3,733 men and 5,757 women participating in the Greek EPIC cohort. In Figure 1, the percentage of subjects with a BMI above 28 kg/m^2 is presented according to age and sex (Greek EPIC Newsletter, 1996). Findings are valid for the specific study population and they are considered important since these are derived from a big, non-hospitalized sample, including subjects who reflect a wide range of social-demographic parameters. The presented values can not be considered as reference values but values that give a picture of the specific sample.

Figure 1. Prevalence of obesity in 9,480 Greek EPIC volunteers

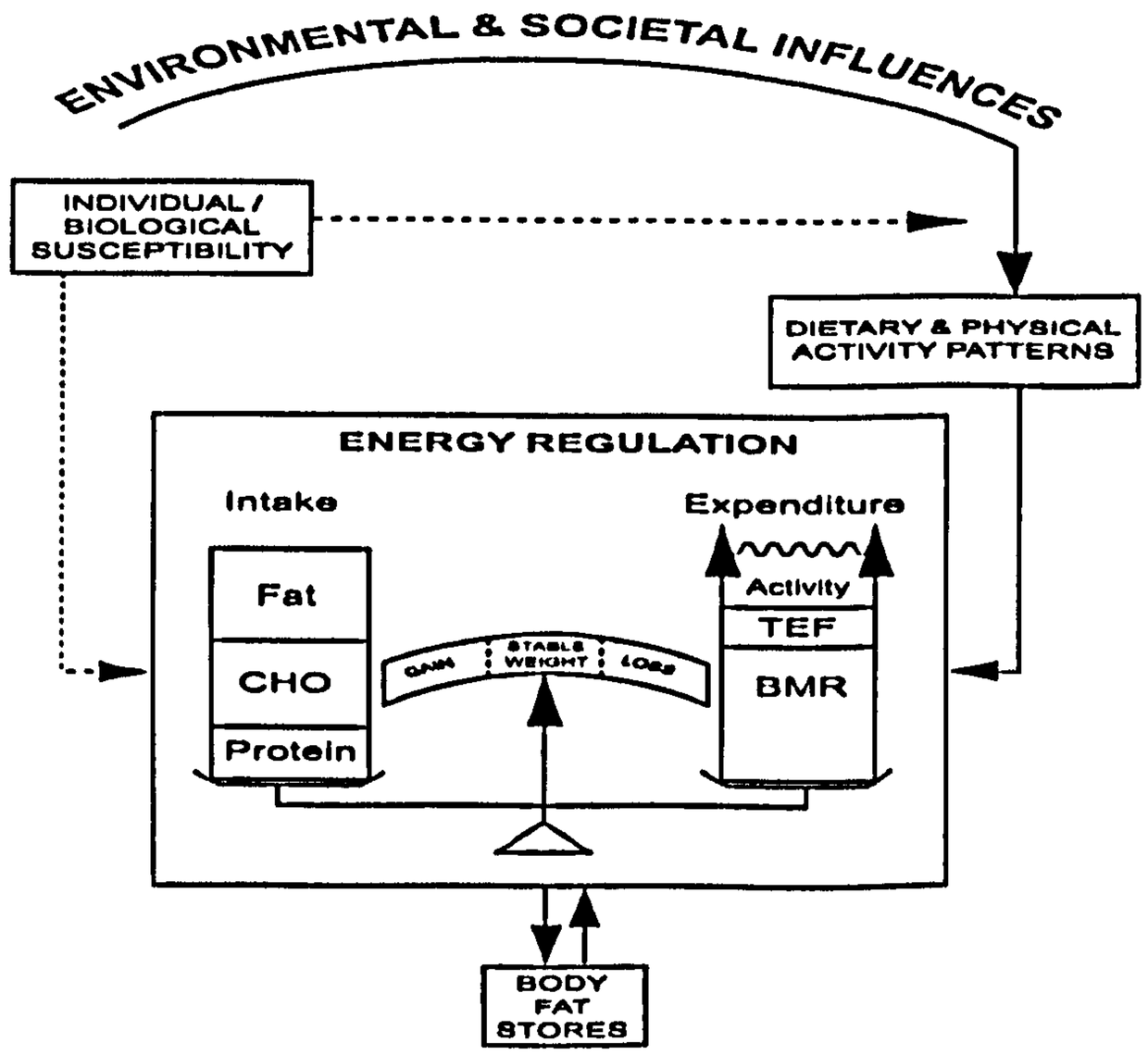


1.4 Etiology of obesity

Obesity is commonly recognized as a complex multi-factorial disease; it is a condition resulting from a lifestyle which promotes a chronic, positive balance between energy intake and expenditure, but also one that becomes manifest more readily in people who have an inherited susceptibility to be in positive energy balance. Susceptibility may be mediated through a wide range of metabolic and behavioral traits (Brownell, 1984, Danforth, 1985, Bray, 1992, Garrow, 1993, Bray, 1996, WHO Report on Obesity, 1997, Rosenbaum et al, 1997, Bjorntorp, 1997, Ravussin & Tatarrani, 1997, Jequier, 1997, Prentice, 1999a).

This chapter attempts to summarize the current knowledge on the causes of obesity. Figure 2 (adapted from WHO Report on Obesity, 1997) clearly demonstrates the role of multiple physiological, biological, societal as well as genetic factors which may act and promote positive energy balance and subsequently weight gain.

Figure 2. Influences on energy balance and weight gain



However the fundamental principle of energy balance, upon which any theories must and have been based is:

Changes in body energy stores = Energy intake - Energy expenditure

That is to say that all potential mechanisms implicated in the etiology of obesity must act either through increasing energy intake (EI), decreasing energy expenditure (EE) or both (Prentice, 1999b).

Energy balance fluctuates between meals, days and weeks, but in most circumstances and assuming weight stability, overall energy intake (EI) equals energy expenditure (EE). In broad terms, however, the spectrum of energy regulation ranges from positive energy balance (ultimately leading to obesity) to negative energy balance (ultimately leading to wasting). Between these two extremes and across the lifespan, critical periods such as growth and development, pregnancy, lactation, aging, physiological and psychological factors, social and environmental conditions as well as genetic background, may all act and affect energy balance. The way that energy balance is (re)achieved may have short or longer term consequences for the health of individuals and populations (Flynn et al, 1999).

Energy intake, the first component of the energy balance equation, refers to all metabolizable energy provided by food and beverages. Table 5 presents the energy provided per unit weight of each macronutrient; fat is the most energy dense macronutrient in the diet, providing approximately 9 kcal per g as opposed to 4 kcal per g for carbohydrate or protein (WHO Report on Fats and Oils in Human Nutrition, 1993).

Table 5. Metabolisable energy content of macronutrient

Macronutrient	Energy contribution	
	kcal/g	*kJ/g*
Fat	9	37
Alcohol	7	29
Protein	4	17
Carbohydrate	4	16

In recent years there has been an interest in examining whether the source of energy in the diet has any influence on weight control. There has been strong scientific evidence suggesting that high fat diets have a strong influence on the energy balance equation. Various potential mechanisms have been proposed to explain why fat intake should lead to increased relative weight (Lissner, 1997, Prentice, 1999c). These include mechanisms implicating 'passive overconsumption' because of the greater density of a high-fat diet and/or the weaker effect of fat on satiation and satiety, 'active overconsumption' due to greater flavor and palatability of high-fat foods. Finally there has been evidence for more efficient metabolism of this nutrient relative to other macronutrients; fat, is more likely to be stored rather than oxidized whilst storage costs are lower for fat than for carbohydrate. Evidence relating to the association between dietary fat and obesity is extensively reviewed in Chapter 2.

Energy expenditure, the second component of the energy balance equation, has three main components: basal metabolic rate (BMR), thermogenesis, and activity (Willett & Stampfer, 1998, Prentice, 1999b).

Basal metabolic rate (BMR) represents the fundamental requirements of a living organism and it comprises functions such as breathing, heart rate, body temperature, and involuntary organ activities. Basal metabolic requirements are quantitatively the most important, representing approximately 60-75 % of total energy expenditure in sedentary individuals. BMR is generally higher in overweight or obese people

because weight gain increases lean tissue mass as well as fat (Prentice, 1999b, Willett & Stampfer, 1998).

Thermogenesis consists of a) the thermo-regulatory thermogenesis (the production of heat for body temperature maintenance) b) the diet induced thermogenesis (the metabolic cost of absorbing and processing macronutrients) and c) the adaptive thermogenesis (the capacity of an individual to conserve or expend energy in response to variable intake of food). Thermo-regulatory thermogenesis is considered to be of minor importance in humans. Diet induced thermogenesis consists of only about 10% of the total energy expenditure. The role of adaptive thermogenesis, which is defined differently by various investigators, has been controversial for its importance in the regulation of human energy balance (Willett & Stampfer, 1998, Prentice, 1999b).

Finally, physical activity consists of the basic modifiable factor of energy expenditure level. In a moderately active individual, physical activity accounts for approximately 25-30 % of energy expenditure. It is important to note that the energy expended in fixed activities, especially in weight-dependent ones, is always higher in people carrying extra weight. However, the energy expended on physical activity, although it is somehow related to factors such as fitness, it is mostly affected by behavioral choices which relate to lifestyle patterns (Willett & Stampfer, 1998, Prentice, 1999b).

The second component of the energy balance equation, that is energy expenditure, first attracted attention, in the attempt to explain possible mechanisms for the etiology of obesity, during the 1970s. At that time, and many years after, several studies were consistently showing that the diet of overweight and obese people provided the same or even less energy than those of their lean counterparts. Although these findings were difficult to reconcile completely with observations that BMR was

usually higher, in obese subjects, they provided the stimulus for research to identify defects and differences in energy expenditure so as to be able to give an explanation to the apparent paradox. Many years were spent seeking the existence of energy sparing defects which would explain why reported energy intake in obese subjects were similar to, or even lower than, those measured in lean controls. Possible explanations for differences in the ability to regulate body weight have suggested specific mechanisms to spend excess energy which were gifted in fortunate people at one extreme, to enhanced efficiency and energy-sparing defects (low BMR, thermogenesis, reduced energy expenditure) possessed by the less fortunate at the other extreme (Prentice, 1999b).

A great amount of published work has demonstrated that there is no really convincing evidence to support a role for defects in energy expenditure in obese (Prentice et al, 1986) or formerly obese subjects (Black et al, 1991). Together with energy intake data, it was shown that the apparently low intakes of obese people that had influenced decades of research, were clearly demonstrated to be a feature of under-reporting (Prentice et al, 1986, Black et al, 1991). Some researchers however, provide relevant evidence and claim that a low resting metabolic rate (RMR) is more frequent among obese or formerly obese subjects than among normal weight subjects (Astrup , 1999).

As far as the role of physical activity in the regulation of body weight and adiposity is concerned, cross-sectional data reveal an inverse relationship between BMI and physical activity indicating that obese and overweight subjects are less active than their lean counterparts (Schultz & Schoeller, 1994, Prentice & Jebb, 1995, Ferro-Luzzi & Martino, 1996, Williamson, 1996, Haapanen et al, 1997, Westerterp & Goran, 1997). In particular, there has been evidence that energy requirements, and consequently total energy expenditure, increase immediately after the initiation of physical activity, and the increase persists for the duration of the activity. These

relationships have been extensively reviewed in the literature and tables providing approximations of the energy costs of various physical activities are widely available (Hill et al, 1995). There has been some evidence suggesting that further to the intrinsic effect of physical activity patterns on total energy expenditure, the latter also affects fat balance and food intake. One of the most important adaptations to regular exercise seems to be the increased capacity to utilize fat (at equivalent levels of energy expenditure), relative to carbohydrate during moderate levels of physical activity. These differences become considerable when the exercise is maintained over a longer period of time (WHO Report on Obesity, 1997).

Beyond all lifestyle factors discussed above, genetic factors are also involved in the etiology of obesity (Bouchard, 1990, Lancet editorial, 1992, Kopelman, 1999). Epidemiological, genetic and molecular studies suggest that there are people who are more susceptible than others to becoming overweight and obese. While it is possible that single or multiple gene effect may cause overweight and obesity directly, and do so in some individuals with certain genetic disorders, this does not appear to be the case in the majority of people. Instead, it is currently considered that the genes involved in weight gain increase the susceptibility or risk of an individual to the development of obesity when exposed to an adverse environment. Evidence to suggest that genetic factors are partially responsible for such differences in the sensitivity of individuals to gain fat when chronically exposed to a positive energy balance comes from studies in both animals and humans (Heitmann et al, 1995). Possible mechanisms through which a genetic susceptibility may be operating include: low RMR, low level of lipid oxidation rate, low fat-free mass as well as a poor appetite control. Concerning heritability, there has been well documented evidence indicating that obesity tends to run in families, with obese children frequently having obese parents. Further to that a large number of twin studies have provided additional support on the heritability of obesity (Bouchard, 1996).

As shown is Figure 2, the energy balance equation may be further influenced by several other biological or societal forces which act to promote weight gain. A number of biological factors have been shown to influence an individual's susceptibility to weight gain and the development of obesity. The most important are sex and age. There are a number of physiological processes that are believed to contribute to an increased fat storage in females; such fat deposition is considered essential in ensuring female reproductive capacity (Lovejoy, 1998). Further to that, females have a tendency to divert extra energy into fat storage while males utilize more of this energy for protein synthesis (WHO Report on Obesity, 1997). The effects of aging on energy balance have been studied by several research groups. As far as energy requirements are concerned there is well documented evidence that advancing age is associated with a remarkable number of changes in body composition, including reduction in lean body mass and increase in body fat; decreased lean body mass occurs primarily as a result of losses in skeletal muscle mass ('sarcopenia'). Loss in muscle mass (about 15% loss between the third and eighth decade of life), accounts for the age-associated decreases in basal metabolic rate, muscle strength, and activity levels, which, in turn are the cause of the decreased energy requirements of the elderly. Weight gain is promoted, when declining energy needs are not matched by an appropriate decline in energy intake (Evans & CyrCampbell, 1997, Kotz et al, 1999).

Other biological factors for which there is concern that may act and turn a subject more vulnerable in weight gain are parity and menopausal status. Concerning parity, it has been claimed that a woman's BMI increases with successive pregnancies (Bray, 1997, Pettigrew & Hamilton-Fairley, 1997). However, recent evidence suggests that this contribution is rather minor (1 kg per pregnancy), although the range may be wide depending on total weight gain during pregnancy (Ohlin & Rossner, 1990, WHO Report on Obesity, 1997). Also, there is doubt to whether menopausal status makes women more vulnerable to weight gain (Poehlmean &

Tchernof, 1998, Astrup, 1999). There has been evidence indicating that menstrual cycle loss does affect food intake and reduces metabolic rate slightly, although most of the weight gain has been attributed to reduced activity (Wing et al, 1991). However, evidence of the menopause transition on body fat distribution is rather more conclusive. Both cross-sectional and prospective studies have suggested that menopause is related to increased intra-abdominal adipose tissue accumulation that exceeds changes normally attributed to the aging process (Tchernof & Poehlman, 1998).

Concerning societal influences, studies have repeatedly shown that high sosio-economic status (SES), a composite index commonly combining income, education and occupation, is negatively correlated with obesity in developed countries (Seidell & Flegal, 1997). Even in less developed countries which currently attain higher levels of affluence, the positive relationship between SES and obesity is slowly being replaced by the negative correlation seen in developed countries (Popkin, 1998). Further to SES, level of education appears to be inversely associated with body weight in industrialized countries. The observed inverse relationship between education and body weight may be partly attributed to the fact that individuals with higher educational level are more likely to be aware and follow dietary recommendations as well as to adopt other risk-avoidance behaviors (Sorensen, 1995, Stunkard, 1996, James et al, 1997).

Finally, there are also some factors related to treatment with drugs promoting weight gain as a side effect, or 'personal' choices, such as smoking habits and alcohol consumption, which interact to determine an individual's susceptibility to weight gain. The most important are briefly considered below.

Despite popular belief, there is little evidence that classical endocrine or hormonal disorders 'cause' obesity. In general, endocrine alterations in obesity are secondary

as they can be induced be overfeeding and reversed by weight loss. Even when an endocrine disorder is causally associated with obesity, the clinical presentation is usually related to specific consequences of the hormonal state rather than weight gain. There are several clear endocrine abnormalities that cause obesity. However, they are very rare and although they should be considered as part of the diagnostic process they are unlikely to be the cause of obesity in any one individual (Finer & Prentice, 1999).

Smoking causes an acute rise in metabolic rate and tends to reduce food intake relative to non smokers (Dalosso & James, 1984). It may also cause a longer-term increase in RMR although evidence for this is conflicting (Hofstetter et al, 1986, Warwick et al, 1995). Smoking and body weight are inversely related (Grunberg, 1986), and smokers frequently gain weight when giving up the habit. Notwithstanding the risk of gaining weight, it is important to understand that smoking cessation should be a higher priority than weight loss in smoking obese patients; a large number of prospective studies have shown that smoking has a larger impact on morbidity and mortality than any small rise in BMI (Willett et al, 1987). The beneficial effects of giving up smoking are unlikely to be cancelled by the weight gain that may follow.

Concerning alcohol consumption, it is important to note that since the body is unable to store alcohol, oxidation of ingested alcohol is given priority over oxidation of other macronutrients. Alcohol consumption therefore meets some of the body's energy needs, and allows a greater proportion of energy from other foods eaten to be stored. Alcohol intake is associated with an increased risk of abdominal fat, although in epidemiological studies, those with the greatest alcohol intakes tend to be thinner (Gruchow et al, 1991, Colditz et al, 1991). This latter paradox may be the result of these people eating less and having much of their energy requirements met by alcohol (Prentice, 1995).

2. DIETARY FAT AND OBESITY – LITERATURE REVIEW

Research relating dietary fat intake and obesity comes from a variety of sources: observational studies (including population, prospective, cross-sectional and case-control studies), intervention trials, clinical studies and animal experiments. Chapter 2.1 describes some of the main features of dietary fat intake. Chapter 2.2 provides a review of the observational evidence relating dietary fat intake and overweight/obesity in humans and discusses some of the methodological difficulties involved in interpreting the results from such studies. Chapter 2.3 summarises the main evidence from the human clinical and experimental literature.

2.1 Dietary fat intake - general concepts

Dietary fat is mainly a mixture of triglycerides, each triglyceride being a combination of a unit of glycerol with three fatty acids. Depending on the number of double bonds they contain, fatty acids are classified as saturated (no double bonds), monounsaturated (one double bond), and polyunsaturated (two or more double bonds). Unsaturated fatty acids can be changed into saturated fatty acids and into a mixture of cis and trans monounsaturated fatty acids by controlled treatment with hydrogen (hydrogenation); trans fatty acids behave in some respects like saturated fatty acids. All fats contain all types of fatty acids but in widely varying proportions depending on the source. Types of fats and oils are defined on the basis of the type of fatty acids that is prevailing in their composition.

Dietary fat includes not only 'visible fat', such as butter and margarine, cooking fats and oils, and the visible fat from meat, but also 'invisible fat' which can be found in milk, nuts, lean meat and other animal and vegetable foods. Fat is the most concentrated source of energy (9 kcal/g) compared to carbohydrate, protein (4 kcal/g) and alcohol (7 kcal/g).

2.2 Evidence from observational studies

This chapter reviews evidence from population, prospective, cross-sectional and case-control studies relating dietary fat intake and overweight/obesity in humans and discusses some of the methodological difficulties underlying such studies.

Published reports of observational studies relating dietary fat intake or fat-rich foods to overweight/obesity were identified through a MEDLINE search (back to 1980) conducted for any combination of obesity and dietary fat related keywords (obesity, energy balance, nutrient balance, dietary fat), through previous reviews, and by personal contact and discussion with investigators in the field. Studies reviewed were in the English language and based on humans; animal data and studies which only explored mechanisms were excluded. An abstract of each reference was checked and if the study met the inclusion criteria, a copy of the paper was obtained. In addition, for each paper the associated reference list was checked for further relevant papers. Information on the following issues was abstracted from each paper and tabulated: year, study-design, sample size, study population (including age and sex), measurement and analysis of obesity, measurement and analysis of fat intake, adjustment for confounders, results, methodological problems concerning validity of the dietary assessment method, multi-collinearity, under-reporting, and method used to adjust for energy in design or analysis. This approach resulted in a thorough review of classical and more recent studies and abstraction of important relevant data to be discussed and further considered.

It should be noted that only cross-sectional (case-control) and cohort studies were included in the detailed review presented in this report, since methodological issues involved in these studies are more relevant to the present study. However, all approaches investigating the association between dietary fat intake and obesity were reviewed and included in the bibliography.

2.2.1 Population studies

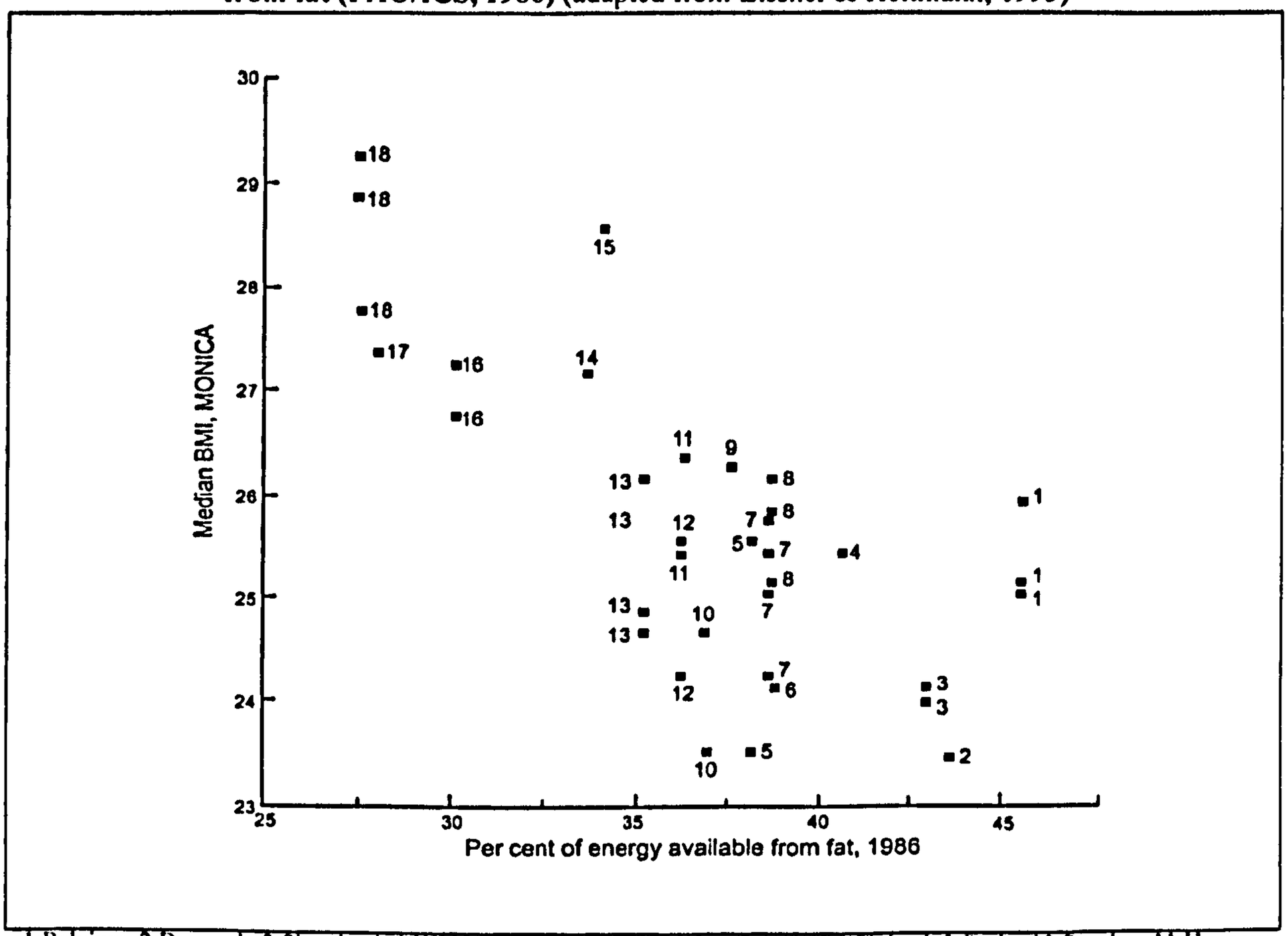
The most basic level of epidemiological evidence describing dietary fat intake and obesity at the population level comes from ecological studies.

2.2.2.1 Correlation (multi-country) studies

Primarily, in multi-country studies, a higher prevalence of overweight and obesity was observed in more affluent countries, where the consumption of dietary fat intake tended to be higher, rather than in poorer regions of the world with lower fat consumption (Lissner & Heitmann, 1995). This led to the suggestion that dietary fat intake may be involved in the development of obesity. However, Willett (Willett, 1998a, Willett, 1998 b) claimed that, since such multi-country observations may be exceedingly confounded by extreme variations in affluence, and subsequently food availability, as well as physical activity level, more essential are comparisons within regions of the world with similar degree of economic development. In this regard, percent of energy available from fat, derived from national food balance data, from 18 European countries, was not found to be positively associated with the median BMI of adults in the same countries, as measured by the WHO-MONICA surveys. No associations or trends were seen in men, while in women higher fat diets were associated with lower rather than higher BMI (Figure 3 adapted from Lissner & Heitmann, 1995).

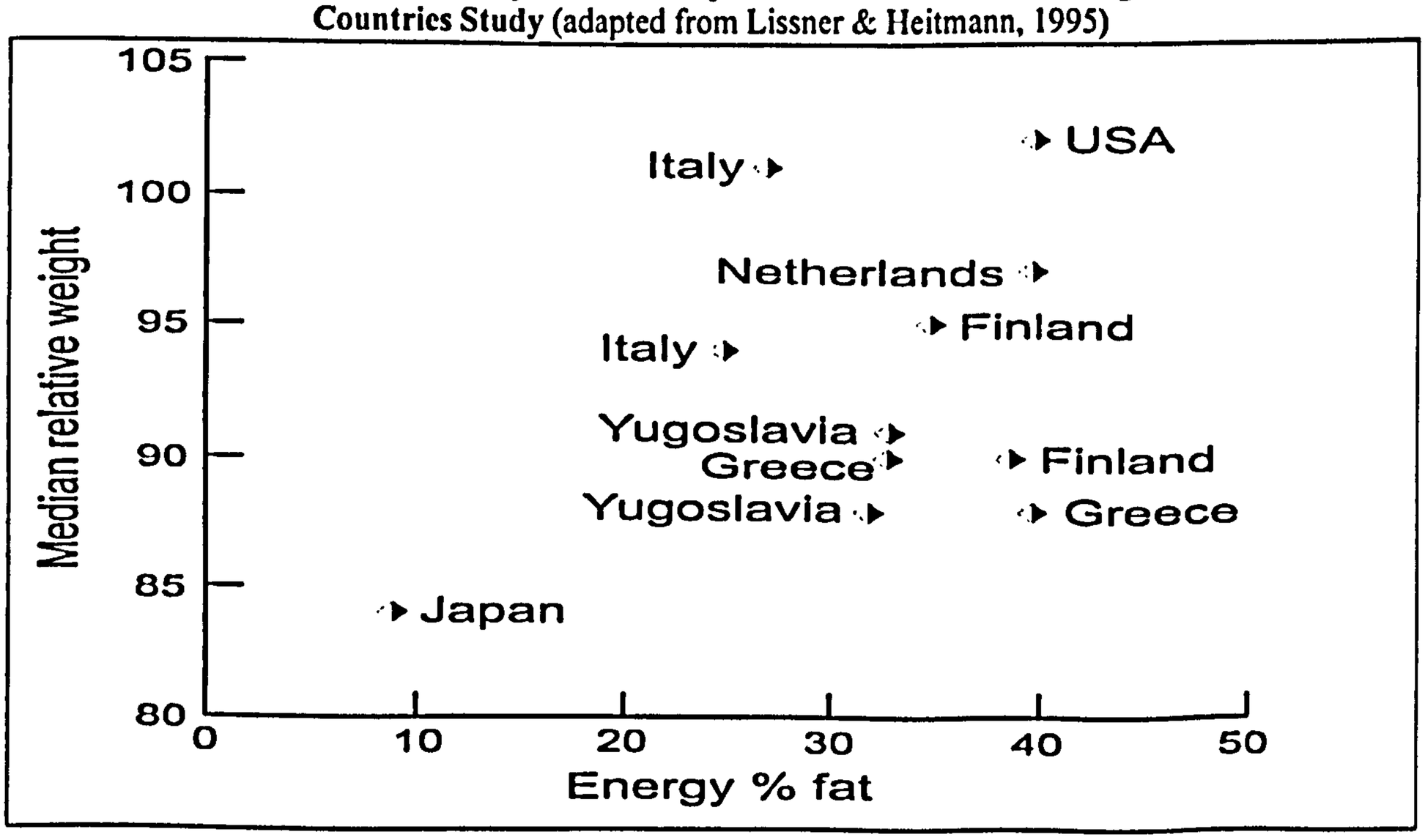
Also, dietary survey intake data from the classic Seven Countries Study (Keys, 1980), produced no clear associations between dietary fat and relative weight among populations, whose food intake were directly surveyed (Figure 4 adapted from Lissner & Heitmann, 1995). Furthermore, in a within-country comparison study, conducted in 1983, among 65 counties in China, no correlation was found between dietary fat intake and body weight (Chen et al, 1990).

Figure 3. Median BMI of women in MONICA surveys (1982-1986) versus % of energy available from fat (FAO/ICS, 1986) (adapted from Lissner & Heitmann, 1995)



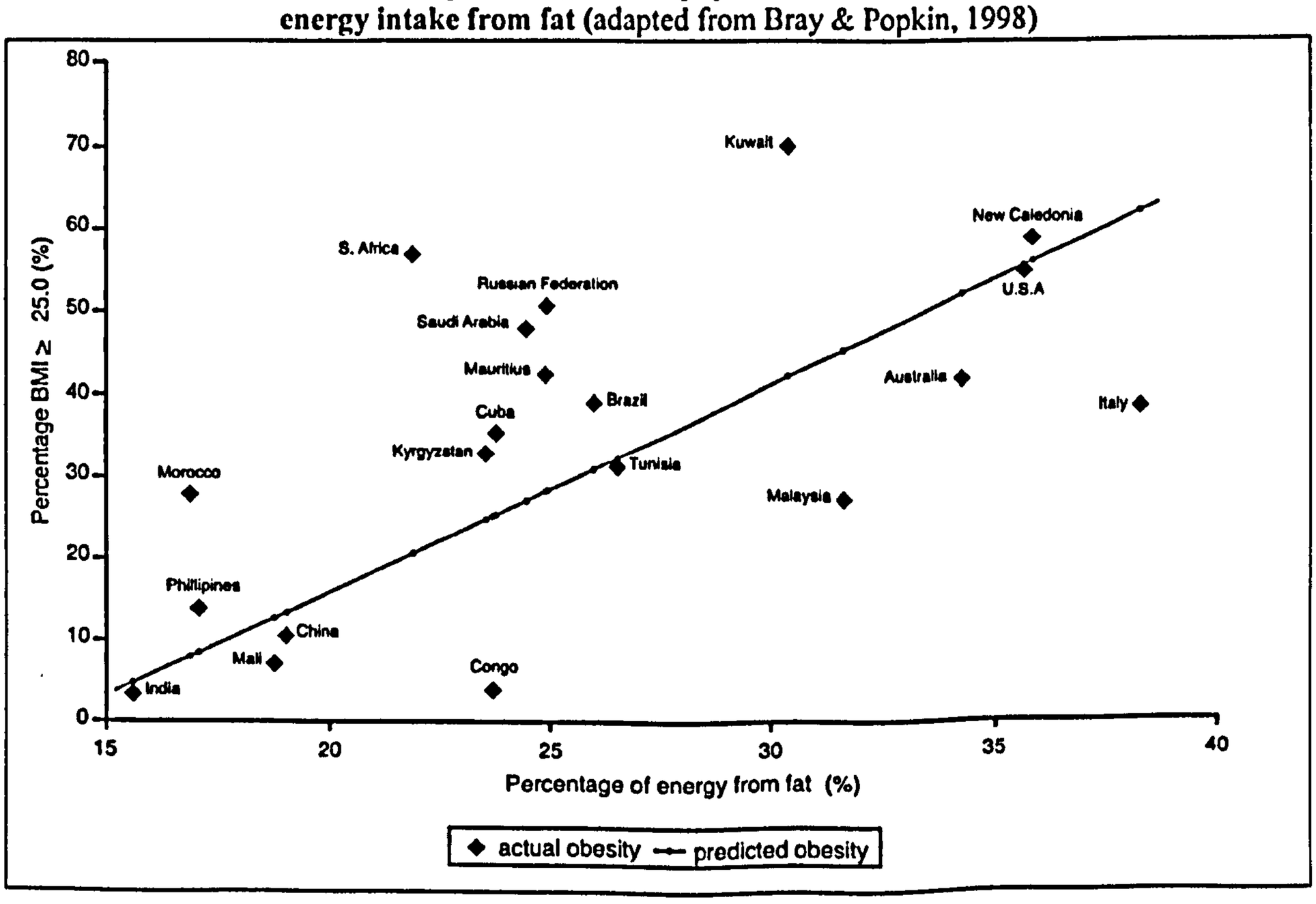
1:Belgium, 2:Denmark, 3:Sitzerland, 4:UK, 5:France, 6:Iceland, 7:West Germany, 8:Finland, 9:Spain, 10:Sweden, 11:Hungary, 12: Italy, 13:East Germany, 14:Chechoslovakia, 15:Malta 16:Poland, 17: Yugoslavia, 18:USSR

Figure 4. Association between percent dietary fat and median relative weight in the Seven Countries Study (adapted from Lissner & Heitmann, 1995)



A recent report on the world wide phenomenon of obesity epidemic, prepared by Popkin and Doak, does not seem to support the above mentioned findings. The authors used a large set of nationally representative surveys providing adult weight and height data, conducted in 20 several low and middle income countries from different regions of the world (Popkin & Doak, 1998). The proportion of energy from fat was derived from national food balance data. An ordinary least squares regression analysis that weighted each country with each population was used to relate the BMI (Figure 5 adapted from Bray & Popkin, 1998) to the proportion of dietary energy from fat. The authors concluded that there was a large significant, positive association between dietary fat consumption and the proportion of the population that was overweight (BMI≥25 kg/m^2). However, the interpretation of the results was challenged by Willett (Willett, 1998a, Willett, 1998b) who claimed that with a more careful look at data, one might notice that nearly 60% of the South African population is overweight, with an intake of about 22% of energy from fat (Figure 5) suggesting that a massive obesity problem may occur even with fat intakes that are generally considered to be low.

Figure 5. Relation between the percentage of the population that is obese and the proportion of energy intake from fat (adapted from Bray & Popkin, 1998)



2.2.2.2 Secular trends

Secular trends provide another epidemiological approach using aggregated data. It has been suggested that the rising prevalence of obesity in many wealthy countries over the past 50-100 years has occurred in parallel to an increasing proportion of energy derived from fat and a decreasing proportion derived from carbohydrate. Since the gene pool has remained fairly constant in this short period, environmental and behavioural changes are considered to represent the largest contributors to the rise in obesity (Prentice & Poppitt, 1996).

The trend towards a fattier diet has been particularly well documented in the United Kingdom, where the National Food Survey has been conducted on an annual basis for over 50 years (Prentice & Poppitt, 1996). Similarly, in a study of 377,200 Danish military recruits (1943-1974) (Lissner & Heitmann, 1995), there was a marked parallel increase in the percentage of energy from fat and the subsequent increase in obesity (defined as BMI>31 kg/m^2). In a similar set of studies conducted among the Pima Indians in the United States (Price et al, 1993), parallel changes were observed between the prevalence of obesity and the proportion of energy from fat. Consistent are also results from research on the nutrition and lifestyle transition in China, Brazil, South Africa, and other countries (Popkin et al, 1995, Bray & Popkin 1998, Popkin & Doak, 1998). In each case, increases in consumption of proportion of energy from fat, have been parallel with increases in obesity.

It has been argued, however, that although correlations in time are generally informative, time trends in dietary and body fat within countries, making a transition from poverty or agrarian lifestyles to greater affluence, are likely to be confounded by changes in food availability and level of physical activity, since higher fat intake typically accompanies newly gained affluence. The results have been further challenged by what is called 'the American Paradox', according to which over the last 25 years, in the US (and several other northern European countries), the

prevalence of obesity has increased despite reductions in dietary fat; the reason might be that in these countries, fat intake and affluence have been disassociated by conscious efforts to reduce fat intake (Heini & Weinsier, 1997, Willett, 1998a, Willett, 1998b). Of course, one may not exclude the potential that if dietary fat intake was increasing in these countries, the prevalence of obesity might increase more rapidly, or that the fat intake may have increased in those segments of society in whom the prevalence of obesity has increased, while decreasing in other segments or finally that obesity is caused by many underlying biological factors (Bray & Popkin, 1998).

2.2.2.3 Migration studies

A special design of the ecological study that avoids confounding by ethnic differences is the migration study. Curb and Marcus showed that Japanese men living in Japan had a lower incidence of obesity (expressed by mean BMI and sub-scapular skinfold thickness) and a lower dietary fat intake compared to first and second generation Japanese immigrants living in Hawaii or California. Presumably, the adoption of Western lifestyles and diet by Japanese in the USA has been associated with adverse effects on body weight (Curb & Marcus, 1991).

2.2.2.4 Conclusions

In conclusion, the evidence from ecological studies is rather inconclusive and seems to vary with the quality of the dietary data collected. The use of national food consumption data is an important limitation of this epidemiological approach, since it cannot be assumed that figures used for comparison reflect the population's dietary consumption with the same accuracy in all regions. Different methods (cut-off points used) for describing obesity in populations can create further uncertainties in interpreting ecological studies. Furthermore, inconsistencies between different studies may be due to various potential ecological fallacies due to both confounding

by unmeasured variables such as food availability, activity levels, smoking and cultural attitudes towards body fat as well as unknown data quality factors that may differ systematically across the populations studied.

2.2.2 Prospective studies

The prospective study is generally considered to be the strongest observational epidemiological design since it provides information on the temporal association between the hypothesised cause and effect. According to this approach an individual's weight change over time is typically studied as a function of dietary fat intake at the beginning of the study.

Six published reports of prospective studies relating dietary fat intake or fat rich foods to obesity were identified. Four were from the USA, one from Europe and one from Asia. One report comes from the US National Health and Examination Survey (Kant et al, 1995) and one from the Nurses' Health Study (Colditz et al, 1995). Four reports focused on males and females, while two included females only. Sample size varied widely from 294 (Klesges et al, 1992) to around 31,940 in the US Nurses' Study (Colditz et al, 1995). Details on study design and relevant issues are given in Table 6.

One of the most recent studies, published in 1997, investigated prospectively the relationship between baseline diet composition and weight gain among 465 adult men and women from southeastern New England in the USA. Participants had completed a Food Frequency Questionnaire (FFQ) at baseline (1986-1987) and were re-interviewed four years later. Multiple regression analysis revealed that after adjusting for age, smoking status, baseline BMI, physical activity level and total energy, none of the macronutrients were significantly related to weight gain (Parker et al, 1997).

Also, recent nation-wide data from the China Health and Nutritional Survey, an ongoing longitudinal survey of 8 provinces, published in 1995, related individual dietary changes over time to obesity in Chinese adolescents and adults (Popkin et al, 1995). A multistage, random, cluster sampling procedure was used to draw the sample from each province. Dietary data were collected using three 24-hour recalls for each individual completed in the 1991 and 1993 surveys. Weight, height and physical activity data were also measured. Data for adults were collected between 1989 and 1993. The authors examined changes in other confounders (e.g. physical activity and smoking in adults) that may vary over time. The hypothesis that energy from fat has an independent effect on body fatness, was tested after controlling for total energy intake as well as for the effect of changes in energy from all non-fat sources. In both cases, the authors concluded that there is a significant independent effect of energy from fat on BMI.

A study by Kant (Kant et al, 1995) published in 1995, involved 7,147 subjects aged 25-74 years, who had their nutrient intakes and body weight recorded at baseline in 1971-1974 and who had body weight re-measured in 1982 as part of the National Health Epidemiologic Follow-up Survey (1982-1984). The purpose of the study was to investigate the association of percent energy intake from fat with subsequent weight change. Weight change was defined as the difference between the follow-up weight and the baseline weight. Fat intake was estimated from a 24-hour dietary recall obtained at baseline. The multivariate model included percentage of fat energy, energy intake, age, smoking status, race, physical activity, education, length of follow up, baseline BMI, parity, alcohol intake, special diet status, and morbidity. The authors concluded that, baseline percent fat energy intake and subsequent weight change, over 8-10 years of follow-up, were inversely related in women aged <50 years, but positively associated in men without any morbidity.

In the context of the Nurses' Health Study, Colditz (Colditz et al, 1995) used a combined prospective and retrospective design in which prior and subsequent weight

gain in 31,940 women aged 30-55 years were examined in relation to fat intake. The women were followed-up for eight years. Each woman reported her weight every two years on questionnaires, whilst diet was assessed at baseline with a semi-quantitative FFQ. Cross-sectional analyses indicated a positive association between total fat intake and BMI. In the prospective analysis, fat intake was positively associated with previous rather than subsequent weight changes. Interestingly in this study, distinct differences were seen between the various types of dietary fat in the prediction of subsequent weight gain in female nurses. Thus, although total fat was not a significant risk factor for weight change in this study, animal and vegetable derived fats respectively were positively and negatively associated with weight change in an energy-independent fashion. The authors concluded that it was not clear whether this contrast reflected chemical properties of dietary fat or confounding by healthier lifestyles among consumers who prefer vegetable-based fat. Unfortunately, important potential confounders, such as level of physical activity, were not adjusted for in these analyses.

A six-year longitudinal study of 361 women aged 38-60 years in Gothenburg, Sweden addressed the issue of potential interaction between dietary and genetic influences in the prediction of weight gain (Heitmann et al, 1995). Women were first examined in 1968-1969. Dietary intake was estimated by diet history interview and parental fatness by questionnaire. Results suggested that fat intake at the time of the initial examination, adjusted for total energy intake, smoking habits, physical activity and menopausal status was an independent risk factor for weight gain over the following six years only in those relatively overweight women with at least one obese parent (predisposed) but not in those without a genetic predisposition. This suggestion of a genetic predisposition to weight gain with exposure to high fat diets could be of considerable importance in evaluating such longitudinal studies.

In a study by Klesges (Klesges et al, 1992) data on dietary intake, measured using the Willett's FFQ, physical exercise and body weight were collected on 142 adult men and 152 adult women over a three-year period. Weight gain over the latter two years was compared with baseline variables in a multivariate model controlling for energy intake, leisure and occupational physical activity, alcohol consumption and smoking. Results indicated a different pattern of predictors of weight change for men and women. It was found that the proportion of fat- derived energy was a positive predictor of weight change both in men and women. However, in women, energy percent fat at baseline was an independent risk factor for subsequent weight gain, while the strongest predictor among men was current change in fat intake.

There have also been studies, which although not primarily investigating the association between dietary fat and obesity, do provide relevant data. Thus, in 1991, Rissanen (Rissanen et al, 1991) using a basic prospective approach studied sociodemographic and behavioral factors as predictors of weight gain in 12,669 adult Finns who were examined twice with a median interval of 5.7 years. The authors reported a 70% excess risk of substantial weight gain over five years among women (but not men) with dietary fat intakes in the highest quintile. However, similar associations were seen with high intake of the other macronutrients, making it unclear whether the fat concentration in the diet was specifically associated with weight gain. Furthermore, in the context of the San Antonio Heart Study, a population based study of diabetes and cardiovascular disease in 1,030 Mexican-Americans and 580 non-Hispanic whites, there was no convincing evidence for the effect of dietary fat (and other macronutrients) in predicting future obesity in Mexican Americans (Haffner et al, 1991).

In conclusion, relatively few prospective studies of diet in relation to subsequent weight change have been published and the results have been rather equivocal; three longitudinal studies out of 8 reviewed, clearly report weight gain in individuals consuming a diet with a higher fat content (Klesges et al, 1992, Popkin et al, 1995, Colditz et al, 1995).

The observed inconsistency may be due to several methodological issues which are common for both prospective and cross-sectional studies and will be discussed below (see Chapter 2.2.4). In addition to those, however, behavioral factors, involving dieting in response to weight gain, may have similar implications. Since individuals are aware of the dependent variable, body weight, and also have conscious control over its primary determinants, physical activity, total energy intake, as well as the percentage of energy from fat, their initial dietary practices may be irrelevant to the cause of their weight fluctuations. Once more, longitudinal analysis of dietary fat intake and weight gain during periods of transition from poverty to a modern society is problematic, since dietary fat intake may have increased concurrently with increases in wealth and food availability, and declines in physical activity. Finally, this type of study has rarely taken into account the possible interaction between genetic predisposition and dietary fat in promoting weight gain.

2.2.3 Cross-sectional (and case-control) studies

In dietary surveys cross-sectional studies may also be considered retrospective, since usual or previous dietary exposures are often studied in relation to body weight (outcome). Cross-sectional studies cannot distinguish a temporal sequence between diet and obesity and merely establish a non-directional relationship. Thus, a significant association between dietary fat intake and obesity could be the result of one or more of the following conditions: a) obesity could cause increased dietary fat consumption; b) dietary fat intake and obesity could each be related to other factors, resulting in spurious causal associations between fat consumption and body fat; c) high dietary fat intake could cause increased body fat. Furthermore, cross-sectional studies of diet and body weight may also well be complicated by the possibility that persons who consider themselves to be overweight may have altered their diets to reduce weight during the survey period.

Twenty-one published reports of cross-sectional studies relating dietary fat intake to obesity were identified. Fourteen were from Northern America, five from Europe one from Australia and one from Asia. Two reports also included a longitudinal component (Klesges et al, 1992, Colditz et al, 1995). Nine reports focused on males and females, seven on males and five on females only. Sample size varied widely from 78 (Miller et al, 1994) to around 31,940 in the US Nurses' Study (Colditz et al, 1995). Details on study design and results are given in Table 6.

The most recent study of Richards (Richards et al, 2000) addressed the issue of potential interaction between dietary and genetic influences in the prediction of weight gain by exploring the association between functional status and emotional well-being with energy and nutrient intake in 145 sibling pairs of twins (290 individuals) raised together in the same family. It was shown that the severely obese siblings (BMI≥30 kg/m^2) had a higher energy and percent fat intake (3% higher) and a lower physical activity energy expenditure, compared with normal weight siblings (BMI≤27 kg/m^2).

Samaras (Samaras et al, 1998), investigated the relationship between dietary fat intake (total and subtype) and total body and central abdominal fat in middle-aged female twins. Evidence of interaction between diet and genetic predisposition was further explored in 90 monozygotic pairs. Measurements in 436 female twins included dietary intake assessed through a FFQ, BMI, total body and central abdominal fat, and other environmental factors (smoking status, hormone replacement therapy and physical activity). Cross-sectional analyses demonstrated no relationship between dietary fat and body fat, particularly after controlling for genetic and environmental factors included in the study protocol. There was no evidence of a gene-environment interaction between fat intake contributing to greater body mass in subjects genetically predisposed to obesity.

The determinants of central obesity (expressed as waist circumference/hip circumference) were explored in a cross-sectional design in the context of the Indian Women's Health Study (Singh et al, 1998). 3,212 women aged 25-64 years were randomly selected from different parts of India. They provided dietary data by determining 7-day food intake and they went through a physical examination and anthropometric measurements of weight, height, waist and hip circumferences. Physical activity was also assessed. Logistic regression analysis revealed that excess intake of fat (>21% energy intake/day) was weakly associated with central obesity after controlling for age, BMI, sedentary lifestyle and a family history of obesity.

In the context of the Leeds Fat Study, a re-analysis of data from the Dietary and Nutritional Survey of British Adults was undertaken, in order to explore the different characteristics of high and low fat consumers, in particular their macronutrient intake and body mass index. 1,240 subjects were classified as high or low fat consumers on the basis of 7-day weighed food records. Comparisons revealed that the frequency distribution of BMI in individuals who consumed a high fat diet was higher compared with those who ate a low-fat diet. However, as the authors noted, there were large numbers of individuals eating the high fat diet whose BMI was entirely normal, suggesting that there are important underlying physiologic factors, probably related to a genetic basis, that influence whether there was an increase in BMI in consumers of a high fat diet (Macdiarmid et al, 1996).

Larson (Larson et al, 1996) evaluated the association between dietary fat and adipose tissue stores in 135 men and 214 adult white men and women. Dietary intake was estimated from 3-day food records, body composition from hydrostatic weighing, intra-abdominal and sub-cutaneous adipose tissue by computed tomography and physical activity by using the Baecke questionnaire. Analysis revealed that after adjustment for fat-free mass, sex, age, physical activity and non-fat energy, fat intake plays a minor role in increasing overall adiposity and does not specifically influence fat accumulation in the intra-abdominal region (Larson et al, 1996).

In a study by Andersson (Andersson & Rossner, 1996) the energy and macronutrient intake was compared in 86 obese and 61 normal weight men. Dietary intake was assessed through 12 telephone-administered 24-hour dietary recalls conducted over a period of three months. Obese men reported a higher protein and a lower alcohol intake compared to that of the lean subjects. However, when separated according to tertiles of reported energy intake, the obese men in the highest tertile reported a higher intake from fat and a lower intake from protein compared to men in the lowest tertile. The same pattern was observed in lean men.

Nelson and Tucker (Nelson & Tucker, 1996) evaluated whether usual diet, especially intake of fat, carbohydrate and fiber, was related to body fat percentage in 203 healthy men aged 21 to 71 years. Dietary intake was estimated from a food frequency questionnaire. Percentage body fat was determined using three-site skinfold measurements. Finally a submaximal treadmill test was used to estimate aerobic fitness. In multiple linear regression analysis dietary fat intake was not significantly related to body fat after controlling for energy intake, fitness level, body weight and age. However, multivariate analysis of variance revealed that when subjects were separated into low-, moderate-, and high-body-fat groups, the fattest subjects reported eating significantly more dietary fat compared to the leanest subjects.

In a study by Tremblay (Tremblay et al, 1995) the association between adiposity, lipid and alcohol intake and physical activity participation was explored in a sample of 358 male subjects who participated in the first phase of the Quebec Family Study. Dietary intake was determined using a 3-day dietary record. Significant positive correlations were observed between the percentage of dietary energy as fat and adiposity indicators. Accordingly, subjects classified as low fat consumers displayed significantly lower levels of fat mass and subcutaneous adiposity compared to high fat consumers.

A study by Miller (Miller et al, 1994) explored the relationships between dietary fat and carbohydrate and body fatness in a sample of 23 lean men, 17 lean women, 23 obese men and 15 obese women. Body composition was determined by hydrostatic weighing at residual volume. Dietary intake was assessed using a three-day food diary and a FFQ. Multivariate analysis revealed that obese subjects derived a greater proportion of their energy from fat. Percent of fat intake for saturated, mono-unsaturated and poly-unsaturated fats was not different among groups.

The study by Tucker and Kano (Tucker & Kano, 1992) examined the association between fat intake and body fat percentage in 205 adult females aged 22-47 years recruited from seven businesses in western United States. Subjects completed a written questionnaire regarding lifestyle factors (including physical activity and smoking habits), demographic data, whereas the National Cancer Institute's Health Habits and History Questionnaire was used to assess the usual, self-selected diets of individuals over the past year. Skinfold-thickness measures were used to ascertain the percentage of body fat. Results showed that increased dietary fat intake was related significantly to increased adiposity, with and without control for potential confounders: age, total energy intake, total exercise time per week, years of regular physical activity, consumption of other macronutrients and smoking.

The association between body fat and its distribution with dietary fat intake was not supported in a study by Slattery (Slattery et al, 1992) who investigated the association between body fat and several environmental factors in 5,115 young black and white adults. Dietary-intake data were obtained using a detailed diet history questionnaire. Multivariate regression analyses, used to further explore the association between body fat and dietary intake, physical activity, energy yielding nutrients and different intensities of energy expenditure, suggested that as a percentage of kilojoules (kilocalories), fat was not associated with body fat or its distribution.

Miller (Miller et al, 1990) examined the relationships between body fat, diet composition, energy intake and exercise in 216 males and females aged 18-71 years. Body fat was determined by hydrostatic weighing. Data on diet and exercise behaviours were investigated using questionnaires. Diet was assessed by a variety of methods including: an eating behaviour questionnaire evaluating the antecedents to eating, eating behaviour, and consequences of eating, a dietary recall performed for the 24 hours before testing; a 2-day food dairy used to record all food and beverage intake over one weekday and one weekend day; and finally an extended diet history that records specific food frequency intake over periods of up to one month. According to the results, as body fat increased, percent of energy intake derived from fat increased whereas the percent from carbohydrate decreased. When subgroups of lean and obese subjects were compared, the lean subjects derived about 29% of their energy from fat and 53% from carbohydrate vs 35% and 46% respectively for the obese subjects.

The relationship between dietary fat intake and regional adiposity was studied by George in 344 men and 335 women aged 35-55 years (George et al, 1990). Three-day food records were used to estimate total energy intake and percent intake of all macronutrients. Subjects were categorised as having an energy intake from fat that was either low or high; categories of low and high fat consumers were determined from the lower and upper quartiles of percent intake from fat. Results showed that in both genders, subjects with a higher percentage of energy as fat weighed significantly more, had a higher BMI and had higher fat mass indices than subjects with a low fat intake.

In a three-component study performed by Tremblay (Tremblay et al, 1989) to assess the effects of a high-fat diet and exercise-induced changes on fat oxidation and energy intake in humans, a cross-sectional design was used to evaluate the effects of a high fat diet on adiposity. The study sample consisted of 244 healthy adult males. Food intake was measured by the use of a 3-day diet diary. Results indicated

significant positive correlations between the percentage of energy from fat and body fat mass, percent body fat, and sum of subcutaneous skinfold thicknesses.

In the study of Dreon (Dreon et al, 1988) the relationship between obesity, nutrient intakes and dietary habits were examined. Nutrient intakes from 7-day diet diaries were compared with hydrostatically determined body composition in 155 sedentary obese men aged 30-59 years. Univariate analysis indicated that percent body fat correlated positively with the density (g/1000 kcal) of total, saturated and monounsaturated fatty acids, and inversely with carbohydrates and plant protein. Physical activity level was controlled for in the design of the study by including only subjects with sedentary lifestyles.

In the context of a study conducted to validate a self administered semi-quantitative food-frequency questionnaire, Romieu (Romieu et al, 1988) investigated the relationships of relative weight to energy intake and physical activity in a sample of 141 women aged 34-59 years. Dietary intake data was collected using four one-week weighed records of food intake. After adjustment for energy intake, age, physical activity, alcohol and smoking, obese women were found to report higher intakes of total fat. Relative weight was significantly correlated with intakes of total fat and saturated fatty acids.

The determinants of body mass index were further explored in a study investigating the associations between smoking habits, social class, body mass index and diet (Fehily et al, 1984). The study was conducted on a sample of 493 men aged 45 to 59 years. Dietary intake data were collected by completion of a 7-day weighed dietary record. In univariate analysis no association was found between dietary fat intake and BMI.

In the cross-sectional analysis conducted using the Zutphen Study's data (Kromhout, 1983), relationships between dietary variables and indicators of body weight and

adiposity were examined in a cohort of 871 middle aged men. Food intake data were collected using the diet history method. In univariate analyses saturated, mono-unsaturated and polyunsaturated fat were significantly inversely related to BMI. In multivariate analyses, however, saturated fat intake was significantly positively related to BMI after energy intake per kg body weight was taken into account. In addition polyunsaturated fat was significantly positively related to the sum of skinfold thicknesses (measure of adiposity) and monounsaturated fat to BMI.

Most cross-sectional studies reviewed have examined the association between macronutrient intake and obesity. The association between the fat to carbohydrate ratio and obesity has rarely been examined. Bolton-Smith and Woodward (Bolton-Smith & Woodward, 1994), using existing dietary survey data from the Scottish Heart Health Study, analysed the prevalence of obesity in 11,600 Scottish men and women after dividing them into fifths according to their intakes of sugars, fat and the fat to sugars ratio. Neither starch non intrinsic sugar (percent energy) were found to be significantly associated with obesity. Both extrinsic sugar and fat were significantly associated with the prevalence of obesity. Significant inverse associations were found between sugar intake (as a proportion of total energy) and obesity and direct associations were reported between fat (as a proportion of total energy) and obesity. The effect was significant in both men and women, when data analysed separately. These data appear to support the importance of the ratio of fat to carbohydrate in the diet for determining the prevalence of overweight and obesity.

In conclusion, seventeen cross-sectional studies out of the twenty-one reviewed show that obese individuals consume a diet higher in fat compared to their lean counterparts (Richards et al, 2000, Singh et al, 1998, Macdiarmid et al, 1996, Larson et al, 1996, Nelson & Tucker, 1996, Tremblay et al, 1995, Colditz et al, 1995, Bolton-Smith & Woodward, 1994, Miller et al, 1994, Tucker & Kano, 1992, Klesges et al, 1992, George et al, 1990, Miller et al, 1990, Tremblay et al, 1989, Romieu et al, 1988, Dreon et al, 1988, Kromhout, 1983). Although most of the studies controlled

Table 6. Dietary fat intake and obesity: evidence from observational studies

First author	Country	Design	Sample	Age	Dietary Method	Validity Reproducibility	Outcome Measurement	Under-reporting	Energy adjustment	Confounders	Results
Richards et al, (2000)	USA	XS	290 (145*2) M & F Twins	Adults	FFQ	Discussed	BMI	Discussed	Nutrient density method	Physical activity, alcohol intake, smoking, Socioeconomic status	(+)
Samaras et al, (1998)	Australia	XS	436 F (213*2) twins	48-68	FFQ	(+)	BMI % body fat % abdominal fat	Exclusion of under-reporters	Nutrient density method	Smoking habit, Hormone Replacement Therapy, physical activity	(-)
Singh et al, (1998)	India	XS	3,212 F	25-64	7-d food record	-	WHR	-	Nutrient density method	Age, BMI, sedentary life style, predisposition to obesity (alcohol, smoking)	(+)
Parker et al, (1997)	USA	CH	176 M 289 F	18-64	FFQ	Not in the same population	Weight change (10 yrs)	-	Standard multivariate method	Age, smoking, baseline BMI, physical activity, (pregnancy)	n.s. + men n.s + women
Macdiarmid et al, (1996)	UK	XS	169 M 156 F	16-64	7-d weighed food record	Discussed	BMI	Exclusion of under-reporters	Nutrient density method	Age, sex, alcohol and smoking	Men (+) Women (+)
Larson et al, (1996)	USA	XS	135 M 214 F	31-59	3-d food record	-	Fat mass IAAT SCAAT TAAT	Discussed	Energy decomposition model	Fat-free mass, sex, age, physical activity, menopausal status, (pregnancy, morbidity)	Fat mass (+) TAAT (+) SCAAT (+) IAAT (+)
Anderson et al, (1996)	Sweden	XS	86 obese 61 normal weight M	20-60	12* 24h recalls	Discussed	BMI	Discussed	Nutrient density method	Age (matched), (morbidity)	Obese men in tertile 3 of reported EI, reported higher % fat. The same observed in normal – weight subjects
Nelson et al, (1996)	USA	XS	203 M	28-53	FFQ	(+)	% body fat	Discussed	Standard multivariate method	Age, body weight, fitness level (morbidity, alcohol, smoking)	(+)

XS: cross-sectional; CH: Cohort ; FFQ Food Frequency Questionnaire; (-): significant negative association; (+): significant positive association; n.s.: non significant; TF: Total fat; SAT: saturated fat; PUF: polyunsaturated fat; MUF: monounsaturated fat ; BF: Body fat; IAAT: Intra-abdominal adipose tissue, SCAAT: subcutaneous abdominal adipose tissue, TAAT: Total abdominal adipose tissue

Table 6. Dietary fat intake and obesity: evidence from observational studies (continued)

First author	Country	Design	Sample	Age	Dietary Method	Validity Reproducibility	Outcome Measurement	Under-reporting	Energy adjustment	Confounders	Results
Tremblay et al, (1995)	Canada	XS	358 M	30-60	3-d food record	(+)	BMI Fat mass (kg) % body fat	-	Nutrient density method	Physical activity, alcohol intake, household income	(+)
Popkin et al, (1995)	China	CH	1,830 M 2,071 F	20-45	3*24h recalls & (weighted household consumptio data)	(+)	BMI	Discussed	Nutrient density Method Energy decomposition method	Physical activity, alcohol	(+)
Heitmann et al, (1995)	SW	CH	316 F	38-60	- Diet history - 24h recall	(+)	Weight change (6yrs)	Discussed	Standard multivariate method	Genetic predisposition, baseline BMI, age, smoking, physical activity, energy intake, menopausal status	(+) Ow/Op - n.s. Ow/Np - n.s. Nw/Op - n.s. Nw/Np
Colditz et al, (1995)	USA	CH * XS	31,940 F	30-55	FFQ	(+)	Weight gain (4 yrs) BMI	Exclusion of women with implausibly high or low food score, based on foods items eaten per day	Standard multivariate method	sex, age, smoking, alcohol intake, special diet, pregnancy, morbidity BMI at baseline	Weight change (+) BMI (+)
Kant et al, (1995	USA	CH	2,580 M 4,567 F	25-74	24h recall	-	Weight change (8-10 yrs)	Exclusion of unsatisfactory 24-h recalls based on interviewer's judgement	Nutrient density method Standard multivariate method Analysis of fat intake within EI strata	Race, sex, age, education, alcohol, smoking, physical activity, pregnancy, length of follow up, baseline BMI, parity, special diet status, morbidity	(+) males without any morbidity (-) females <50 yrs

XS: cross-sectional; CH: Cohort ; FFQ Food Frequency Questionnaire; (-): significant negative association; (+): significant positive association; n.s.: non significant; TF: Total fat; SAT: saturated fat; PUF: polyunsaturated fat; MUF: monounsaturated fat ; BF: Body fat; IAAT: Intra-abdominal adipose tissue, SCAAT. subcutaneous abdominal adipose tissue, TAAT: Total abdominal adipose tissue

Table 6. Dietary fat intake and obesity: evidence from observational studies (continued)

First author	Country	Design	Sample	Age	Dietary Method	Validity Reproducibility	Outcome Measurement	Under-reporting	Energy adjustment	Confounders	Results
Bolton-Smith & Woodward (1994)	UK	XS	5,768 M 5,858 F	25-64	FFQ	(+)	BMI	EI/BMR quartiles	Nutrient density method	Sex, alcohol intake, slimming diet, physical activity, smoking, menopausal status	(+) males (+) females
Miller et al, (1994)	USA	XS	23 lean, 23 obese, M 17 lean, 15 obese F	Adults	3-d food diary FFQ	-	% body fat	Discussed	Nutrient density method	(metabolic disorder0	Men Fat (+) MUF (+), PUF,SAT n.s. Women Fat (+) MUF (+), PUF n.s. SAT (+)
Tucker et al, (1992)	USA	XS	205 F	22 –47	Diet history	(+)	% body fat	Discussed	Standard multivariate method Nutrient density method	Sex, age, pregnancy Physical activity, macronutrients, smoking	(+)
Slattery et al, (1992)	USA	XS	5,115 M & F W & B	18-30	Diet History	(+)	- BMI - WHR - % body fat	-	Nutrient density method	Race, sex, age, education, smoking, alcohol intake, physical activity, macronutrients, BMI	White: BMI: n.s. + men (+) women WHR: n.s. + men n.s. + women Black: BMI: n.s + men n.s. – women WHR: n.s. –men n.s. + women
Klesges et al, (1992)	USA	CH * XS	142 M 152 F	24-52	FFQ	(+)	Weight gain (3-yrs) BMI	Discussed	Nutrient density method Standard multivariate method	sex, physical activity, alcohol, smoking, family risk of obesity, pregnancy	CH: (+) males (+) females XS: (+) males (+) females

XS: cross-sectional; CH: Cohort ; FFQ Food Frequency Questionnaire; (-): significant negative association; (+): significant positive association; n.s.: non significant; TF: Total fat; SAT: saturated fat; PUF: polyunsaturated fat; MUF: monounsaturated fat ; BF: Body fat; IAAT: Intra-abdominal adipose tissue, SCAAT: subcutaneous abdominal adipose tissue, TAAT: Total abdominal adipose tissue

Table 6. Dietary fat intake and obesity: evidence from observational studies (continued)

First author	Country	Design	Sample	Age	Dietary Method	Validity Reproducibility	Outcome Measurement	Under-reporting	Energy adjustment	Confounders	Results
George et al, (1990)	Canada	XS	344 M 335 F	35-55	3-d food record	(+)	Body weight BMI % body fat Fat mass (kg)	Discussed	Standard multivariate method Nutrient density method	Age, sex, physical activity, morbidity	Body weight: (+) men (+) women BMI: (+) men (+) women % BF: + n.s. men + n.s. females Fat mass: + n.s. men + n.s. females
Miller et al, (1990)	USA	XS	107 M 109 F	18-71	- Diet history - 2-d food record - 24h recall	(+)	% body fat	Discussed	Nutrient density method	sex (physical activity used in an independent univariate analysis)	(+) men (+) women
Tremblay et al,(1989)	Canada	XS	244 M	30-50	3-d food record	Reproducibility assessed	% body fat	-	Nutrient density method	-	(+)
Dreon et al, (1988)	USA	XS	155 M	30-59	7-d food record	(+)	BMI % body fat	-	Nutrient g /1000kcal	Physical activity	- BMI: TF (+) SAT + n.s. MUF + n.s. PUF + n.s. - % BF: TF (+) SAT (+) MUF (+) PUF + n.s.
Romieu et al, (1988)	USA	XS	141 F	34-59	4*1-wk food records	-	BMI	Discussed	Energy adjusted method	age, height, physical activity, alcohol intake, smoking	TF (+) SAT (+)
Fehily et al, (1984)	UK	XS	493 M	45-59	7-d food record	(+)	BMI	-	-	Social class Smoking habits	(-)
Kromhout et al, (1983)	NL	XS	871 M	Adults	Diet history	-	BMI % body fat	-	+	Macronutrients	BMI: (+) SAT, (+) MUF ST: (+) SAT

XS: cross-sectional; CH: Cohort ; FFQ Food Frequency Questionnaire; (-): significant negative association; (+): significant positive association; n.s.: non significant; TF: Total fat; SAT: saturated fat; PUF: polyunsaturated fat; MUF: monounsaturated fat ; BF: Body fat; IAAT: Intra-abdominal adipose tissue, SCAAT: subcutaneous abdominal adipose tissue, TAAT: Total abdominal adipose tissue

for energy intake by expressing macronutrient intake relative to energy, many studies did not control for other potential confounding factors.

2.2.4 Comments and Conclusions

The observational epidemiologic evidence that a high-fat diet promotes the development of obesity is considered suggestive but not definitive.

Reports of observational studies identified were conducted in different adult populations, including males, females or both. Both cross-sectional and prospective study designs have been utilized. The majority of the observational studies do not appear to have been designed specifically to test the hypothesis that dietary fat intake is associated with obesity but rather are analyses of existing datasets. Sample size ranged from 78 to 31,940 (Table 6).

In the reports reviewed various dietary methodologies, both 'meal' and 'list' based were used: the 24-hour recall (Andersson & Rossner, 1996, Popkin et al, 1995, Kant et al, 1995, Heitmann et al, 1995, Haffner et al, 1991, Miller et al, 1990); the food records (Singh et al, 1998, Macdiarmid et al, 1996, Larson et al, 1996, Tremblay et al, 1995, Miller et al, 1994, George et al, 1990, Miller et al, 1990, Tremblay et al, 1989, Dreon et al, 1988, Romieu et al, 1988, Fehily et al, 1984); the diet history method (Heitmann et al, 1995, Tucker & Kano, 1992, Slattery et al, 1992, Rissanen et al, 1991, Miller et al, 1990, Kromhout et al, 1983); and the Food Frequency Questionnaire (FFQ) (Richards et al, 2000, Samaras et al, 1998, Parker et al, 1997, Nelson et al, 1996, Colditz et al, 1995, Bolton Smith & Woodward, 1994, Miller et al, 1994, Klesges et al, 1992). In some studies more than one dietary assessment methods were used (Heitmann et al, 1995, Miller et al, 1990).

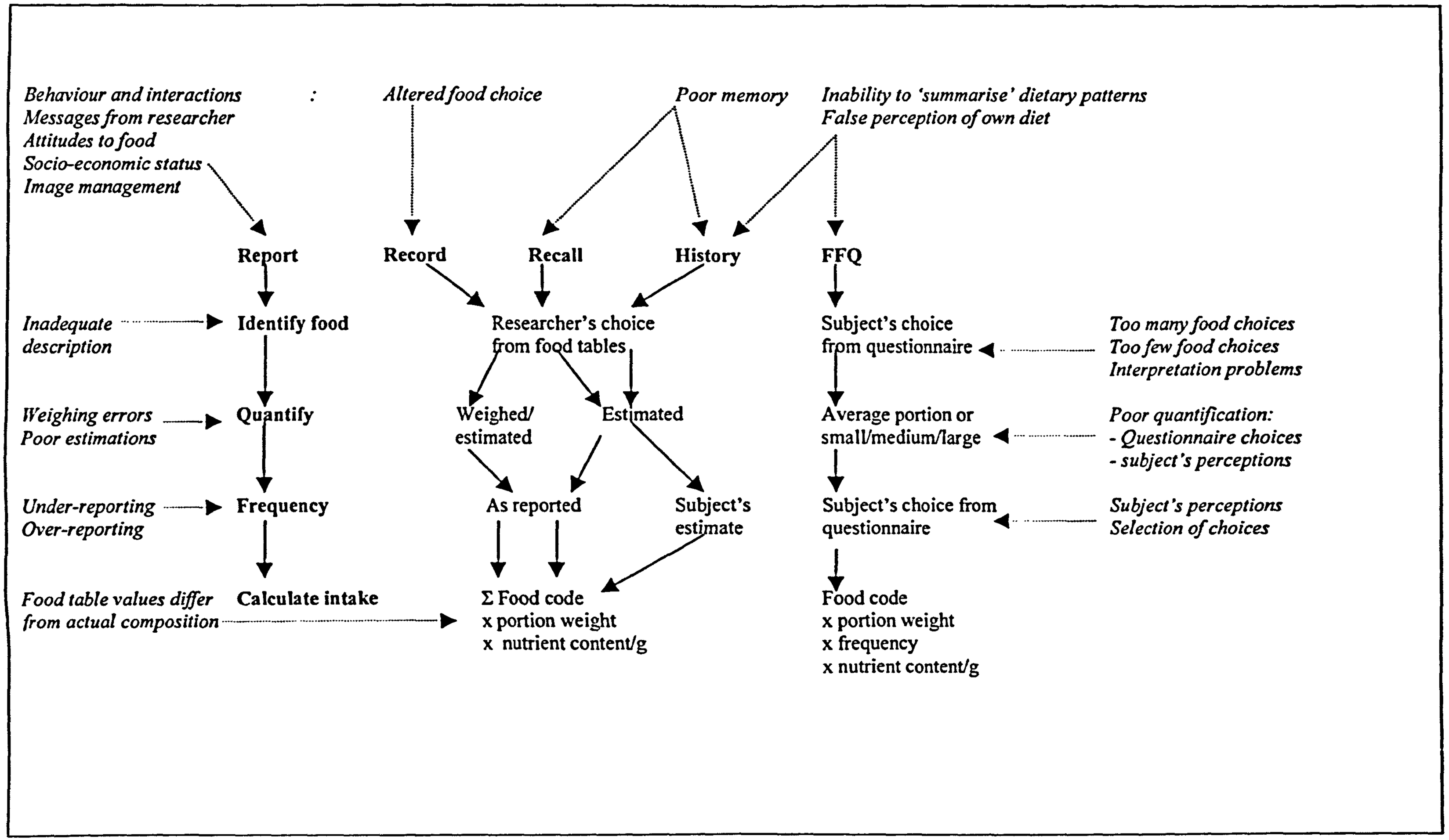
Figure 6 (adapted from Black, 1999) summarizes in a comprehensive way the process of different dietary assessment methods (bold), the variations according to different methods (plain) and most importantly the major sources of error inherent in each of these methods (italic).

'Meal' based methods (recalls and records) may provide accurate, quantitative information on recent intake. However, precision in the assessment of usual or habitual diet is dependent on the number of days of dietary data collection (Nelson et al, 1989, Nelson & Bingham, 1997); in some of the studies reviewed, 'meal' based methods were simply inadequate to classify individuals within the population distribution of true (habitual) intake due to high within to between person variation in fat consumption. Due to high within/between person variance in dietary fat intake in western populations, a limited number of 24-hour recalls or diet records is inadequate in terms of its ability to rank individuals according to their dietary fat intake within a population. It has been estimated that 6-7 days of diet records are required to rank dietary fat intake with desired precision ($r \geq 0.9$) (Nelson et al, 1989, Nelson & Bingham, 1997).

The effect of using an inadequate measure of fat intake is that the tail of the nutrient's distribution is artificially broadened (since the standard deviation is increased). It is thus possible that some individuals will have a higher than average intake on the day sampled, compared with their long-term average, whereas others will have a lower than average intake on the day sampled compared with their long-term average. In univariate analysis, this results in misclassification and subsequently attenuation of the results (regression dilution).

In multivariate analysis, if the variable of interest has measurement error but the covariates do not, the association is attenuated. If both the variable of interest and the covariates have measurement error, the association can be either attenuated or spuriously strengthened (Liu, 1989).

Figure 6. The four basic procedures for dietary assessment (adapted from Black, 1999)



Concerning 'list' based methods, these are supposed to estimate 'habitual' energy intake. Since complete diet history is particularly expensive and time consuming, FFQ has been more frequently used. Actually, FFQ may provide sufficiently valid estimates of usual diet, although it is less quantitative and subject to problems of recall and seasonality, and have become established as the primary method for assessing diet in epidemiologic studies of disease occurrence. It is considered by its users to be a well validated diet survey method. The argument is that several well-designed validation studies that have been conducted (Willett et al, 1985, Willett, 1994, Willett 1998c, Block et al, 1990, Block et al, 1992), have proved that they perform well especially for ranking of individuals within populations rather than estimates of absolute intakes. As a result, measurement error in the dietary data is often not taken into account in the interpretation of the results. Recently, however, re-analyses of the data in the validation studies of these questionnaires suggest that the measurement error associated with the questionnaires may seriously bias the results. In this context, Liu (Liu, 1994) reported that the attenuation factors for the semi-quantitative FFQ may be similar to those associated with a 2-day or 3-day food records or recalls and strongly suggested that validation studies should be conducted to evaluate semi-quantitative FFQ's performance in the specific population to be studied (Liu, 1994).

Concerning data analysis, all studies reviewed controlled for age and sex either in the design or in the analysis. Some reports treated dietary fat intake and/or obesity as continuous variables and others as categorical variables. Analyses have been conducted by employing different definitions (cut-off points) of overweight/obesity. Risk was usually assessed across levels of exposure (sometimes in thirds, fourths or fifths). Control for other important covariates which may confound the association between dietary fat intake and obesity, such as, physical activity, smoking, alcohol consumption, race, educational status, parity, predisposition to obesity, morbidity varied between studies (Table 6). It might therefore be potentially misleading to compare directly, risk estimates from different studies using different cut off points and definitions.

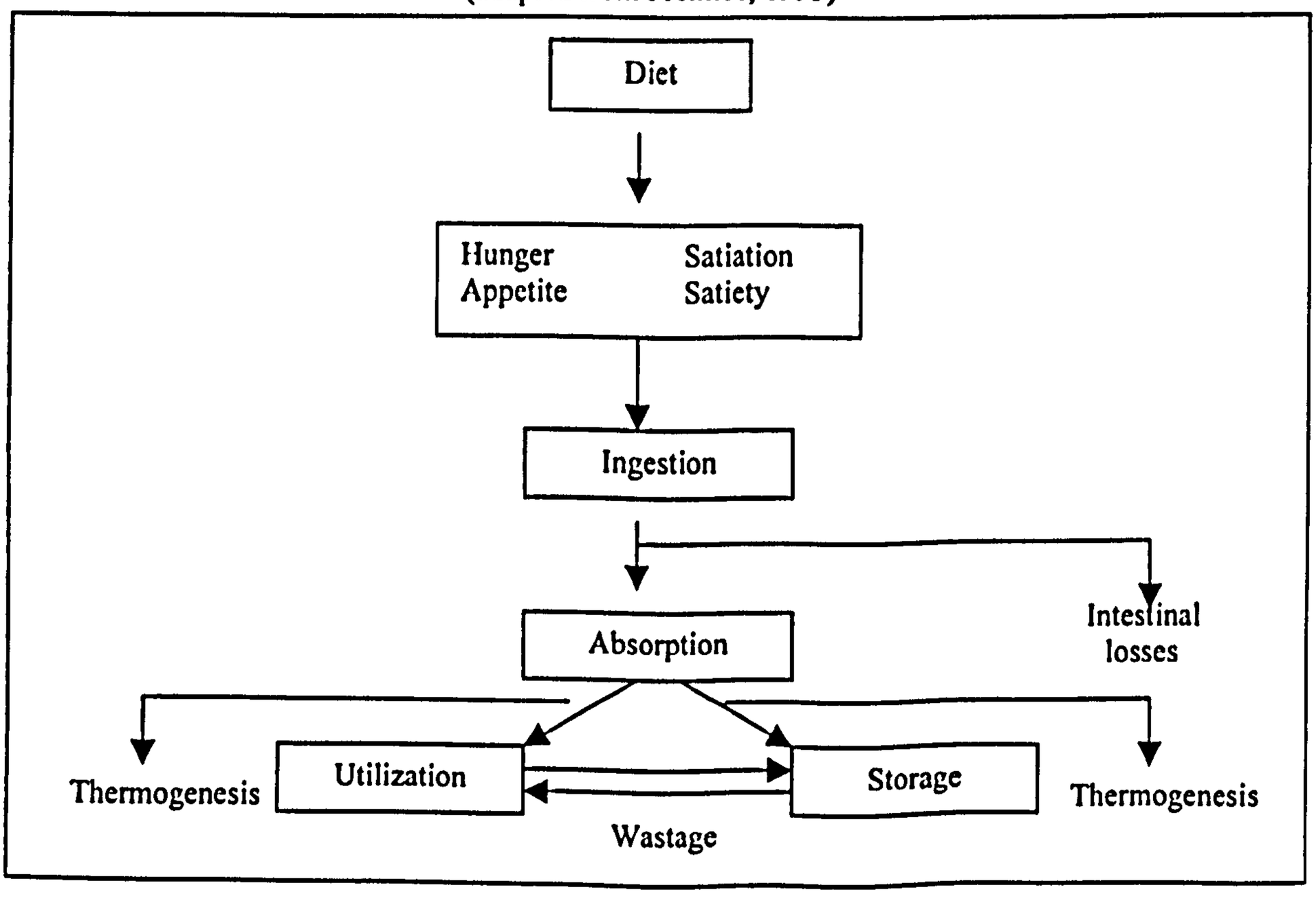
The methodological problem of a high degree of multicollinearity between different nutrients and energy intake was approached using the strategy of adjusting risk estimates for level of energy intake, although the approach adopted was not consistent between studies (Table 6).

The problem of underreporting (see Chapter 7) was addressed in the majority of the papers reviewed (Table 6). This is of particular importance, since it is not so long ago that epidemiological studies were simply based on the premise that obese individuals report their dietary intake as accurately as non-obese, an assumption that has been challenged in several studies (Black et al, 1991, Black et al, 1993, Prentice et al, 1986, Hulten et al, 1990, Heitmann, 1993, Lissner & Lindroos, 1994). It is of primary interest to note that most of the studies comparing diet and body weight have failed to detect a relationship between energy intake and body weight (Macdiarmid et al, 1996 Andersson & Rossner, 1996, Miller et al, 1994, Romieu et al, 1988, Slattery et al, 1992, Miller et al, 1990, Dreon et al, 1988). Only six studies have reported the energy intake relative weight relationship (Richards et al, 2000, Nelson et al, 1996, Colditz et al, 1995, Miller et al, 1994, Tucker & Kano, 1992, George et al, 1990, Tremblay et al, 1989). Concerning analytical action taken to cope with the problem, two of the most recent of the reviewed studies examined the prevalence of underreporting by BMI status and undertook analyses by excluding under-reporters (Samaras et al, 1998, Macdiarmid et al, 1996). Another two attempted to cope with the problem by excluding records considered to provide implausible data. The criterion for the exclusion were either subjective judgement (Kant et al, 1995) or implausibly high or low food score based on food items eaten per day (Colditz et al, 1995). However, none of the studies performed analysis by both including and excluding under-reporters to enable an assessment of the potential effect of such a bias on the results.

2.3 Evidence from human experimental studies

An important criterion used when judging the plausibility of epidemiological findings has been consistency with clinical and human experimental studies, in which the problem of uncontrolled confounding by known, unknown or unmeasurable confounding factors is resolved through appropriate randomization to produce a similar distribution of confounding factors in treatment and control groups. Experimental studies in humans have been conducted using different approaches and study designs to explore a number of points in the physiological pathways leading from energy intake to ultimate storage, at which macronutrients may differ (Figure 7 adapted from Prentice, 1995). They may have different effects on hunger and satiety and thus on the regulation of energy intake; they may be absorbed from the gut more or less efficiently; they might invoke different levels of diet-induced thermogenesis or they may be utilized and stored with different levels of metabolic efficiency. These different possibilities are briefly examined below:

Figure 7. Points at which macronutrients may exert different effects on energy balance
(adapted from Prentice, 1995)



2.3.1 Studies investigating short-term effects of dietary fat on energy intake

This section focuses on the impact of dietary energy density and/or macronutrient composition on the intake side of the energy balance equation. It has been frequently suggested that high fat foods are readily overeaten. This could be because fat intake enhances the palatability and texture quality of foods or because fat has a low satiety value compared with carbohydrate or protein.

2.3.1.1 Dietary fat effects on satiety

Much of the information regarding the satiating power of macronutrients has been derived using the classic preload experimental design (Stubbs, 1995). This involves providing subjects with fixed amount (preload) of a given nutrient, food or meal, in which the energy content and/or nutrient composition has been covertly manipulated. After a predetermined delay, depending on the goal of the study, subjects are generally required to fill in scores of hunger and satiety, before receiving an ad libitum test meal. This allows investigators to assess both motivation to eat and actual consumption and hence their ability to compensate for the hidden preload on the subsequent test meal (Rolls & Hammer, 1995).

On the basis of preloading studies, it has been frequently stated that the satiety power is in the order of protein<carbohydrate<fat with the position of alcohol as yet undetermined (Stubbs, 1995, Prentice & Poppitt, 1996). However, when focused on the question whether fat has a weaker effect on satiety compared to carbohydrate, when palatability and energy density are matched, there are numerous studies which fail to confirm this hierarchy, indicating that there is little or no difference in the effects of fat and carbohydrate on satiety (Foltin et al, 1990, de Graaf et al, 1992, Foltin et al, 1992). Some studies claim that the effectiveness of compensating for ingested energy in diets high in fat by reducing the subsequent intake of energy is somehow impaired in women (compared to men) as well as in individuals who

consciously restrain food intake. It has also been suggested that the low satiating power of fat is most pronounced on subjects with a predisposition to obesity (Lawton et al, 1993, Rolls et al, 1994).

At this point it should be noted that the pre-load paradigm has limitations. One particular problem is that the test meals are provided at a fixed interval after the preload, thus obscuring possible differences in the time course of satiety effects. Their relevance to the control of total daily food intake is therefore unknown. A number of studies have partially overcome this by asking subjects to continue recording their food intake for the remainder of the day (Prentice & Poppitt,1996).

2.3.1.2 Sensory preferences for fat

There is little doubt that dietary choices are strongly influenced by the taste as well as the texture of foods (Drewnowski, 1997). Thus, presence or easy access to 'palatable' foods has a causal role in overeating since individuals tend, at least in the short term, to eat greater amounts of better-liked food. Since fat-rich foods tend to be more palatable, it has been suggested that they may greatly contribute to overweight and obesity (Drewnowski, 1998).

A variety of mechanisms by which the sensory hedonic value of foods could alter short term energy intake and expenditure have been proposed and explored. Cornell (Cornell et al, 1989) has suggested that positive sensory stimulation can easily prompt a desire to consume food, even when individuals are not hungry. Such a mechanism lead to what is called 'active overconsumption', since it increases total energy intake within a meal. This finding is not surprising since it is in accordance with everyday experience. Furthermore, it has been suggested that sensory stimulation may influence some post- prandial metabolic events, some of which modulate hunger and nutrient utilization (Teff & Engelman, 1996). The effect, however, of sensory stimulation on post-ingestive events such as appetite rating is

not clear. There have been studies that have found that consumption of a more palatable and appealing food may either stimulate (Hill et al, 1984) or suppress (Warwick et al, 1993) later feelings of hunger.

The essential question to be answered is whether the sensory appeal of fat-rich foods exerts an independent effect on energy balance, or simply contributes to overeating and obesity given its high energy density which may actually be the root of its hedonic value (Mela & Rogers, 1998).

There have also been short-term feeding experiments investigating whether there is an association between sensory preference for fat and body fatness. In general, these studies have suggested that obese subjects select food with much more attention to palatability than do normal weight individuals. Consequently, obese people and formerly obese people seem to prefer high-fat stimuli (Drewnowski & Kurth, 1991, Mela & Sacchetti, 1991, Mela, 1996) and overconsume high-fat foods. However, studies on food choices and taste preferences of the obese are difficult to interpret since results from tests of short-term eating behavior do not provide an answer to the question of what the obese eat over the medium or long term.

2.3.2 Studies investigating long-term effects of dietary fat on energy intake

There have also been a number of long-term feeding experiments lasting 2-11 weeks, in which subjects were offered diets with different proportions of fat and spontaneous food intake was measured to assess the effect of dietary fat on daily energy intake. In the Dunn Clinical Nutrition Center, Stubbs (Stubbs et al, 1995a, Stubbs et al, 1995b, Stubbs & Prentice, 1993) performed a series of three experiments of two to three weeks duration each, in order to examine the impact of macronutrients and energy density on appetite. The subjects in these studies were all lean men who, by inference, were competent regulators of energy balance. In each

study, they were given ad libitum access to one of three covertly manipulated diets. In the first two studies (Stubbs et al, 1995a, Stubbs et al, 1995b) the energy density was allowed to differ as would occur in normal diets. In both studies, subjects displayed 'passive overconsumption' and were in positive energy balance on the high fat diet. These two studies conducted in Cambridge were consistent with similar trials performed in the US over a period of two to eleven weeks (Lissner et al, 1987, Kendall et al, 1991). These studies compared an ad libitum diet composed of high-fat foods (45-50% or 35-40% of energy from fat) to low fat diet (15-20% or 20-25% of energy from fat) of similar palatability and showed spontaneous overconsumption of energy on high-fat, low carbohydrate diets and underconsumption on low fat, high carbohydrate diets.

In the third study employed by Stubbs and Prentice, the energy density was held constant across diets, in order to dissociate potential macronutrient effects from energy density effects. This resulted in a virtual abolition of the high fat hyperphagia observed in the first two studies where energy density was allowed to vary with fat content (Stubbs & Prentice, 1993). This last study is analogous to the classic feeding experiment by van-Stratum et al (van Stratum, 1978) in which spontaneous energy intakes were compared among adult females (Trappist nuns) who for two weeks were fed liquid diets of high (47%) or low (24%) fat contents, not varying in energy density or palatability. Mean energy intakes were measured daily and found to be constant between the two diets. On the basis of these results, dietary fat content per se could not be shown to promote hyperphagia or weight gain. However the isoenergetic experimental design is not directly analogous to normal feeding conditions in human populations, where dietary fat content has a direct relation to energy density and often to palatability as well.

One should note that to some extent the 'energy density vs fat fattening' issue is a rather specious one, since in most food there is close correlation between fat content and energy density. The latter is further supported by a tendency to maintain the

volume or weight of food consumed at a constant level; in all of the studies in which the fat content of the total diet was covertly manipulated (Lissner et al, 1987, Tremblay et al, 1989, Kendall et al, 1991), subjects adopted a strategy of eating similar portions of foods across the conditions (Rolls & Shide, 1994). Thus the weight of food consumed was maintained at a more constant level than energy intake. One interpretation of these results is that most adults have become habituated to consuming a certain quantity of food measured in terms of weight, bulk or appearance. This notion seems to be rather powerful since it remains virtually unaffected by extreme manipulations of the fat content of the diet or by the environment in which the foods are consumed.

2.3.3 Studies using fat substitutes

There have also been studies using fat substitutes to facilitate research on the degree to which covert changes in energy density alter total energy intake and macronutrient selection. In short-term studies, lasting up to two days, the substitution of indigestible fat substitutes for fat in the diet showed 2 patterns of adaptation. When olestra was substituted for fat in a single breakfast meal, there was energy compensation over the next 24-36 hours in healthy young men (Rolls et al, 1992, Burley & Blundell, 1992). When fat intake was lowered from 40% to 30% of energy by substituting fat for olestra in the noon or evening meal, there was no energy or nutrient compensation over the next 24-hours (Cotton et al, 1996). However, when substitution with olestra lowered the fat intake from 30% to nearly 20% of energy over 3 meals, healthy subjects felt less satisfied at the end of the substitution and compensated for nearly 75% of the energy deficit over the next day (Cotton et al, 1996).

In longer-term experiments, lasting from 2 weeks to 3 months, the substitution of olestra for about 33% of the fat in a diet containing 40% of energy from fat, reduced

energy consumption by about 15% (2,000 kcal) reduced to (1,750 kcal) for the same weight of food. In each experiment there was only partial compensation for energy, suggesting that when the energy density of the diet did not change, the subjects continued to eat the same volume of food, even though it provided less metabolizable energy. Weight loss in the 2-weeks experiment was 1.5 kg and in the 3-months experiment was nearly 5 kg, which was significantly greater than the amount of weight loss in the control group (Bray et al, 1995, Roy et al, 1997).

In these studies, subjects compensated for the reduction in energy but did not eat proportionately more fat to make up for the fat reduction. It was clear that the energy manipulation was detected, which supports the hypothesis that fat affects satiety mechanisms.

2.3.4 Studies investigating short-term macronutrient effects on energy expenditure

Several experiments have been employed in order to measure the impact of diet's macronutrient composition on changes in energy expenditure and substrate oxidation. There has been evidence suggesting that when energy is in excess, the human body processes nutrients according to an oxidative hierarchy (Flatt et al, 1985, Ravussin & Tarratani, 1997). Excessive carbohydrate and protein intakes are disposed of by increased oxidation. In contrast, excess fat intake does not promote its own oxidation in the short- and mid-term (Flatt et al, 1985). This, in the long-term, could lead to an increase in fat stores.

Inter-individual differences in lipid oxidation, probably genetically determined, that may protect from or predispose to obesity have been reported. Thus, Astrup (Astrup & Buemann, 1994) using 24-hour calorimetry found that carbohydrate oxidation was lower and fat oxidation higher in overweight and obese subjects compared to normal

weight controls. These experiments supported the hypothesis that obese subjects may select a diet with a lower carbohydrate and a higher fat content and/or that dietary fat is preferably metabolized by obese people. In the same experimental context, the possibility that dietary fat is a risk factor for obesity in a susceptible subpopulation was further examined. It was suggested that post-obese individuals with a positive family history of obesity, had an impaired ability to increase the fat to carbohydrate oxidation ratio in response to a high-fat diet compared to non-obese controls.

2.3.5 Weight loss trials

In longer term randomised trials, whose primary purpose was not to reduce body fat but to decrease chronic disease risk, Kazim and Martino (Kazim & Martino, 1993), Hunningale (Hunningale et al, 1993), Levitsky and Strupp (Levitsky & Strupp, 1994), observed that those receiving advice to eat a low-fat diet (15%-20% of energy from fat) had mean weight losses of 3.4 kg over 3 months, 1.4 kg over 9 weeks, and under 1 kg over 6 weeks respectively. However, the available long-term results were not encouraging. After the first three months of the one-year trial of Kazim and Martino (Kazim & Martino, 1993) subjects experienced no further weight reduction in spite of reported adherence to the fat-reduced diet. Also, in the studies of Lee-Han and Boyd, twelve months after consuming the low-fat diet, several of the women had regained most or all of the weight they had lost (Lee-Han et al, 1988, Boyd et al, 1990). Furthermore, at the time of the two years follow-up examination in the Women's Health Trial Feasibility Study of Sheppard (Sheppard et al, 1991) women had sustained 90% of the original dietary fat reduction (20% of energy from fat) and 94% of their energy reduction, but only 60% of their weight loss.

This implies that the subjects may have been unable to maintain their dietary changes in the long term, although they appear to have reported the prescribed diet on the days chosen for dietary recording. Alternatively, adaptation to the low-fat diet could

have occurred in these studies, possibly consisting of changes in appetite signals and/or energy expenditure after long-term exposure to these diets.

However, since most of these studies did not focus on obesity but rather on cardiovascular disease or cancer, there has been argument that weight loss with the low-fat diet may be confounded by several factors that usually accompany preventive treatment of these diseases, such as exercise and smoking cessation (Fehily et al, 1984).

2.3.5.1 Effect of diet composition on weight loss in overweight subjects

Several trials have examined the effects of a low-fat diet either with or without energy restriction on overweight subjects.

In a recent review of studies focusing on reductions in dietary fat without restricting energy intake, Bray and Popkin (Bray & Popkin, 1998) reported a consistent decrease in energy intake ranging from 11% to 31% compared to the one of the usual diet. Moreover, a regression analysis has shown a biologically plausible weight loss of 1.6 g/day per each percentage unit reduction in energy from fat. This finding supports the fact that, even in studies in which the goal is not to restrict energy intake, the latter may be often reduced unintentionally when a low-fat diet is consumed (Puska et al, 1983).

In a similar context, remarkable weight loss of 7.8 kg was observed in 19 obese native Hawaiian men and women who were fed a traditional low-fat ad libitum diet for 3 weeks (Shintani et al, 1991); the percentage of fat in their diet was reduced from 32% to 7% of energy. Similarly, in a study of men and women at a Danish work site, a 12-weeks low fat diet resulted in a 4.2 kg weight loss as opposed to a 0.8 kg weight loss in the control (usual diet) group (Siggard et al, 1996).

There have also been intervention studies of overweight women, lasting up to 6 months, in which an ad-libitum low fat diet was compared with a low energy diet. These studies demonstrated that both the low energy and the ad-libitum low-fat, high-carbohydrate, diets resulted in weight loss but that the low energy diets have resulted in more significant amounts of weight losses than did the ad-libitum low-fat diets (Hammer et al, 1989, Schlundt et al 1993, Harvey-Berino, 1998).

At this point it is important to note that even modest reductions on body weight on low fat diets, could potentially be extremely important if they were cumulative over periods of years. Thus, longer-term studies are critical.

In the context of studies lasting more than 6 months, it has been frequently demonstrated that, the group assigned to the low-fat diet lost slightly more weight than the group assigned to the low energy diet (Shah et al, 1994, Jeffery et al, 1995). By 18 months later, most of the weight that had been lost had been regained. However the low-fat group did not gain as much weight as the low- energy group; although the difference was not significant. The authors pointed out that the low-fat group indicated higher satiety scores, subsequently compensation for the decrease in energy content was not complete, and significantly higher palatability and quality of life scores, which would presumably enhance compliance. Nevertheless, this effect was not reflected in lower long-term weight, and might even have contributed to overeating of low fat foods.

It has been suggested that despite the modest weight loss and poor dietary compliance associated with long term trials of low fat diets, these diets may still be important for weight maintenance (Westerterp et al, 1996, Toubro & Astrup, 1997). Toubro and Astrup (Tubro & Astrup, 1997) randomly assigned patients who had lost weight by means other than a low-fat diet, into a group that received a low fat diet ad libitum or a fixed energy (weight maintenance) diet. After 1 year, the low fat group was 3.5 kg lighter than the fixed energy group. At the 1 year follow-up, the fixed

energy group has regained 11.3 kg, whereas the low-fat group has regained only 5.4 kg. These data coupled with the findings of De Graaf et al (De Graaf et al, 1997) that long-term consumption of reduced fat products leads to lower energy and fat consumption than does the consumption of full fat products, suggest that low fat intakes may make it easier to maintain a low-energy diet and thus slow down the rate of weight gain or weight regain.

One hypothesised reason to expect lower body fat on a iso-energetic diet with a lower percentage of calories from fat, is the difference in metabolic efficiency of processing fat compared with carbohydrate or protein. Three studies of weight loss have been employed to investigate this particular mechanism (Alford et al, 1990, Powell et al, 1994, Golay et al, 1996). In the larger of these studies, Powell provided 1,200 kcal diets to women randomised to 10%, 20%, 30% and 40% of energy from fat. No significant differences were seen in weight change at 6 and 12 weeks, although the magnitude of reduction was actually somewhat less (although non significant) on the lowest fat intake. Similarly, the 6-weeks and 10- weeks studies employed by Golay (Golay et al, 1996) and Alford (Alford et al, 1990) respectively, also found no significant effect of the fat composition of the diet on weight loss. These data indicate that under realistic circumstances, the theoretical differences in metabolic efficiency associated with different levels of fat intake, when energy intake is held constant, do not account for important differences in rate of weight loss.

The results of these studies investigating this particular mechanism, have been challenged by arguments that total energy deficit was the primary factor responsible for weight loss, which was independent of the percentage of fat consumed. Moreover, it has been noted, that it is rather impossible to test whether there was a differential effect between fat and carbohydrate on weight loss, because no excess energy was available for storage in the adipose tissue.

2.3.6 Comments and conclusions

In summary, human experimental research investigating factors promoting overweight-obesity has shown a limited effect of a high fat diet under conditions of controlled energy-density and palatability but strong effects of high fat, energy dense diets. This has been demonstrated both in normal-weight and over-weight subjects. One may conclude that the experimental evidence on this relationship is more conclusive, although biological mechanisms remain controversial. Nonetheless, in some cases the experimental constraints imposed in such studies may not reflect the cause of obesity in free-living populations.

2.4 General conclusions

In conclusion the review of the observational epidemiological evidence for an association between dietary fat intake and obesity suggests inconsistency in results both within and between studies. This may be due to several reasons, both methodological and behavioural ones. Methodological issues refer to the variation in statistical power due to a wide range of sample sizes, variation in the assumptions made, differences between exclusion criteria used, and a wide variation in the outcome and exposure definition which all complicate the interpretation of results across studies.

Furthermore, observational studies of diet and body weight may also be complicated by behavioral factors, involving the possibility that persons who consider themselves to be overweight may alter their diets to reduce weight during the survey period. Since individuals are aware of the dependent variable, body weight, and also have conscious control over its primary determinants, physical activity, total energy intake, as well as the percentage of energy from fat, their initial dietary practices may no longer be relevant to the cause of their weight fluctuations.

Further observational studies on this issue should ensure adequate statistical power, adequate control for confounding variables, the use of dietary assessment methods which have been validated for measurement of the nutrients in question to reduce the regression dilution problem and focus on potential interactions between fat intake and other nutrients and energy intake. A quantitative overview may resolve some of the uncertainties regarding the potential benefit of a change in dietary fat intake and its possible public health relevance.

Finally, evidence from human experimental research has shown a limited effect of a high fat diet under conditions of controlled energy-density and palatability but strong effects of high fat, energy dense diets. This has been demonstrated both in normal-weight and over-weight subjects. One may conclude that the experimental evidence on this relationship is more conclusive, although biological mechanisms remain controversial.

3. MAIN HYPOTHESIS AND OBJECTIVES

3.1 Hypothesis

There is a positive association between total dietary fat intake and overweight/obesity among Greek men and women aged 30-75 years that is independent of energy intake and other confounding factors.

3.2 Objectives

- To review the epidemiological evidence pertaining to the association between overweight/obesity and dietary fat intake

- To explore the association of BMI in relation to energy intake and expenditure among adults in Greece

- To evaluate the hypothesis that there is a direct association between dietary fat intake and relative weight (increased BMI) in Greek adult men and women. In particular, to elucidate the sub-hypotheses: a) that the ratio of g from fat to g from all energy generating nutrients (ethanol included) is higher among persons with higher BMI; and b) that the concentration of fat in the diet per se, independent of total calorie intake, is positively associated with increased BMI after adjusting for potential confounding factors

- To explore the association of WHR and WC in relation to energy intake and expenditure among adults in Greece

- To evaluate the hypothesis that there is a direct association between dietary fat intake and increased WHR and WC

SECTION II. METHODS

The hypothesis will be addressed using a cross-sectional design. The opportunity to assess the relationship between nutrient intake and obesity in a very large non-hospitalised sample of Greek adults was presented by the availability of data collected in the Greek component of the European Prospective Investigation into Cancer and Nutrition (EPIC) Study.

4. THE EPIC STUDY

The EPIC Study is a large prospective epidemiological study on diet and other lifestyle and environmental factors in relation to the incidence of, and mortality from cancer and other chronic diseases. It involves nine European countries (France, Italy Spain, The Netherlands, United Kingdom, Germany, Greece, Sweden and Denmark) and is coordinated by the International Agency for Research on Cancer (IARC). The study is supported by the 'Europe against Cancer' Program of the European Commission and various national sources (for Greece the Ministry of Health). The study has collected data on diet, other lifestyle and environmental factors and anthropometry as well as biological samples in a cohort of about 470,000 healthy European adults. The subjects are followed up and scientific results are expected to be produced within the next 10-15 years (Riboli, 1992, Riboli & Kaaks,1997).

In Athens, Greece, EPIC is organized by the Department of Hygiene and Epidemiology of the Athens Medical School, the National Center for Nutrition of the National School of Public Health and the Greek Society of Nutrition and Foods. In the Greek component of the EPIC study men and women 25-82 years of age are recruited into the cohort. They are volunteers who either a) respond to the publicity made through media; b) are personally invited by letters or c) are contacted, in the

context of massive recruitment efforts, in working places, institutions, communities and municipal authorities all over Greece. In Greece the recruitment process took place between January 1994 and March 1999; almost 28,000 subjects have been included in the study.

4.1 Data collection methods

Eligible participants from all over Greece, who agreed (by signing informed consent) to participate in the EPIC study, were expected to provide blood samples, anthropometric data and to complete a full series of questionnaires.

4.1.1 Questionnaires

The three main questionnaires were all interviewer-administered at the local examination center. These questionnaires are described below:

4.1.1.1 Semi-quantitative food frequency questionnaire (FFQ)

This was designed to assess intake of a variety of nutrients hypothesized to be associated with cancer and heart disease. These include protein, specific types of fat, cholesterol, total carbohydrate, sucrose, crude and dietary fiber; preformed and provitamin A, vitamins E, C and B_6. It was developed using previous experience from several case-control studies conducted to investigate dietary habits in the Greek population (Trichopoulos et al, 1990) and from dietary methodology obtained in the large cohort studies conducted in the USA (Willett et al, 1985, Rimm et al, 1992). The questionnaire is divided into four parts:

The first part includes questions relevant to general dietary habits (consumption of vitamins/supplements) as well as questions on current and previous dieting practices (e.g. adherence to special diets, fasting for religious reasons). The second part is considered to be the main part of the questionnaire in which questions on the average consumption during the previous year of foods selected from a list containing 165 food items and dishes as well as beverages. For 15 items there are separate questions concerning the seasonal differences in consumption. Portions are estimated by either using natural units where appropriate (e.g. egg), standard quantities (e.g. 1 teaspoon) or photographs of 3-6 portions, -all taken under standard scale conditions, of various typical dishes which contribute significantly to the Greek dietary profile (Kampman & Kolias, 1988). The frequency of response categories is non overlapping and consists of: never or rarely; 1-3 times per month; 1-2 times per week; 3-4 times per week; 5-6 times per week; or daily with exact times per day recorded. In the third part there are questions concerning alcoholic beverage consumption, separately for wine, beer, brandy, whiskey, other cordials, ouzo and other alcoholic drinks. In the final section the type of fat used for cooking or added to the foods (e.g. salads) is further explored.

Since subjects are asked to consider their average diet, the FFQ is assumed to measure subjects' "habitual" food intake.

Validity and reproducibility of the FFQ:

The conduct and analytical results of the validation study have been described in detail elsewhere (Gnardellis et al, 1995, Katsouyianni et al, 1997). In the pilot phase of the study 480 letters inviting subjects to participate in the EPIC pilot phase were sent, and 170 positive replies received (35% response rate). From these, one hundred subjects were randomly chosen and finally ninety-eight of them, living in the Greater Athens area, agreed to participate in the study. Eighty volunteers, 42 men and 38 women aged 25 to 67 years of age, complied with the dietary methods-requirements

of the protocol and slightly fewer provided adequate plasma and urine for biochemical assessment.

Fieldwork was carried out between 1991-1992. All subjects were asked to visit the study site monthly. At their first visit they completed the first FFQ, while in each visit, a 24-hour dietary recall was completed by interview. The visits of each subject were arranged on different days of the week (including Saturdays and Sundays) to account for differences in daily dietary habits. During their final visit participants returned a recently completed second FFQ. Two venous blood and three 24-hour urine samples were also collected at randomly selected periods during the year between the two administrations of the 2 FFQs.

The use of the twelve repeated 24-hour recalls, as a reference method, has several advantages in the context of the Greek cohort: 1) they are easier to use and less expensive; the additional resources needed to train participants to weigh and record their food intake would make the use of diet records considerably more complicated and expensive; 2) the requirements of the validation study, in terms of participants' time and effort, would be far greater if diet records were used, thus leading to a selected sub-group of subjects and consequently to results possibly unrepresentative of the cohort; 3) the habits and lifestyle of residents in Athens involve frequent meals outside the home, rendering weighed records difficult to use or alternatively leading to substantial changes in eating habits in order to use them;. and 4) although it is likely that 24-hour recalls are less reliable compared to diet records (Nelson & Bingham, 1997, Buzzard, 1998) which have been used in other validation studies (Willett, 1985, Rimm et al, 1992) it is also conceivable that they generate estimates of relative validity and reproducibility closer to the true cohort values.

Validity: The relative validity of the questionnaire was tested against twelve 24-hour diet recall questionnaires, two venous blood and three 24-hour urine samples. Mean values for intake of most nutrients assessed by the two different dietary methods

were calculated. Pearson correlation coefficients assessed the validity of nutrient values derived from the first and the second FFQ against the mean of the 24-hour recalls. To take into account within-person variation due to day-to day fluctuations and seasonal variation, within and between person components of variation from the 24-hour recalls were used to 'deattenuate' the Pearson correlation coefficients. Analytical results are given in Table 7.

Table 7. Pearson Correlation Coefficients between FFQs and the average of twelve 24-hour recalls calculated for unadjusted and energy adjusted nutrients (adapted from Gnardellis et al, 1994)

	Correlation Coefficients					
		With Questionnaire 1			With Questionnaire 2	
	Unadjusted	Energy Adjusted	Deattenuated	Unadjusted	Energy Adjusted	Deattenuated
			Men			
Calories	0.63		0.66	0.43		0.45
Proteins (g)	0.67	0.52	0.56	0.51	0.47	0.50
SAT (g)	0.68	0.44	0.47	0.52	0.37	0.39
MUF (g)	0.64	0.26	0.29	0.27	0.25	0.28
PUF (g)	0.56	0.13	0.15	0.39	0.23	0.26
Cis-Linoleic acid (g)	0.62	0.42	0.50	0.23	0.32	0.38
Cholesterol (g)	0.65	0.38	0.44	0.63	0.52	0.60
Mono-di- saccharides (g)	0.26	0.31	0.35	0.38	0.42	0.47
Polysaccharides (g)	0.54	0.33	0.36	0.43	0.57	0.62
Dietary fibre (g)	0.27	0.36	0.41	0.18	0.25	0.28
Alcohol (g)	0.79	0.76	0.90	0.71	0.69	0.82
Calcium (mg)	0.57	0.64	0.69	0.54	0.42	0.45
Phosphorous (mg)	0.66	0.69	0.73	0.57	0.45	0.47
Iron (mg)	0.42	0.29	0.33	0.31	0.28	0.32
Zinc (mg)	0.69	0.63	0.70	0.46	0.41	0.46
Retinol (A) (mg)	0.46	0.22	0.24	0.36	0.27	0.30
Beta carotene (mg)	0.16	0.25	0.31	0.43	0.46	0.57
Ascorbic acid (mg)	0.26	0.29	0.34	0.30	0.39	0.46
			Women			
Calories	0.46		0.50	0.32		0.34
Proteins (g)	0.54	0.40	0.44	0.34	0.27	0.29
SAT (g)	0.49	0.45	0.49	0.34	0.59	0.64
MUF (g)	0.41	0.36	0.41	0.26	0.39	0.44
PUF (g)	0.24	0.18	0.21	0.24	0.32	0.37
Cis-Linoleic acid (g)	0.48	0.58	0.68	0.23	0.46	0.54
Cholesterol (g)	0.50	0.35	0.43	0.37	0.23	0.28
Mono-di- saccharides (g)	0.40	0.32	0.35	0.49	0.24	0.26
Polysaccharides (g)	0.39	0.29	0.32	0.41	0.46	0.51
Dietary fibre (g)	0.31	0.17	0.19	0.25	0.17	0.19
Alcohol (g)	0.48	0.52	0.58	0.53	0.58	0.64
Calcium (mg)	0.47	0.55	0.60	0.41	0.49	0.54
Phosphorous (mg)	0.51	0.53	0.58	0.36	0.31	0.34
Iron (mg)	0.42	0.16	0.18	0.31	0.25	0.29
Zinc (mg)	0.56	0.27	0.31	0.37	0.33	0.38
Retinol (A) (mg)	0.15	0.14	0.17	0.58	0.63	0.78
Beta carotene (mg)	0.21	0.12	0.16	0.04	-0.04	-0.05
Ascorbic acid (mg)	0.35	0.27	0.33	0.21	0.14	0.17

In general the correlation coefficients were moderate to weak. Nevertheless, average correlation coefficients of ≥ 0.50 were noted for alcohol, calcium, phosphorous, and cis-linoleic acid, whereas coefficients between 0.40 and 0.50 were found for total energy intake, saturated fat, proteins, cholesterol, polysaccharides and zinc. The remaining correlation coefficients were lower with the lowest correlation coefficient noted for β-carotene among women. There were no clear differences between men and women, or between the correlations of the 24-hour recalls with the first and second FFQ.

It is important to note that energy adjusted interclass correlations were not systematically higher than unadjusted values. This is not what would have been expected according to previous experience in some American validation studies (Willett et al, 1985, Rimm et al, 1992). One possible explanation for this is that energy adjustment removes both nutrient variation due to variation in energy intake and reduces the range of variation generated by over- or under- reporting. It was actually found that these two effects of energy adjustment were equally important, with one or the other predominating for each nutrient, so that energy adjusted coefficients were not very different from the crude ones (Gnardellis et al, 1995). A similar situation was evident in a Spanish EPIC validation study (Martin-Moreno et al, 1993).

Plasma levels of cholesterol, carotene and vitamin C were compared to dietary intake using partial correlation coefficients after controlling for BMI, gender and age. Similarly dietary protein was compared to the average of three assessments of urinary nitrogen. These comparisons were confined to non-smokers, because smoking has been inconsistently reported to affect the levels of some biological markers including vitamin C. Results are summarized in Table 8.

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Table 8. Partial correlations adjusted for BMI, age, gender and plasma cholesterol, between dietary intake of nutrients with comparable nutrients found in plasma or urine (adapted from Katsouyianni et al, 1997)

	FFQ1 (n)	FFQ2 (n)	24-hour recalls (n)
Plasma			
Cholesterol	-0.07 (59)	-0.09 (61)	0.00 (61)
β-carotene	0.03 (59)	-0.11 (58)	0.22 (61)
Vitamin C	0.30 (54)	0.14 (54)	0.33 (54)
Dietary protein vs urinary nitrogen	0.36 (79)	0.30 (79)	0.47 (79)

Among the non-smokers, dietary cholesterol, β-carotene, and vitamin C were only marginally associated with plasma levels of these nutrients. The correlation between diet and plasma was highest for Vitamin C ranging from 0.14 for FFQ2 vs plasma levels to 0.33 for recalls vs plasma levels. Little correlation was found between dietary intake of cholesterol and plasma levels. A significant correlation was found between dietary protein intake and the average of three assessments of urinary nitrogen excretions. The correlation between dietary intake of protein and urinary nitrogen was 0.36 for FFQ1 and 0.30 for FFQ2.

Reproducibility: The reproducibility of the questionnaire has been tested against a second semi-quantitative FFQ administered one year later to 42 men and 38 women aged 25 to 67 years of age. Mean values for intake of most nutrients assessed by the two FFQs were calculated and intraclass correlation coefficients for nutrient intakes assessed the repeatability of nutrient values derived from the first and the second FFQ. Analytical results are given in Table 9.

Agreement between mean levels in the two FFQ is moderate to high and is not systematically different between sexes.

Table 9. Intraclass correlation coefficients (r) between the two Food Frequency Questionnaires (adapted from Katsouyianni et al, 1997)

	Men		Women	
	Unadjusted	Energy adjusted	Unadjusted	Energy adjusted
Calories	0.62		0.56	
Proteins (g)	0.67	0.73	0.59	0.57
Saturated fat (g)	0.69	0.60	0.54	0.44
Monounsaturated fat (g)	0.56	0.45	0.58	0.70
Polyunsaturated fat (g)	0.66	0.31	0.44	0.58
Cis-Linoleic acid (g)	0.52	0.46	0.61	0.73
Cholesterol (mg)	0.77	0.75	0.72	0.56
Mono-di-saccharides (g)	0.38	0.44	0.57	0.45
Polysaccharides (g)	0.53	0.50	0.60	0.67
Dietary fibre (g)	0.55	0.66	0.57	0.70
Alcohol (g)	0.71	0.72	0.78	0.79
Calcium (mg)	0.67	0.70	0.49	0.47
Phosphorous (mg)	0.66	0.71	0.59	0.66
Iron (mg)	0.50	0.39	0.54	0.54
Zinc (mg)	0.58	0.68	0.58	0.49
Retinol A (mg)	0.70	0.68	0.42	0.30
Beta-carotene (mg)	0.65	0.75	0.39	0.24
Ascorbic acid (mg)	0.46	0.44	0.62	0.66

In conclusion, the correlation coefficients were generally fairly similar to or slightly lower than those observed in studies of other populations (Willett et al, 1985, Piettinen et al, 1988a, Piettinen et al, 1988b, Rimm et al, 1992, Martin-Moreno et al, 1993, Bloemberg, 1993). The intraclass correlation coefficients of energy adjusted nutrient between the 2 administrations of the questionnaire were similar or somewhat better than coefficients from other reproducibility studies (Willett et al, 1985, Rimm et al, 1992, Martin-Moreno et al, 1993).

The validation study results were subsequently used as the solid basis on which the main study's data collection tools and procedures were formulated. Initially, the approximately 1,200 days of dietary intake recorded by participants in the validation study were used to confirm that important foods were not missed by the baseline FFQ list. Since the food items in the 24-hour recalls were coded in much finer detail compared to those appearing in the FFQ, we first collapsed food codes into broader categories corresponding as closely as possible to our baseline FFQ questionnaire items. Foods not corresponding to an item of the baseline questionnaire were further

evaluated in terms of their importance as sources of nutrient intake. We finally reviewed the lists and confirmed that our baseline list was already complete. Changes related mostly to the format of the questionnaire.

The fact that the validation study was conducted among a highly motivated and educated group of individuals, that is the primary school teachers, strengthened the Greek EPIC team's subsequent decision to interview administer the FFQ. In spite of the impracticalities, cost and increased human effort that such a decision would imply, it was considered critical; it would help to overcome problems related to incomplete and incorrectly filled FFQs by less educated and motivated people, and thus improve the quality of the dietary data.

4.1.1.2 Health status and life-style questionnaire

This has been developed by a) following generalized protocols for inter-country comparability purposes; and b) ensuring the applicability and appropriateness of the questionnaire for local habits. The questionnaire is divided into 4 main parts:

The first part includes questions concerning socioeconomic and demographic data as well as occupational history. Information is collected on: occupation of the subject; occupational position of the father and spouse; education; home ownership; size of house; date of first purchase of car and refrigerator by the family.

The second part addresses questions on physical activity during work and leisure time. Information on physical activity was measured with a questionnaire prepared for the EPIC study (Pols et al, 1997). The development of the questionnaire on physical activity was based on a complete review of previous methods used in epidemiological studies, particularly in large prospective studies on cardiovascular disease risk factors (EPIC protocol, 1991). The EPIC physical activity questionnaire

was originally designed to cover large intra- and inter-individual variation in physical activity and to estimate usual individual daily energy expenditure. However, the EPIC Steering Committee decided to change the goal of measuring physical activity in the EPIC study from estimating exact energy expenditure to ranking subjects according to physical activity. Only those activities expected to account for major within- and between-person variability were selected to be included in the 'short' core questionnaire.

The questionnaire used in the Greek EPIC is actually a combination of these two versions. Specifically the questionnaire is structured according to main categories of physical activity: sleeping time; resting time; work activity; transport (walking, driving, cycling etc.); housework; sports, (regular or seasonal); do-it-yourself jobs; and gardening. Information is requested on frequency, duration and intensity of the different activities.

Studies on the validity and reproducibility of the physical activity questionnaire have been centrally conducted in the Netherlands in the period October 1991-October 1992, in a population of 126 Dutch adults (64 men, 62 women). For assessing validity, a four times repeated 3-day activity diary was used as a reference instrument. The administration of the questionnaire three times during the course of 1 year was used to assess repeatability. Final results of the validation study have been published by Pols (Pols et al, 1997). According to these, differences in mean energy expenditure among repeated administrations of the questionnaire were small and not significant. The correlation coefficients between the questionnaire and the diary was 0.66 (95% CI 0.49-0.78) for men and 0.43 (95% CI 0.18-0.63) for women. Spearman's correlation coefficients for 5 and 11 months, respectively, were 0.76 [95% confidence interval (CI) 0.63-0.85], and 0.70 (95% CI 0.54-0.81) for men, and 0.58 (95% CI 0.36-0.74) and 0.71 (95% CI 0.54-0.82) for women. The authors

concluded that this questionnaire is a useful tool for estimating energy expenditure in epidemiological studies.

Although physical activity questionnaire has not directly been validated to the Greek population, the fact that the calculation of METS is the grand sum of several weighted activities makes it unlikely there would be biases towards the same direction throughout, particularly because the total time of the average day provides a built-in constraint.

In the third part questions on health status, health problems, reproductive history and medication prescribed or consumed by the subject are included. Information is collected on previous respiratory, cardiac, circulatory, skin, kidney and urinary diseases, diabetes, hepatitis, gastrointestinal problems, cancer and tumors, surgical treatments, and the age at which these occurred. A number of risk factors related to reproductive history and sexual activity, oral contraceptive and hormone use are identified for inclusion in the core questionnaire. The factors included are age at menarche and menopause, regularity of cycles, cycle length, parity, fertility, oral contraceptive use and hormone replacement therapy, and history of sexual activity.

In the fourth part particular attention has been given to tobacco smoking habits. There is a flow of questions on the intensity of tobacco smoking, by starting with present smoking habit and going back to the past.

4.1.1.3 Family health history questionnaire

In this questionnaire health status of first degree relatives is further explored. The questionnaire consists of a predefined list including: cause and date of death (if occurred) and family history of cardiovascular disease; cerebrovascular disease; diabetes mellitus; osteoporosis; cancers (asking for site); and obesity. The scope of

this section is to contribute to the scientific knowledge concerning genetic predisposition to several chronic diseases.

4.1.2 Anthropometry

The core protocol includes the following measurements: height, weight, waist circumference, hip circumference, seated height. All measurements are made according to standard instructions and their reproducibility (intra- and inter-observer) has been assessed during the study. Height, weight, waist and hip are measured without shoes and in light clothes and without any restrictive underwear. Waist measurements are taken at the end of a normal expiration, midway between the lowest rib and iliac crest, while the subject was standing erect, with the arms at the side and the feet together. Hips are measured horizontally at the level of the greatest lateral extension of the hips. All circumferences were measured using an inelastic tape without compressing the skin and were recorded to the nearest 0.1cm (EPIC protocol, 1992).

4.1.3 Collection of biological samples

Laboratory measurements on biological samples collected from the study subjects have been one of the key features of the study. Following a common protocol and using identical equipment, blood samples are collected; 1 blood sample (30 ml), once per subject. After collection, blood is kept in the dark until centrifugation and then is aliquoted into plasma, serum, white blood cells and erythrocytes. Blood samples (kept in "straws" of 0.5 ml) are stored in liquid nitrogen containers at -196 degrees C for future laboratory analyses on cancer cases and matched healthy controls (Riboli & Kaaks,1997).

4.2 Field operation and quality control procedures

4.2.1 Field operations

The recruitment procedure consisted of two main approaches which had been developed after the experience gained in the context of the pilot phase conducted between 1991-1992. The first approach was to recruit volunteers at the premises of the study center in Athens, where three dietitians and one doctor were working full-time for data collection purposes. The second approach involved travelling and making field visits throughout Greece, to villages, health centers, occupational sites etc. For this purpose, two external working teams (consisting of 1 doctor and 3 dietitians each) were formed. The administrative staff had and still has the responsibility for the planning and organization of the work and is located at the premises of the study center in Athens.

At this point, it is important to state that ongoing training had been provided in all the tasks performed by the survey staff at regular intervals. Those included methods for establishing good communication with subjects, importance of observer impartiality, instructions on the amount of detail required, methods for checking consistency in responses and finally practical experience in data collection under real survey conditions. In the same context, a regular control and analysis of systematic variation between interviewers in questionnaire responses were developed and elaborated.

4.2.2 Data management and quality control procedures

Extensive work has been conducted on the quality control aspect of the data collection and management. A client-server Data Base Management System (DBMS)

was found to be the best architecture, based in terms of higher concurrence in accessing, retrieving and updating the information collected. CUPTA, a client-server data management system, is utilized to structure the DBMS. Tight security in accessing the database files has been enforced due to the sensitive nature of some data. Quality control of the data is a major factor and although several options were considered it was finally decided that quality control was to be enforced on-line. Extensive programs have been developed to perform checks for:

- reading and/or transcription errors
- wrong or improbable amounts
- numerical inversions of digits resulting in wrong codes or amounts
- amounts or codes being entered twice
- consistency in responses.

4.3 Statement of responsibility of the author in the context of the Greek segment of the EPIC study

This work was undertaken whilst the author was a member of the Greek EPIC team working group at the Athens School of Public Health. Within the group, I was primarily responsible for the development of the Greek EPIC-soft version, a computerized 24-hour recall software, which has been used as the primary tool for the collection of dietary data in the context of the Greek calibration study. In addition, I was responsible for the conduct of the calibration study's field work. I participated also in the recruitment of volunteers, the supervision of the collection of dietary data by other group members, the correction of dietary intake data as well as the data management and preliminary analysis of the data (work still in progress).

5. DIETARY FAT INTAKE &OBESITY: AN EMPIRICAL STUDY IN GREEK ADULTS

5.1 Sample size calculation

Fat intake was divided into appropriate percentiles (quartiles) and the prevalence of obese subjects in each quartile was estimated. The comparison of the prevalence of obese subjects in the highest quartile of dietary fat intake (exposed) vs that in the lowest quartile of dietary fat intake (unexposed) was the approach used to calculate the appropriate sample size (Hennekens & Buring, 1987).

All the calculations made were based on the assumption that the prevalence of obesity in the Greek population is about 20% based upon epidemiological data in countries with similar health profile to that of Greece (Tavani et al, 1994). We used the formula to compare two proportions to explore different scenarios of obesity prevalence rates among exposed and unexposed groups. Significance level has been kept constant at 0.05, whereas statistical power varied between 80% and 90% (EPI-Info).

Prevalence of obesity among exposed	*Prevalence of obesity among unexposed*	*Power*	*Significance level*	*No in each group*	*Total no of 4 groups*
30%	*10%*	*80*	*0.05*	*71*	*284*
30%	*10%*	*90*	*0.05*	*92*	*368*
35%	*15%*	*80*	*0.05*	*82*	*328*
35%	*15%*	*90*	*0.05*	*106*	*424*
30%	*15%*	*80*	*0.05*	*133*	*532*
30%	*15%*	*90*	*0.05*	*174*	*696*
25%	*10%*	*80*	*0.05*	*112*	*448*
25%	*10%*	*90*	*0.05*	*146*	*584*
25%	*15%*	*80*	*0.05*	*270*	*1080*
25%	*15%*	*90*	*0.05*	*354*	*1416*

Therefore a sample size of 700 subjects (or 584 for the analysis) will have at least 90% power of detecting a difference in prevalence of obesity between 25% and 10% even if one assumes that 20% of the sample are lost (missing values or to allow for multivariate analysis). All analyses will be performed separately for males and females. Therefore, we aim to recruit a sample size of 1,400 (700 men, 700 women).

The study would have a higher power to detect a significant effect if fat and obesity were analyzed as continuous variables.

5.2 Study population

In order to evaluate the association between fat and obesity it would be valuable to select a population characterized by wide variation in the exposure (dietary fat) and in the outcome (obesity) measures. The study population for the present study consists of the total EPIC sample from the olive producing island of Mytilini (total number of participants 638) and the total EPIC sample from the urban area of Thessaloniki (total number of participants=919). These two areas may be or may not be representative of Greece as a whole, but representativeness is not a prerequisite for validity. These two areas were carefully chosen so as to cover the requested sample size, and to assure a good participation rate of both men and women. The final sample consisted of 596 men and 961 women aged from 23 to 78 years old, people with a broad range of socio-economic characteristics, who voluntarily participated in the study during the period 1994-1997.

In the analysis we pooled data from the two populations. Although in theory, two populations should not be pooled when they are different with respect to a known or unknown factor, which cannot be controlled for in the analysis and may interact with one of the study variables, for a country as small and as homogeneous as Greece, it is

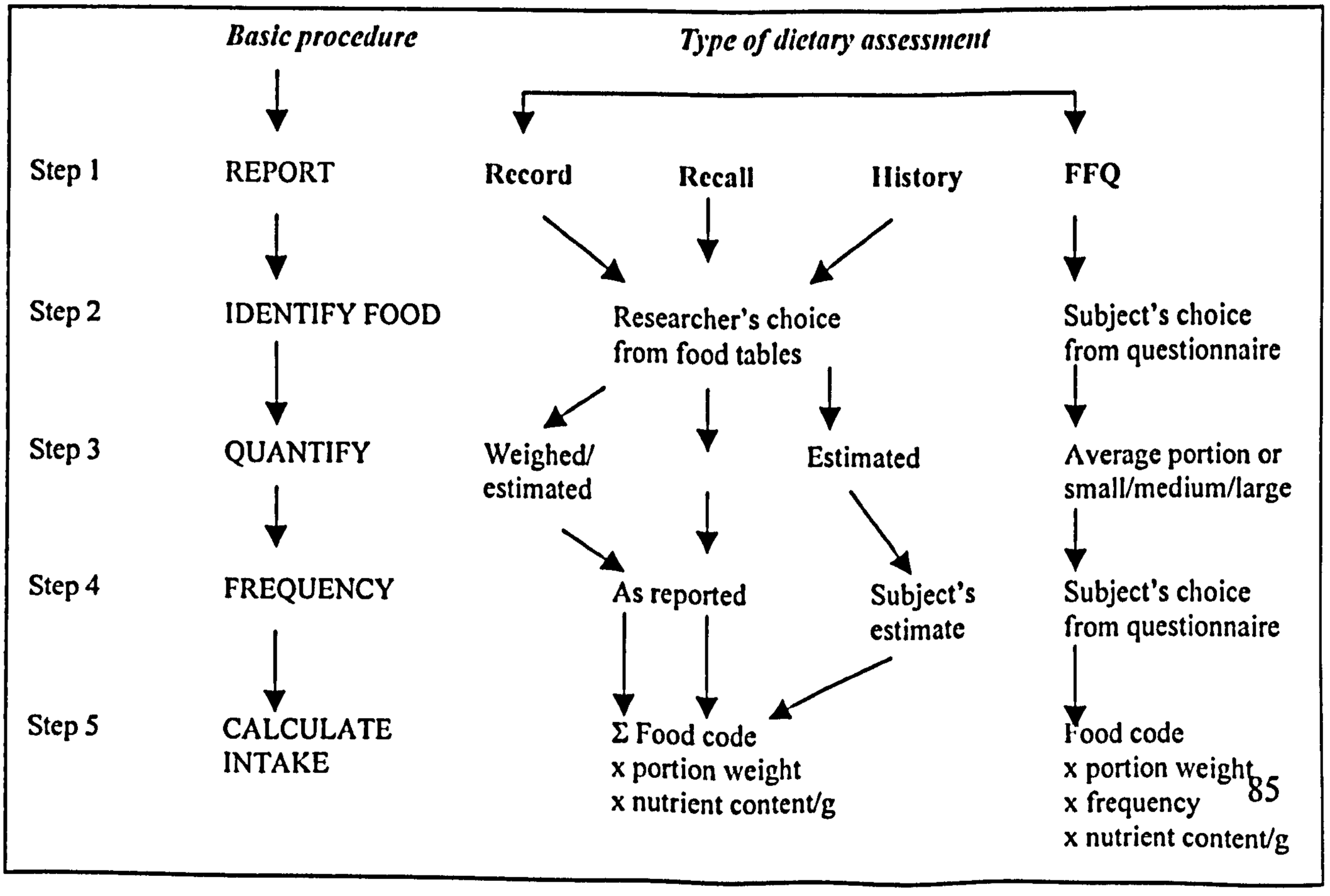
difficult to think of such a difference – particularly since data were collected by the same team. This statement was further supported by univariate analysis results.

5.3 Data handling and classification

5.3.1 Dietary intake

Figure 8 adapted from Black (Black, 1999) summarizes in a comprehensive way the basic procedure of assessing dietary intake in the context of epidemiological studies. The first step is to obtain a self-report of all the items, foods or beverages, consumed by the subjects. The second step is to quantify the weight of each item consumed. The third step is to determine the frequency of consumption of each item. The fourth step is to correctly identify all items consumed, with respect to their assignment in an appropriate food code from the food composition tables. Finally, the fifth step is to combine the information and calculate the nutrient intake provided by the foods that were reported as consumed.

Figure 8. The basic steps in assessing dietary intake (adapted from Black, 1999)



The steps followed and the assumptions made for the handling of dietary intake data derived from the FFQ are briefly described below; the result of the whole procedure, is the estimation of the mean daily nutrient intake.

a) Frequency of consumption

"Ready to eat" foods: The average frequency of consumption of foods which do not require any preparation or cooking in order to be consumed, was estimated according to pre-coded levels of intake, namely, never or rarely, times per month, times per week or times per day. The frequency of consumption of different food items was eventually quantified in terms of the number of times per month the food was consumed. Thus, a value of 30 was assigned to food items consumed once almost every day; a value of 22 to those consumed about five to six times per week; a value of 14 to those consumed about three to four times per week; a value of 6 to those consumed about once to two times per week; a value of 2 to those consumed at least once to three times per month but not every week; a value of 0.8 to those items consumed rarely; and a value of 0 was assigned to food items never consumed.

For some food items (such as some vegetables, fruits, soups from pulses, or ice-cream), which are consumed only for some months per year -either due to seasonal availability or preference for consumption due to climatic conditions- the estimation was done by considering that very fact (e.g. grapes were assumed to be consumed 3 months per year or pulses soup 6 months per year).

Mixed dishes: There are also, however, foods like certain types of meat or vegetables which in order to be consumed, are mixed with other ingredients (e.g. added dietary lipids) and further cooked. In order to study these foods, we took into account information derived from specific questions included in the last part of the FFQ. These questions refer to the frequency that different ways of cooking are applied, as well as to the frequency that different added dietary lipids are used. Various ways of cooking (i.e. boiled, stewed, grilled/roasted and fried) as well as different types of

added dietary lipids (i.e. butter, margarine, olive oil and vegetable oils) have been included in well pre-defined lists, following what is considered to be typical in the traditional as well as contemporary Greek culinary habits. The average frequency was estimated according to pre-coded frequency levels such as never, sometimes, often and always. In order to calculate the frequency of consumption by way of cooking as well as by added dietary lipid type used, we considered all the possible frequency combinations that may appear in the questionnaire and we assigned weighing factors i.e. 0 for "never", 0.25 for "sometimes", 0.50 for "often" and 1 for "always"; all these should add up to 100% of consumption. Following the previously described procedure, an expanded version of the food list originally appeared in the FFQ was created; the latter incorporated simple food items commercial products, mixed dishes and beverages.

The subsequent step was to estimate the mean daily consumption of each of the food items in terms of standard/typical food portions. Portion sizes were estimated by various methods, namely natural units, standard quantities or photos. Foods that may be consumed as natural units were estimated as such (e.g. apple, egg etc). Several others were estimated with the help of standard portions adopted from the portion sizes stated in the 1993 Market Regulations for restaurants (e.g. souvlaki, fruiting vegetables stuffed with rice etc. Finally photos were used for those foods habitually consumed in Greece which could not easily be quantified by one of the above mentioned methods. In order to limit the size of the questionnaire, only one series of photographs was used for different foods which resemble each other (e.g. different soup dishes with similar density). Photographs presented small, medium and large portion sizes of twenty four different food items. Most of the series consist of three to six pictures, depending on the range of portion sizes to be presented; portion sizes are not always presented in a growing order. The portions presented in the pictures can be used flexibly, giving the subject the possibility to select his/her portion as a fraction of one of the portions presented. The medium portion size presents the usual

weight of the food as it is "normally" served. In most cases, the small and large portions were respectively one and a half to twice as small or large as the medium portion size. Foods were photographed on the same dish with a knife beside as a reference; both commonly used in Greece. The photographs were taken from the same angle and with a similar presentation of foods. (To avoid visual differences interfering with the estimation, all photographs were taken by a professional photographer, using a NIKON FE camera with an 50 mm objective and a 100 ASA Kodak Gold film.)

The mean daily consumption was estimated by multiplying the reported quantity by the reported frequency of consumption per month and then dividing their product by thirty.

b) Analysis at the nutrient level

When the information on mean daily consumption, in terms of standard/typical portions, had became available for all simple foods, commercial products as well as mixed dishes that had been consumed by each participant, the next step was to combine this information by food composition data in order to produce the daily nutrient intake of each subject participating in the study. The food composition data referred to energy and nutrients which are derived from a computerized food composition database that has been especially developed to accommodate the characteristics of the Greek diet. This database is based on the edition "Food Composition Tables - Raw foods and Greek recipes" (Trichopoulou, 1992), in which composition of the Greek recipes has been performed using the UNIDAP program (Barrow et al, 1988), whereas the composition of raw foods is based mainly on the British Food Composition Tables (McCance & Widdowson 1993). The nutrients estimated for the purposes of the dietary fat intake and obesity study are: total fat, saturated, mono- and poly-unsaturated fatty acids, total carbohydrate, protein, in grams, ethanol in grams and total energy intake in kcal.

5.3.2 Measurement of obesity

For the purpose of defining and classifying obesity three simple indicators have been used in this analysis. General adiposity will be expressed and determined by the use of Body Mass Index (BMI) calculated as measured weight (kg)/measured height squared (m^2). Fat distribution data will be determined by both WHR, because of its popularity, and WC alone, because it generates more understandable results (Molarious & Seidell, 1998). WHR is calculated as measured waist circumference (cm)/measured hip circumference (cm) and WC as measured waist circumference (cm).

Concerning general adiposity, there is a relative consensus about its classification based on categories of BMI. In this study we use the round BMI value of 30 to define obesity; this classification is convenient and it fits the data well. Similar universal recommendations are not available for WHR and WC. As mentioned previously (Chapter 1.2) several attempts have been made and various cut-offs have been suggested in the literature (Molarius & Seidell, 1998). In this study, cut-off points have been selected based on the evidence provided in the WHO Report on Obesity (1997). A 'high' WHR, WHR>1.0 in men and >0.85 in women, and a 'high' WC (WC > 102 cm in men and > 88 cm in women) have become accepted as the cut-off points of identifying individuals with abdominal fat accumulation.

5.3.3 Confounders and exclusion criteria

Review of several studies as well as results from univariate analysis demonstrated the need to control for a number of potential confounding factors when investigating associations between dietary fat intake and obesity (Ursin et al, 1993, Baghurst & Record, 1994, Subar et al, 1994). Such variables are total energy intake, physical

activity, socioeconomic status, smoking status, menopausal status, parity and genetic susceptibility to obesity.

All dietary variables have been obtained as already described from the Food Frequency Questionnaire. Physical activity data (see below), three levels of education -considered to be the best proxy of socioeconomic status, at least in the case of Greece (primary school graduate, secondary school graduate and university school graduate) as well as three levels of self reported smoking history (current smoking, past smoking, never smoking) were obtained from special parts of the life-style questionnaire.

In the context of this study it was not feasible to control for the potential confounding effect of genetic susceptibility, menopausal status and parity since these data, although collected, were not available to the author for inlcusion in this thesis.

5.3.3.1 Assessment of physical activity

Of special concern is the appropriate handling and use of physical activity data. Energy expenditure may be assessed using different methods; the most accurate methods are the doubly labeled water method (Schoeller & van Santen, 1982) as well as direct and indirect calorimetry (Laporte et al, 1985, Pols et al, 1998). However, the fact that these methods are very expensive and either technically difficult or even inappropriate, since they require subjects to be incarcerated in a chamber, to employ as routine processes makes it impossible to use in wide-range nutritional epidemiological studies. Several other 'field methods', may be used alternatively in subjects living under normal conditions; these involve job classification, recall or diaries of physical activity as well as occupational and leisure time physical activity questionnaires. From the methods mentioned above, occupational and leisure time physical activity questionnaires are the method which are most commonly used in the

majority of the epidemiological studies. The estimation of physical activity through a physical activity questionnaire may lead to the estimation, at the individual level, of the energy expended by the subject per hour or per day, expressed either in energy units (kcal or kJ) or in "metabolic equivalents". The metabolic equivalent (MET) refers to the energy that is expended by a person in resting conditions that is 1kcal per kg of body weight per hour (Ainsworth et al, 1993); an activity with a metabolic equivalent of 2 METs demands twice the energy to that expended in resting conditions. It is thus clear that the assessment of energy requirements in terms of metabolic equivalents is automatically adjusted for the weight of the subject who conducts the activity (e.g. an activity of 4 METs (e.g. cycling) conducted from a subject of 60 kgs for 40 minutes lead to energy expenditure of: 4 METs * 60 kgs * (40 min /60 min) = 160 kcal, whereas the energy consumption for the same activity from a subject of 80 kgs is 4 METs * 80 kgs * (40 min/60 min) = 213 kcal.

In the context of this work, occupational and leisure time activities were assessed by the special section of the life-style questionnaire, which was also used for an overall evaluation of physical activity level. First, the average time per day spent on job-related work of variable intensity, household activities, walking (including walking to work, during shopping and during leisure time), cycling (including cycling to work and during leisure time), repairing and gardening (separately for summer and winter) was calculated. The average time spent per day was also assessed for the following leisure time sporting activities: volleyball, swimming, basketball, football, jogging, gymnastics, climbing, dancing, exercise cycling, tennis, rowing, skiing, water skiing and wind surfing. Sporting activity assessment was done taking into account whether the activity was performed throughout the year or seasonally. Finally, the average time spent daily for sleeping (including afternoon nap) and commuting was assessed separately for weekends and weekdays. For subjects whose

recorded total hours per day were less or more than 24 hours, the total hours spent daily were proportionately increased (or decreased) so that they summed to 24.

Each activity was assigned a MET value (the ratio of the metabolic rate associated with a given activity to the resting metabolic rate) derived from published tables (Ainsworth et al, 1993). The time spent in each of the above activities was multiplied by the MET value of that activity, and all MET-hour products were summed to give a total MET-hour score for the day.

Thus, by assigning h_i to total hours spent per day for an activity i with MET value MET_i, the MET-hours-per-day score for a set of k activities performed, on the average, by an individual daily, is defined as:

$$\text{MET-hours-per-day score} = \Sigma MET_i \times h_i \text{ where } i=1,2,...k$$

This score essentially corresponds to the amount of kcals per kilogram of body weight, expended by an individual during the day.

5.3.3.2 Exclusion criteria

It should be noted that subjects a) who follow diets for weight-loss purposes, or diets of any type or b) who have metabolic disorders; and/or c) who are under medication which may promote weight gain as a side effect, should either be excluded or treated with caution in the analyses, since these are characteristics which may confound the association of interest.

In the context of the obesity and dietary fat intake study it was not feasible to check for the latter two issues, since relevant data were not readily available. However,

questions concerning systematic adherence to slimming or any type of diet, included in the first part of the FFQ as well as in the health history part of the life-style and health questionnaire, have been used in order to identify and exclude people on diet since the dynamic condition of their energy balance equation might complicate the results as well as their interpretation.

The final sample size of 1,557 participants included 596 men and 961 non-pregnant women.

5.4 Statistical analysis

Statistical analysis was performed using the SPSS statistical package (version 9.0 SPSS Inc., Chicago). Different statistical approaches were used to evaluate the relationship between dietary fat (nutrient) intake, BMI, WHR and WC data. BMI, WHR as well as WC and dietary fat (nutrient) intake -mean daily consumption- were used both as categorical and/or continuous variables. The distribution of all continuous variables (BMI, WHR, WC, energy intake, fat intake (g), carbohydrate (g), protein intake (g), alcohol intake (g), mono-unsaturated intake (g), poly-unsaturated intake (g) saturated intake (g), fat intake (%), carbohydrate intake (%), protein intake (%), alcohol intake (%), mono-unsaturated intake (%), poly-unsaturated intake (%) saturated intake (%), physical activity level), have been checked for non-normality. This was done by comparing descriptive statistics (mean, median, mode, outliers, quartiles) and by generating histograms. No transformations were required since distribution of all variables appeared to be sufficiently symmetric.

5.4.1 Univariate analysis

Initially, univariate analysis of the selected variables was performed for the whole sample and for each sex separately. Subsequently the same types of analysis were performed for the whole sample as well as for each sex separately after excluding the under-reporters, unless otherwise indicated.

In the first approach BMI, WHR and WC were treated as binary variables & dietary fat (nutrient) intake as a categorical variable. Cut-off points of BMI < 30 vs BMI ≥30, WHR < 0.85 vs WHR ≥ 85 (women) and WHR < 1.00 vs WHR ≥1.00 (men) and WC< 0.88 vs WC ≥0.88 (women) and WC < 102 vs WC ≥102 (men), were used to transform the naturally continuous outcome variables to binary ones, while distribution of dietary fat (nutrient) intake was divided into quartiles; appropriate quartiles were estimated for the whole sample as well as for each sex separately. Comparisons of the percentage of obese subjects in different groups of dietary fat (nutrient) intake were assessed by the chi-squared test. Furthermore, the chi-squared test for trend was employed to determine whether the prevalence of increased obesity increases according to increased dietary fat (nutrient) intake. Comparisons of crude odds ratios were also performed.

In the second approach BMI and WHR and WC were treated as binary variables (see above) while dietary fat (nutrient) intake remained as a continuous variable. The t-test was used to perform the comparison of mean dietary fat intake between obese and non-obese groups.

In the third approach BMI WHR and WC were treated as continuous variables, while dietary fat (nutrient) intake became a categorical one (using appropriate quartiles which were estimated for the whole sample as well as for each sex separately).

Comparisons of the mean BMI (WHR, WC) in different dietary fat intake groups were performed using the F test (ANOVA).

Finally, both BMI (WHR, WC) and dietary fat (nutrient) intake were treated as continuous variables. Linear regression analysis was applied to determine the extent of linear association among the principal variables of the study without statistical control for any of the potential confounding factors.

The same types of analyses described above were performed for variables considered as potential confounders of the association between dietary fat intake and obesity.

5.4.2 Multivariate analysis

After sex specific univariate associations were examined, multivariate analysis was subsequently used to determine the association between obesity and dietary fat (nutrient) intake. Initially, regression analysis using BMI, WHR and WC as the dependent variable and dietary fat (nutrient) intake as independent variable was performed in order to determine the effect of different nutrients on obesity. Analysis was conducted for the whole sample as well as for each sex separately. Variables such as age, total energy intake, physical activity level, education level, and smoking status, were assessed in the model to determine the independent effects of each of these variables on obesity. Multivariate analysis was performed as follows:

1) BMI, WHR and WC were treated as the dependent binary variables while dietary fat intake and other nutrients intake as independent variables. Logistic regression was performed to determine the relationship between degree of obesity and dietary fat (nutrient) intake after adjusting for differences in selected variables. The adjusted odds ratios were estimated.

2) Multiple regression analysis with BMI, WHR, WC treated as the dependent continuous variables and dietary fat (nutrient) intake as independent variables. Multiple regression was performed to determine the relationship between obesity and dietary fat (nutrient) intake after adjusting for differences in age, energy intake, physical activity, educational achievement, smoking status.

Although all the above mentioned types of analyses were performed in order to describe the associations between dietary variables and different obesity indices, I considered it more appropriate to present and comment on the analytical results from the linear regression analysis. This would allow to take advantage of the richer information offered by the use of continuous variables. Concerning results from the other types of analyses (logistic regression, t-test, F-test), I can say conclusively that none of the analyses performed in parallel to linear regression analysis draw any statistical associations between dietary and/or socio-demographic variables that were not also significant linearly.

5.5 Nutrient intake data adjusted for total energy intake

Intake of most nutrients in free-living populations tend to be positively correlated with total caloric intake. Therefore, as dietary fat increases, the intake of total energy tends to increase and vice versa. Hence, the possible relation between fat consumption and relative weight may be confounded by differences in total energy intake. There has been some preliminary evidence suggesting that in documented cases of overall underreporting, the energy percent fat is less sensitive than absolute intake of fat to this bias (Lissner & Lindroos, 1994). Therefore, methods to adjust the fat-obesity association for total energy intake will be applied in order to determine the independent effect of dietary fat intake on BMI, WHR and WC. Current methodology used in nutritional epidemiological studies provide different methods to

control for the effect of total energy intake. (Willett & Stampfer, 1998, Willett et al, 1990, Willett & Stampfer, 1986, Mackeras, 1996). The basic alternative methods for addressing the problem of collinearity between specific nutrient intakes and total energy intake in epidemiological analyses are briefly described below: The statistical model for each method is given, where Y denotes the outcome variable (obesity), F denotes nutrient intake (e.g. fat) and X denotes the total energy intake:

5.5.1 Standard multivariate method

The standard multivariate methods in which both energy intake (X) and nutrient intake (F) are included as terms in a multiple regression model with disease outcome as the dependent variable (Willett & Stampfer, 1986, Willett, 1990, Willett & Stampfer, 1998).

$$Y = a + b_1F + b_2 X$$

The standard multivariate method and the nutrient residuals methods give identical results. However, Willett (Willett & Stampfer, 1998) supports the view that the standard multivariate model may complicate the results as well as their interpretation, since when a particular nutrient and total energy intake are entered simultaneously in a model, the coefficient for total energy intake represents energy intake independent of the specific nutrient (total energy intake is a surrogate for non specific nutrient energy). Thus, this approach changes the biological meaning of total energy intake which is not any more interpretable (the coefficient for total energy intake may fail to attain significance even in cases when total energy intake has an important and significant relation to the disease).

5.5.2 Nutrient density method

Nutrient density method in which nutrient intake is divided by total energy intake; in the case of macronutrients, nutrient intake is expressed as a percentage of total energy intake (Willett & Stampfer, 1986, Willett, 1990, Willett & Stampfer, 1998).

$$Y = a + b\,(F/X)$$

Although this approach seems to be simple and practical, it is less intuitive for the average reader, since it introduces an actually complex variable which might complicate the results as well as their interpretation. This is mainly due to the fact that this variable consists of two components, that is the nutrient intake and the inverse of total energy intake. Since the relative contributions of nutrient intake and total energy intake to between-person differences in nutrient densities are related to the ratio of their variability, as between-person variation in the specific nutrient would decrease, the nutrient density value would approach the inverse of caloric intake (multiplied by a constant). Furthermore, in the case where energy intake is itself associated with the disease, as in the current analysis, since the nutrient density variable contains the inverse of energy intake as a component, nutrient densities would tend to be associated with the disease in the direction opposite to that of total energy intake, even when the nutrient itself had no association with disease independently of energy intake.

5.5.3 Multivariate nutrient density method

Multivariate nutrient density method in which the nutrient density is computed and then entered with total energy intake in a multiple regression model.

$$Y = a + b_1 (F/X) + b_2 X$$

In this model, the regression coefficient for the nutrient density term represents the relation of the nutrient composition of the diet with disease whilst total energy intake is held constant. This model overcomes the statistical drawbacks related with the use of nutrient density alone. The regression coefficient for total energy intake in this model is interpretable representing the effect of total energy intake, in the usual biological sense, since the nutrient density term is not anymore part of, or highly correlated with, total energy intake.

5.5.4 Nutrient residuals method

The nutrient residuals method in which nutrient intake is computed as the residual from the regression model with total energy intake as the independent variable and absolute nutrient intake as the dependent variable. Since residuals do not provide an intuitive sense of actual nutrient intake, a constant (usually the predicted nutrient value for the mean energy intake) may be added to the residual intake (Willett & Stampfer, 1986, Willett, 1990, Willett & Stampfer, 1998).

$$Y = a + bF'$$

where: $F' = F + \beta (\bar{x} - xi)$, $F = \alpha + \beta X$

An alternative nutrient residual model is one in which both energy intake (X) and energy adjusted nutrient intake (F') are included as terms in a multiple regression model (Willett & Stampfer, 1986, Willett, 1990, Willett & Stampfer, 1998). This approach is preferable to use when total energy intake is associated with the disease

since: a) the random error (and thus the width of confidence limits for the effect of the nutrient) is reduced; and b) the full effect of energy intake may be observed. The energy adjusted nutrient intakes derived from the energy adjusted model are not associated with total energy intake.

$$Y = a + b_1 F' + b_2 X$$

Nutrient residuals and nutrient densities may lead to similar conclusions in epidemiological analyses; actually energy adjusted nutrient intake have been found to be highly correlated with nutrient densities. However, when it comes to interpretation, in built differences may be important, particularly in instances where confounding of total energy intake is our major concern.

5.5.5 Energy decomposition method

Energy decomposition method is an alternative model in which separate terms for energy from a specific macronutrient (Cal_F) and for energy not from this specific nutrient are entered in the model (Howe et al, 1986, Willett & Stampfer, 1986, Willett, 1990, Willett & Stampfer, 1998).

$$Y = a + b_1 Cal_F + b_2 Cal_{x-F}$$

In this model the regression coefficient for the specific macronutrient represents the full effect of the nutrient unconfounded by the effect of the remaining energy-yielding nutrients. However this model does not directly answer the question of whether energy from the specific nutrient has an association with the disease which is not shared by other sources of energy, that is independently of total energy intake. In

order to answer this question the focus should be on whether the magnitude of the coefficient for the specific nutrient is actually different from the coefficient for other sources of energy (i.e b_1 Cal_F - b_2 Cal_{x-F}).

It is not adequate simply to note that the nutrient coefficient is significant whereas coefficients from other sources are not; even when all sources of energy have the same relation with disease on a calorie for calorie basis, the coefficient for energy from other sources might not be significant simply because of low between person variation of this factor. In fact it has been shown that the difference in these coefficients (i.e b_1 Cal_F - b_2 Cal_{x-F}) and the standard error of this difference is identical to the coefficient and standard error for the nutrient residual (b) in the energy adjusted model.

In conclusion, although the energy decomposition model may provide insight in some instances, its coefficients may be misleading unless interpreted with care.

5.5.6 Energy determinant method

Energy determinant method in which the major determinants of energy intake such as body size, physical activity and metabolic efficiency are entered as separate variables in a multivariate model (Willett & Stampfer, 1986, Willett, 1990, Willett & Stampfer, 1998).

5.5.7 Conclusions

The existence of different methods indicates that the relationship between dietary factors and disease are complex. The use of the most suitable method depends to

some extent on how dependent and independent variables are analyzed, and on how much collinearity there is in the data. In the current study it was considered useful to employ all five methods and compare the results; the energy determinant method was not applicable since data on metabolic efficiency were not available. Careful consideration was given both to the biological assumptions associated with the method that is used, and to the biological implications of the results.

SECTION III. RESULTS

6. DESCRIPTIVE RESULTS

6.1 Study population characteristics

The study sample consists of 961 women with a mean age of 49.9 (±12.9) years and 596 men with a mean age of 50.1 (±13.2) years.

Table 10 shows the age-specific (as well as total) distribution of study subjects by several socio-demographic and behavioural characteristics. There are more women (961) than men (596) in the sample, but adults of all age groups are adequately represented. Approximately 27% men and 47% women were primary school graduates, 34% men and 28% women were secondary school graduates and 38% men and 25% women were university graduates. The majority of women reported that they have never smoked (68%), whilst 39% of men were classified as current smokers, 30% as past smokers and 31% as non-smokers.

Table 10.Age specific distribution (%) of 1557 subjects by socio-demographic & behavioural characteristics

Covariates	MEN (n=596)					WOMEN (n=961)				
	Age groups					Age groups				
	25-39 n=135	40-54 n=250	55-64 n=80	65+ n=131	Total n=596	25-39 n=224	40-54 n=386	55-64 n=180	65+ n=171	Total n=961
Area of residence										
Mytilini	47.4	31.2	35.0	67.9	43.5	41.1	36.5	30.6	53.2	39.4
Thessaloniki	52.6	68.8	65.0	32.1	56.5	58.9	63.5	69.4	46.8	60.6
Education										
Primary school	4.40	8.80	35.0	81.7	27.3	15.6	34.2	76.7	87.1	47.2
Secondary school	42.2	43.6	28.8	12.2	34.4	34.8	37.3	15.0	11.7	28.0
University	53.3	47.6	36.3	6.10	38.3	49.6	28.5	8.30	1.20	24.8
Smoking habits										
Current smoker	51.1	43.2	30.0	22.9	38.8	41.5	26.4	5.60	4.10	22.1
Past smoker	33.3	27.6	43.8	42.7	30.4	12.9	9.60	7.00	7.00	9.30
Non smoker	15.6	29.2	26.3	34.4	30.9	45.5	64.0	88.3	88.9	68.7

In Tables 11 and 12, age-specific (as well as total) mean values and standard deviations of BMI (in kg/m^2), WC (cm), WHR, energy intake (kcal/d), fat intake (g/d and percent of energy), mono-unsaturated (MUF), poly-unsaturated (PUF), saturated fat (SAT) intake (g/d and percent of energy), protein intake (g/d and percent of energy), carbohydrate intake (g/d and percent of energy), daily energy expenditure (in MET hours per day) are shown separately for men and women. Since the distribution of alcohol intake has been skewed (to the right) median, 25th and 75th percentiles are presented instead.

As shown in Table 11, the 596 men had a mean BMI of 28.3 (±3.71) kg/m^2. Mean WHR and WC were 0.95 (±0.07) and 97.1 (±10.3) cm respectively. Men consumed a mean of 2,235 (±621) kcal/d, from which 43.9 (±5.2) % consisted of fat, 39.9 (±5.6) % of carbohydrate, 14.2 (±1.6) % of protein. Mean daily energy expenditure, expressed in MET hours per day, was 33.8 (±6.5).

Table 11. Mean values & SD of obesity indices, dietary and physical activity data for men

Covariates	Age groups									
	25-39 (n=135)		40-54 (n=250)		55-64 (n=80)		65+ (n=131)		TOTAL (n=596)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dietary data										
Energy intake	2584	645	2304	585	2067	445	1845	505	2235	621
Fat, g	131	40.3	112	30.2	98.1	24.9	87.7	26.3	109	34.8
MUF, g	64.1	20.6	56.7	15.0	50.5	12.5	43.6	13.7	54.7	17.4
PUF, g	64.1	20.6	56.7	15.0	50.5	12.5	43.6	13.7	54.7	17.4
SAT, g	38.9	13.9	31.6	10.8	26.5	8.95	24.3	9.28	30.9	12.2
Carbohydrate, g	249	61.5	227	66.1	211	49.8	187	54.6	221	64.2
Protein, g	92.8	23.1	81.1	20.8	72.4	17.9	64.3	17.4	78.9	22.5
Alcohol, g [a]	7.50	3.18	9.00	3.23	8.0	2.23	5.90	2.16	7.80	2.20
Fat, % *	45.3	5.04	43.9	5.07	42.6	4.49	42.9	5.78	43.9	5.23
MUF, % *	22.2	3.46	22.3	3.18	22.0	2.62	21.4	3.86	22.1	3.35
PUF, % *	5.81	1.95	5.38	1.19	5.24	1.02	5.78	2.02	5.55	1.59
SAT, % *	13.4	2.69	12.3	2.38	11.41	2.49	11.8	2.85	12.3	2.65
Carbohydrate %*	38.9	5.46	39.4	5.21	41.1	5.34	40.8	6.41	39.9	5.62
Protein, % *	14.4	1.54	14.2	1.53	14.0	1.66	14.0	1.80	14.2	1.62
Alcohol, % * [a]	2.30	1.50	2.70	2.23	2.60	1.80	2.78	1.60	2.58	1.60
Physical activity **	37.4	6.34	34.97	5.72	33.5	6.48	28.2	3.67	33.8	6.45
Obesity indices										
BMI	27.6	4.22	28.4	3.55	28.9	2.85	28.5	3.79	28.3	3.71
WHR	0.89	0.06	0.94	0.06	0.97	0.07	0.97	0.05	0.95	0.07
WC	91.9	9.89	97.5	10.5	100	9.15	99.8	9.19	97.1	10.3

* percent of energy intake ** MET hours per day a: Median and inter-quartile range

The 961 women had a mean BMI of 29.0 (5.12) kg/m^2. Mean WHR and WC were 0.78 (±0.08) and 84.6 (±12.5) cm and respectively. Women consumed a mean of 1,780 (±492) kcal/d, from which 45.7 (±4.7) % consisted of fat, 41.1 (±5.6) % of carbohydrate, 14.6 (±1.6) % of protein. Mean daily energy expenditure, expressed in MET hours per day, was 34.6 (±4.4).

Table 12. Mean values & SD of obesity indices, dietary & physical activity data for women

Covariates	Age groups									
	25-39 (n=224)		40-54 (n=386)		55-64 (n=180)		65+ (n=171)		TOTAL (n=961)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dietary data										
Energy intake	1984	468	1894	465	1622	444	1420	378	1780	492
Fat, g	104	27.1	97.9	26.3	80.5	25.8	69.9	20.9	90.9	28.6
MUF, g	51.5	12.7	48.9	12.6	41.3	12.8	34.4	10.7	45.5	13.8
PUF, g	12.6	4.70	11.8	4.04	10.2	4.24	9.37	4.45	11.3	4.49
SAT, g	31.0	10.7	28.6	10.4	21.6	9.22	18.8	7.46	26.1	10.8
Carbohydrate, g	194	51.3	190	52.0	172	45.0	155	42.7	181	51.0
Protein, g	72.5	17.3	68.9	17.8	58.1	16.7	51.2	14.5	64.9	18.7
Alcohol, g [a]	2.01	0.16	1.17	0.03	0.61	0.20	0.61	0.01	8.09	2.23
Fat, % *	47.1	4.24	46.5	4.35	44.4	4.50	43.4	5.09	45.7	4.70
MUF, % *	23.5	3.05	23.3	2.86	22.9	3.36	21.7	3.37	22.9	3.16
PUF, % *	5.71	1.43	5.62	1.35	5.64	1.62	6.00	2.74	5.71	1.74
SAT, % *	13.9	2.35	13.4	2.66	11.7	2.44	11.7	2.64	12.9	2.70
Carbohydrate %*	39.1	5.08	40.2	5.21	42.8	5.14	43.9	6.09	41.1	5.63
Protein, % *	14.7	1.54	14.8	1.62	14.4	1.61	14.4	1.80	14.6	1.64
Alcohol, % * [a]	0.71	0.02	0.47	0.01	0.30	0.09	0.32	0.01	2.60	0.78
Physical activity **	35.5	4.29	35.7	4.38	34.2	3.81	31.2	2.92	34.6	4.35
Obesity indices										
BMI	26.2	4.59	28.6	4.91	31.3	4.74	31.4	4.53	29.0	5.12
WHR	0.72	0.07	0.77	0.07	0.83	0.07	0.84	0.06	0.78	0.08
WC	75.98	10.7	82.9	11.4	91.8	10.8	92.3	9.88	84.6	12.5

* percent of energy intake ** MET hours per day a: median and interquartile range

Mean BMI, WC and WHR, all increase with age; however in women the increase in indices of obesity is more profound than it is in men. It is also interesting to note that although for women the increase in obesity indices is continuous, for men there is a 'peak' at the age of 55-64 and then there is a decline. Thus, in women, BMI increases from a mean of 26 kg/m^2 in those aged 25-39 to a mean of 31 kg/m^2 to those aged over 65 years. Accordingly, mean WHR increases from 0.72 in those aged 25-39 to a mean of about 0.84 to those aged over 65 years. Finally, mean WC

increases from a mean of 76 cm in those aged 25-39 to a mean of 92 cm in those aged over 65 years. In men, mean BMI increases with age from a mean of 27.6 kg/m^2 in those aged 25-39 to a mean of 28.5 kg/m^2 in those aged over 55 years. Accordingly, mean WHR increases from a mean of 0.89 in those aged 25-39 to a mean of 0.97 to those aged over 55 years. Finally, mean WC increases from a mean of 92 cm in those aged 25-39 to a mean of 100 cm in those aged over 55 years. However, this is not the pattern observed for energy intake; the latter keeps decreasing as age increases. It is interesting to note that although obesity indices are practically identical between the two last age groups, both for men and women, this is not the case for energy intake which keeps decreasing further. Thus, in both men and women, energy intake decreases with age from a mean of 2,584 kcal for men and 1,984 kcal for women aged 25-39, to a mean of 1,845 kcal for men and 1,420 kcal for women aged over 65 years. Concerning macro-nutrient intake, absolute values of fat and fat fractions, protein and carbohydrate as well as alcohol intake all decrease with increasing age. This is not exactly the case for different macronutrients when these are expressed as percent of total energy intake. Specifically, although mean % fat intake decreases with age, from a mean of 45% for men and 47% for women, aged 25-39 years, to a mean of 43% both for women and men over 65 years of age, % carbohydrate intake increases, both for men and women, whilst % protein intake remains relatively stable across different age groups, both for men and women. Finally, concerning energy expenditure, women appear to have a greater mean energy expenditure of 34.6 (4.4) METs compared to men (33.8 (6.45) METs). Interestingly, men appear to have greater mean values of energy expenditure at the two age younger groups and women at the two older groups.

Tables 13 and 14 show simple correlation coefficients between the principal variables of this analysis. Correlation matrices have been prepared separately for men and women. Briefly, energy intake seems to be inversely correlated to all obesity indices both for men and women, although for men the associations for WC and BMI remained non significant. Physical activity, although positively associated

Table 13. Correlation matrix of key variables – MEN (n=596)

	%Fat	%MF	%PF	%ST	%Cho	%Pro	%Alc	Fatg	MUFg	PUFg	SATg	Cho g	Pro g	Alc g	BMI	WC	WHR	PAL	Age
EI	.08	-.04	-.02	.24**	-.17**	-.09*	.12**	.92**	.87**	.69**	.85**	.87**	.92**	.31**	-.01	-.05	-.16**	.40**	-.40**
% Fat	-	.79**	.31**	.72**	-.60**	.37**	-.49**	.45**	.45**	.29**	.45**	-.21**	.22**	-.43**	-.01	-.07	-.11**	.03	-.16**
%MUF		-	-.04	.29**	-.46**	.16**	-.35**	.26**	.43**	-.03	.13**	-.27**	.02	-.32**	.02	-.04	-.05	.17	-.09*
% PUF			-	.05	-.12**	-.06	-.16**	.11**	-.02	.68**	.02	-.09*	-.04	-.14**	-.03	-.04	-.04	-.06	-.03
% SAT				-	-.50**	.53**	-.37**	.48**	.36**	.21**	.70**	.01	.44**	-.29**	-.04	-.08*	-.14**	.09*	-.21**
% Cho					-	-.24**	-.37**	-.37**	-.37**	-.22**	-.37**	.33**	-.24**	-.38**	-.01	.01	.004	-.03	.13**
% Pro						-	-.43**	.05	-.01	-.10*	.20**	-.18**	.29**	-.41**	.02	.02	.02	-.003	-.07
% Alc							-	-.09*	-.07	-.04	-.13**	-.08	-.07	.94**	.02	.05	.10*	-.001	.05
Fat g								-	.96**	.74**	.93**	.68**	.90**	.09*	-.02	-.08	-.19**	.37	-.42**
MUF g									-	.62**	.82**	.63**	.83**	.10*	-.005	-.07	-.18**	.37**	-.40**
PUF g										-	.61**	.53**	.62**	.11*	-.03	-.07	-.16**	.24**	-.31**
SAT g											-	.62**	.89**	.03	-.04	-.09*	-.20**	.33**	-.40**
Cho g												-	-.76**	.086**	-.01	-.03	-.15**	.37**	.32**
Pro g													-	.12**	-.003	-.04	-.15**	.39**	-.42**
Alc g														-	.01	.04	.07	.06	-.02
BMI															-	.81**	.52**	-.10*	.12**
WC																-	X	-.20**	.28**
WHR																	-	-.25**	.43**
PAL																		-	-.48**
Age																			-

* Correlation is significant at the 0.05 level (2-tailed) ** Correlation is significant at the 0.01 level (2-tailed) X: "illegal analysis"

Table 14. Correlation matrix of key variables – Women (n=961)

	%Fat	%MUF	%PUF	%SAT	%Cho	%Pro	%Alc	Fat g	MUF g	PUF g	SAT g	Cho g	Pr g	Alc g	BMI	WC	WHR	PAL	Age
EI	.23**	.02	-.04	.41**	-.22**	-.05	.11**	.95**	.90**	.69**	.88**	.88**	.93**	.23**	-.13**	-.17**	-.19**	.19**	-.41**
% Fat	-	.72**	.26**	.67**	-.91**	.24**	-.07*	.50**	.50**	.33**	.47**	-.19**	.30**	-.05	-.04	-.10**	-.15**	.11**	-.30**
% MUF		-	-.16**	.15**	-.60**	.03	-.06	.23**	.44**	-.10**	.08*	-.25*	.03	-.07*	.01	-.03	-.09*	.12**	-.18*
% PUF			-	-.03	-.21**	-.06	.01	.04	-.10**	.66**	-.04	-.12**	-.06	.01	.02	-.01	.001	-.05	.04
% SAT				-	-.70**	.41**	-.02	.57**	.43**	.26**	.77**	.07*	.53**	.02	-.11**	-.14**	-.16**	.07*	-.34**
% Cho					-	-.47**	-.24**	-.47**	-.45**	-.29**	-.48**	.24**	-.37**	-.25**	.06	.11**	.15**	-.09**	.33**
% Pro						-	-.12**	.02	-.04	-.09**	.15**	-.26**	.31**	-.12**	.10**	.08**	.06*	-.02	-.07*
% Alc							-	.07*	.07*	.10**	.06	-.01	.06	.97**	-.15**	-.15**	-.14**	.02	-.15**
Fat g								-	.95**	.71**	.94**	.71**	.91**	.18**	-.13**	-.18**	-.21**	.19**	-.44**
MUF g									-	.57**	.82**	.67**	.84**	.17**	-.11**	-.16**	-.20**	.21**	-.43**
PUF g										-	.60**	.54**	.61**	.18**	-.10**	-.14**	-.14**	.11**	-.26**
SAT g											-	.63**	.89**	.16**	-.15**	-.18**	-.21**	.15**	-.43*
Cho g												-	.74**	.10**	-.10**	-.11**	-.12**	.15**	-.26**
Pr g													-	.16**	-.09**	-.13**	-.16**	.17**	-.41**
Alc g														-	-.14**	-.14**	-.14**	.004	-.18**
BMI															-	.84**	.46**	-.16**	.39**
WC																-	X	-.20**	.51**
WHR																	-	-.21**	.53**
PAL																		-	-.32**
Age																			-

* Correlation is significant at the 0.05 level (2-tailed) ** Correlation is significant at the 0.01 level (2-tailed) X: illegal analysis

with total energy intake both for men and women, is significantly inversely associated with all obesity indices both for men and women; the only exception is that of WHR for men for which the association remained non significant. The correlations between different obesity indices and nutrient intakes (%) are rather variable. In women, there are positive correlations with carbohydrate and protein intake, and inverse associations for total fat, saturated fat intake and alcohol intake. These associations are broadly consistent between different obesity indices. The latter, is not the case for men; most of the correlations do not reach statistical significance with the exception of a direct correlation between WHR and alcohol intake and an inverse correlation with WHR and saturated fat intake.

Before going further, it is important to note that the nutritional characteristics of the sample as appeared in the Tables 11-12, are similar to those of the average Greek diet (Trichopoulou et al, 1993a, Trichopoulou et al, 1993b, Trichopoulou et al, 1994).

6.2 Classification of obesity

An interesting observation is that of obesity classification. Table 15 shows the age-specific distribution of study subjects by different obesity indices. As expected, younger men and women had lower BMI, WHR, as well as WC than did older men and women. The increase is particularly profound in women (after the age of 55 years) and may imply menopausal status effects. It is also interesting to note that in both men and women, in the first two age groups, BMI classifies more subjects as obese than WHR and/or WC classifications do. In the older age groups the situation is reversed by BMI classifying fewer subjects as obese than WHR and/or WC classifications do. This might be because of the better physical condition (better stature, more muscle mass and better muscle tone) of younger groups compared to the older ones.

Table 15. Age specific distribution (%) of 1557 subjects by obesity indices

Covariates	MEN (N=596)				WOMEN (N=961)			
	Age groups				Age groups			
	25-39 n=135	40-54 n=250	55-64 n=80	65+ n=131	25-39 n=224	40-54 n=386	55-64 n=180	65+ n=171
BMI (kg/m²)								
<30	76.3	71.2	70.0	66.4	88.3	67.4	41.7	39.8
≥30	23.7	28.9	30.0	33.6	17.0	32.6	58.3	60.2
<25	23.7	16.8	6.3	16.8	46.0	25.4	8.3	6.40
25-30	52.6	54.4	63.7	49.6	37.1	42.0	33.3	33.3
30-40	22.2	28.8	30.0	33.6	14.7	29.5	55.0	55.6
>40	1.5	0	0	0	2.2	3.1	3.3	4.7
WHR								
< 1.00* OR < 0.85**	94.1	80.8	68.8	64.9	99.1	85.8	63.3	57.9
≥ 1.00* OR ≥ 0.85 **	5.9	19.2	31.3	35.1	0.9	14.2	36.7	42.1
WC (cm)								
< 102* OR < 88**	85.9	74.4	65.0	60.3	90.2	73.8	38.3	36.3
≥ 102* OR ≥ 88**	14.1	25.6	35.0	39.7	9.8	26.2	61.7	63.7

* Cut-off point for men **Cut-off point for women

Discrepancies in obesity classification are most likely to be due to the specific cut-off points used to classify individuals. Approximately 29% of men and 39% of women were classified as obese according to BMI classification. These percentages are somewhat less (36% for women and 27% for men) for WC classification, and even less (20% for women and 21% for men) for WHR classification (Table 16).

Table 16. Percentage of obese individuals according to different obesity indices

	BMI>30	*WHR>1.00 (men)* *WHR>0.85 (women)*	*WC>102 (men)* *WC>88 (women)*
Men	29 %	21%	27 %
Women	39 %	20 %	36 %

We proceeded one step further by cross-classifying the subjects according to all three obesity indicators (BMI-WHR, BMI-WC, WHR-WC) in order to identify whether the same subjects were classified in the same way by any two indices. It emerged that BMI and WC classified 85% of men (55% normal weight plus 30% obese) and 85% of women (64% normal weight plus 21% obese) in the same way. Percentages were somewhat less for BMI-WHR (76% for men and 71% for women) and WC-WHR classification (85% for men and 80% for women) (Tables 17-19).

Table 17. Level of agreement in obesity classification between BMI and WHR

	Men		Women	
	WHR<1.00	*WHR>1.00*	*WHR<0.85*	*WHR>0.85*
BMI < 30	63%	8%	56%	6%
BMI > 30	16%	13%	24%	15%

Table 18. Level of agreement in obesity classification between BMI and WC

	Men		Women	
	WC<102	*WC>102*	*WC<88*	*WC>88*
BMI < 30	64%	7%	55%	6%
BMI > 30	8.2	21%	9%	30%

Table 19. Level of agreement in obesity classification between WHR and WC

	Men		Women	
	WC<102	*WC>102*	*WC< 88*	*WC>88*
*WHR<1.00**	68%	11%	62%	18%
*WHR<0.85 ***				
*WHR>1.00**	5%	17%	2%	18%
*WHR>0.85***				

* Cut-off point for men **Cut-off point for women

There has also been an analysis to identify how many subjects have been classified as obese according to all three indices. The analysis revealed that 74 men (12.4%) and 139 women (14.5%) were classified as obese using all three indices. This analysis was also performed by age group. Seven men out of 135 (5.0%) and 2 women out of 224 (0.9%) were classified as obese using all three indices in the first age group (25-39). Thirty-three men out of 250 (13.2%) and 41 women out of 386 (10.6%) were classified as obese using all three indices in the second age group (40-54). Fifteen men out of 80 (18.7%) and 48 out of 180 (26.6%) were classified as obese using all three indices in the third age group (40-54). Nineteen men out of 131 (14.5%) and 48 women out of 171 (28.1%) were classified as obese using all three indices in the fourth age group (65+).

Finally, Tables 20 and 21 show the percentage distribution of obesity indices by education and smoking status. Associations have been tested using the chi squared test.

Table 20. Percentage distribution of 1557 subjects by obesity indices and education level

Covariates	MEN (n=596)				WOMEN (n=961)			
	Education level				Education level			
	Pri/ry n=163	Sec/ry n=205	Univ/ty n=228	P value	Pri/ry n=454	Sec/ry n=269	Univ/ty n=238	P value
BMI (kg/m²)				0.048				<0.001
<30	64.4	71.2	75.9		43.4	72.5	82.8	
≥30	35.6	28.8	24.1		56.6	27.5	17.2	
<25	14.1	19.5	16.7	0.184	9.3	30.9	42.9	<0.001
25-30	50.3	51.7	59.2		34.1	41.6	39.9	
30-40	35.6	28.3	23.7		52.6	24.2	15.5	
>40	0	0.5	0.4		4.0	3.3	1.7	
WHR				<0.001				<0.001
< 1.00* OR < 0.85**	64.4	82.0	86.0		66.3	88.5	95.4	
≥ 1.00* OR ≥ 0.85 **	35.6	18.0	14.0		33.7	11.5	4.6	
WC (cm)				<0.001				<0.001
< 102* OR < 88**	60.7	72.7	81.1		44.7	78.1	86.1	
≥ 102* OR ≥ 88**	39.3	27.3	18.9		55.3	21.9	13.9	

* Cut-off point for men **Cut-off point for women

Table 21. Percentage distribution of 1557 subjects by obesity indices and smoking status

	MEN (n=596)				WOMEN (n=961)			
	Smoking status				Smoking status			
	Current n=231	Past n=181	Non n=184	P value	Current (n=212)	Past (n=89)	Non (n=660)	P value
BMI (kg/m²)				0.147				<0.001
<30	72.7	65.7	74.5		76.9	68.5	55.3	
≥30	27.3	34.3	25.5		23.1	31.5	44.7	
<25	19.5	15.5	15.2	0.148	33.5	33.7	19.1	<0.001
25-30	53.2	50.3	59.2		43.4	34.8	36.2	
30-40	27.3	33.1	25.5		21.2	28.1	41.1	
>40	0	1.1	0		1.9	3.4	3.6	
WHR				0.163				<0.001
< 1.00* OR < 0.85**	77.9	75.1	83.2		92.0	83.1	75.3	
≥ 1.00* OR ≥ 0.85 **	22.1	24.9	16.8		8.0	16.9	24.7	
WC (cm)				0.318				<0.001
< 102* OR < 88**	74.0	68.5	75.0		82.5	70.8	64.3	
≥ 102* OR ≥ 88**	26.0	31.5	25.0		17.5	29.2	35.7	

* Cut-off point for men **Cut-off point for women

Subjects at higher education level are less likely to be obese compared to subjects at lower education level. The association was stronger and more persistent among women than among men. Moreover, female current smokers are less likely to be obese compared to non smokers or past smokers. The association was not significant

for men. It should be noted however, that these descriptive results, although suggestive of the associations, should not be over-interpreted since the critical effect of age has not been taken into consideration. The adjusted effect of education and tobacco smoking will be further explored in the following chapters of this section.

7. REPORTING BIAS

Over the last few years, researchers have gained substantial experience in the evaluation of results derived from large scale nutritional studies. A major methodological issue has been the ascertainment of energy intake data, since most of the studies exploring associations between diet and health have been based on the assumption that the differences between the true and the reported intakes of individuals are random and that an unbiased measure of the group mean intake is obtained. However, the latter assumption has not always been established through independent non-nutritional methods.

If errors in reporting cause both over and under estimation, the mean results are still valid, although the precision of the measurement may be reduced. However, if a large proportion of the population gives misleading information all tending in the same direction, then the mean results will be biased.

7.1 Assessment of under-reporting

Theoretical consideration together with the few existing cross validation studies both suggest that under-reporting is much more prevalent than over-reporting (Goldberg et al, 1991, Black et al, 1991). Under-reporting occurs when the average daily energy intake is appreciably less than the measured or calculated energy expenditure, and it has been shown to be more prevalent and severe among obese rather than lean subjects (Prentice et al, 1986, Hulten et al, 1990, Heitmann, 1993, Gnardellis et al, 1998). Possible reasons for this could be either a distorted concept of food amounts or a guilty conscience. Furthermore, detailed studies indicate that such bias may not be nutrient neutral (Lissner & Lindroos, 1994).

7.1.1 Comparing habitual energy intake with habitual energy expenditure

Since information on energy intake and energy expenditure is available in this study, direct comparison of energy intake (EI) and energy expenditure (EE) becomes possible. The idea of directly comparing individuals' energy intake data with individuals' energy expenditure data lies on the facts that: a) the FFQ is assumed to measure subjects' 'habitual' dietary intake; and b) the physical activity questionnaire claims to measure 'habitual' energy expenditure. Under 'free-error' conditions, and assuming weight stability, this would imply that all subjects whose reported energy expenditure exceeded reported energy intake would be definite under-reporters of energy intake or over-reporters of energy expenditure.

In the Methods Section (chapter 5.3.1) there has been a detailed description of the procedure followed for the estimation of the individual's mean daily energy intake. Individual total energy expenditure was derived by multiplying individual PAL level (METs/24h) by individual BMR (the latter calculated using the Schofield equations (Schofield et al, 1985)). Individual energy expenditure estimates (in kcal) were subsequently subtracted from individual energy intake estimates (in kcal). Negative values were used to distinguish low-energy reporters from regular reporters.

By following this approach 1,044 subjects out of 1,557 (67%) were identified to 'under-report' their energy intake. Table 22 shows the sex and age-specific distribution of study subjects whose reported energy expenditure exceeds reported energy intake. Under-reporting is more extensive in women. It tends to increase with age in women but not in men.

Table 22. Number (% of total) of under-reporters by age and sex groupings

SEX	Age groups				
	25-39	40-54	55-64	64+	TOTAL
Men	91 (67%)	179 (71.6%)	55 (68.7%)	55 (41.9%)	380 (63.7%)
Women	127 (56.6%)	262 (67.9%)	140 (77.8%)	135 (78.9%)	664 (69.1%)
TOTAL	218 (60.7%)	441 (69.3%)	195 (75.0%)	190 (62.9%)	1044 (67%)

* *Percentages refer to % of the total number of subjects in the respective age and sex groups*

However, this approach is crude, since it does not allow for the imprecision which is inherent to measurements of both 'habitual' energy intake and 'habitual' energy expenditure. An effort has been made to estimate how much EI estimates are likely to differ from EE estimates and still provide plausible estimates of both EI and EE measurements, given the constraints and imprecision of the methods used for data collection purposes.

The first step was to plot EI against EE separately for men and women (Figures 9-10). Plotting the data helps to get an impression of the degree of agreement between the two estimates. In men there seems to be a relatively good agreement. However, for the women there is no obvious relation between EI and EE estimates. The latter observation was actually confirmed by calculating respective correlation coefficients. For men the latter was moderate ($r=0.45$) whilst for women it was rather weak ($r=0.26$). Both correlation coefficients were statistically significant but this is rather a feature to be attributed to the large sample size than to the association itself.

The line of equality, on which all points would lie if the methods were in perfect agreement, was subsequently plotted. However, since it is most unlikely that the two estimates would agree exactly by giving identical results for all the subjects, a more realistic approach to the issue was undertaken. According to this, one would expect that a limited number of cases would lie along the equality line but most of the subjects would lie below (under-reporters) or above the line (over-reporters). There has been an assumption that there is approximately 20% imprecision in both EI and EE estimates; the latter assumption is arbitrary, although plausible. We would thus expect that most of the plausible estimates to lie between 2SD of the line of equality. The combined SD of both estimates would equal $\sqrt{(SD_{EI}^2+SD_{EE}^2)}$. In the current data set one SD would equate to $\pm$ 28% and two SDs would equate to $\pm$56%. We have used these data to draw the lines defining the range of how apart our estimates would lie without implying implausible or wrong estimates of EI and EE measurements, given the constraints of the methods used for data collection purposes.

Figure 9. Energy intake vs energy expenditure
Men (n=596)

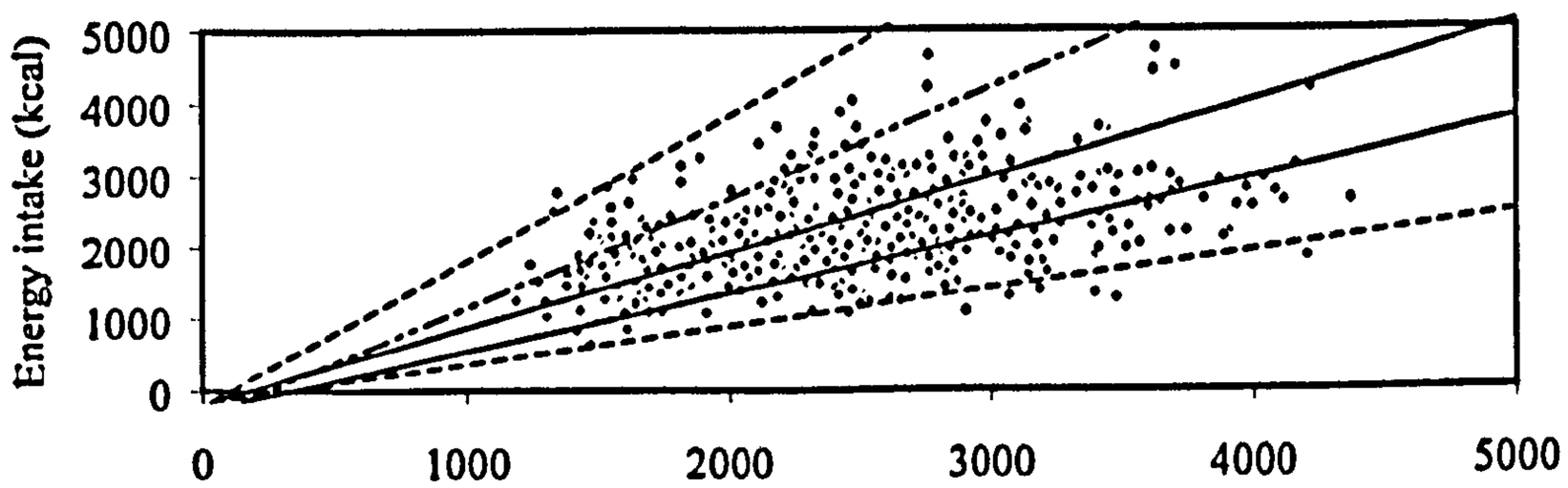
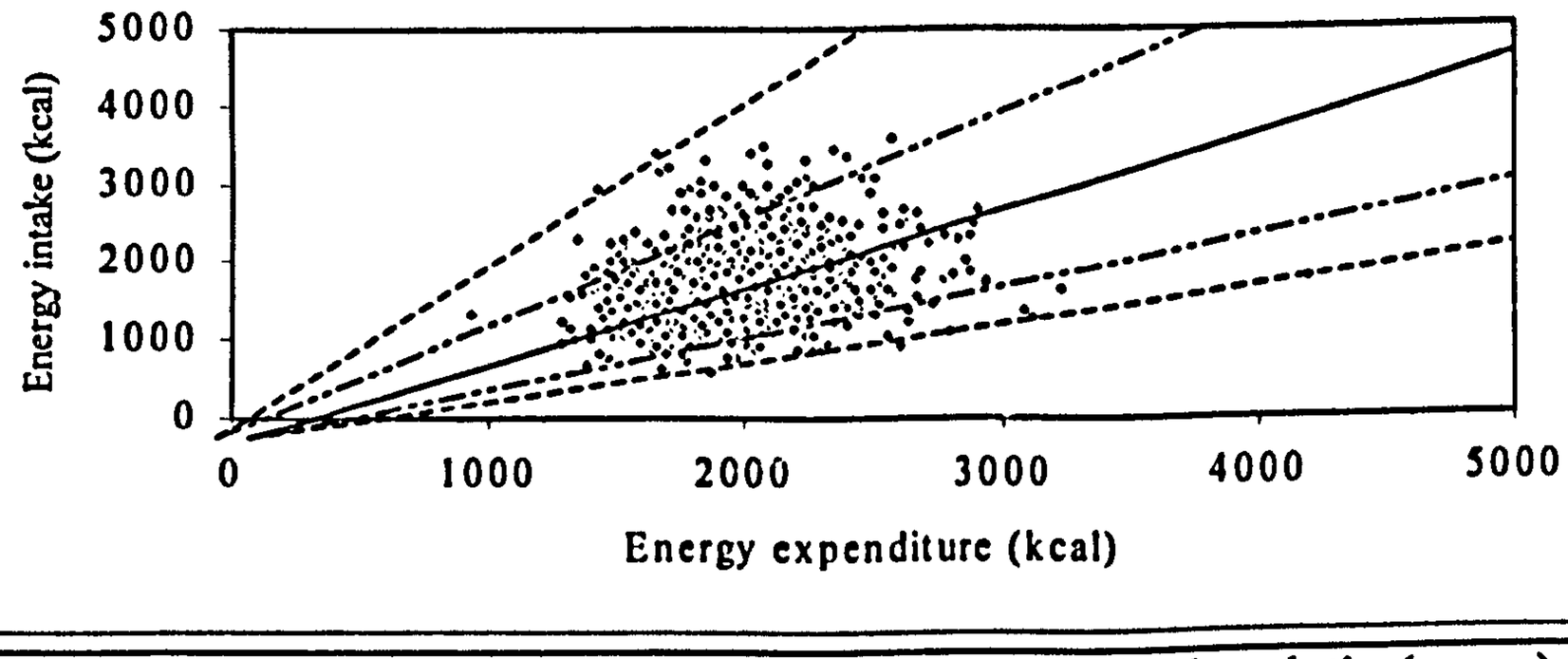


Figure 10. Energy intake vs energy expenditure
Women (n=961)



Dash' (---) lines define the 2 SDs range and 'Long dash, dot, dot' (—·--·—) lines define the 1 SD range.

The same approach was used to calculate the range of accepted values in case of sex and age specific scatterplots (Figures 11-18). Associations between energy intake and energy expenditure data were rather weak for men and even weaker in women. Thus, for men, Pearson correlation coefficients were 0.30, 0.25, 027, and 0.25 for the age groups of 25-39, 40-54, 55-64 and 64+ respectively. For women, there was actually no association (r=-0.001) in the first age group, whereas in the three other groups correlation coefficients were 0.07, 0.12 and 0.23 respectively.

Figure 11. Energy intake vs energy expenditure
Men (25-39 years)

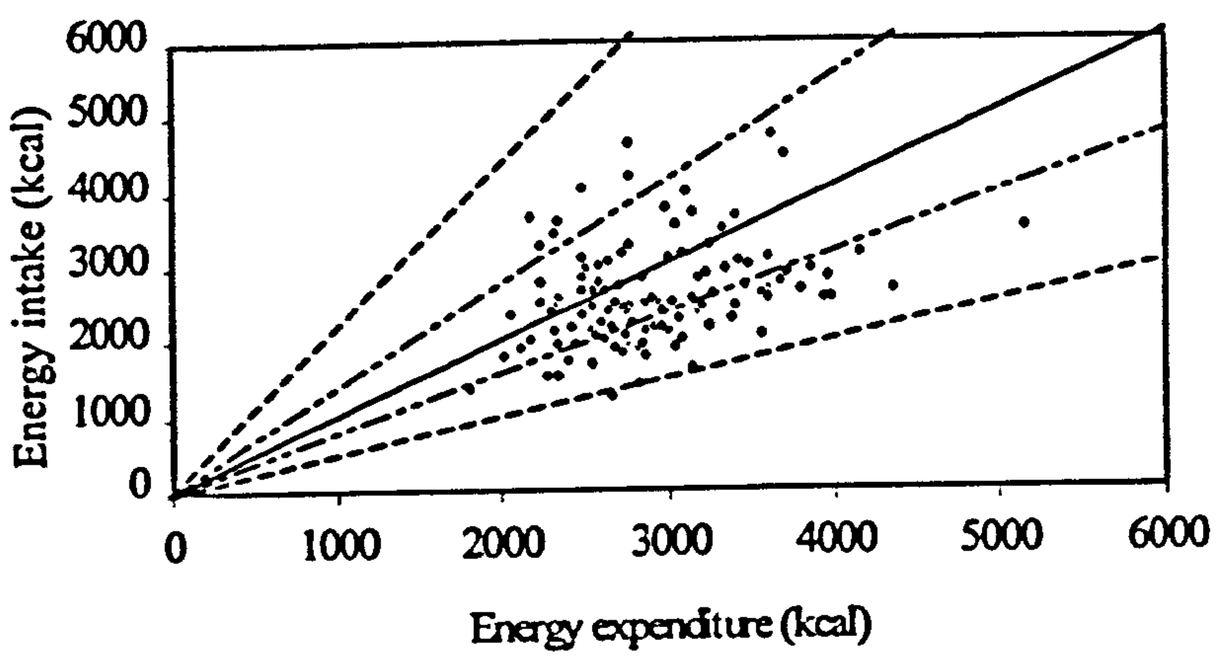


Figure 12. Energy intake vs energy expenditure
Men (40-54 years)

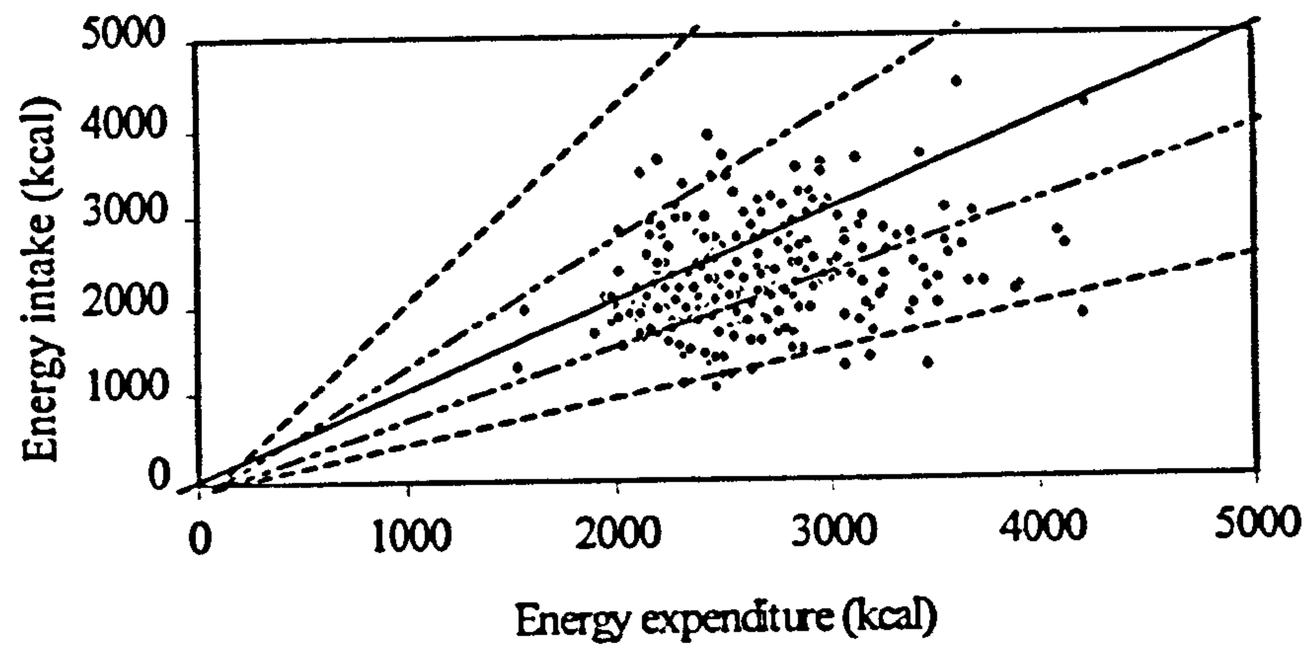


Figure 13. Energy intake vs energy expenditure
Men (55-64 years)

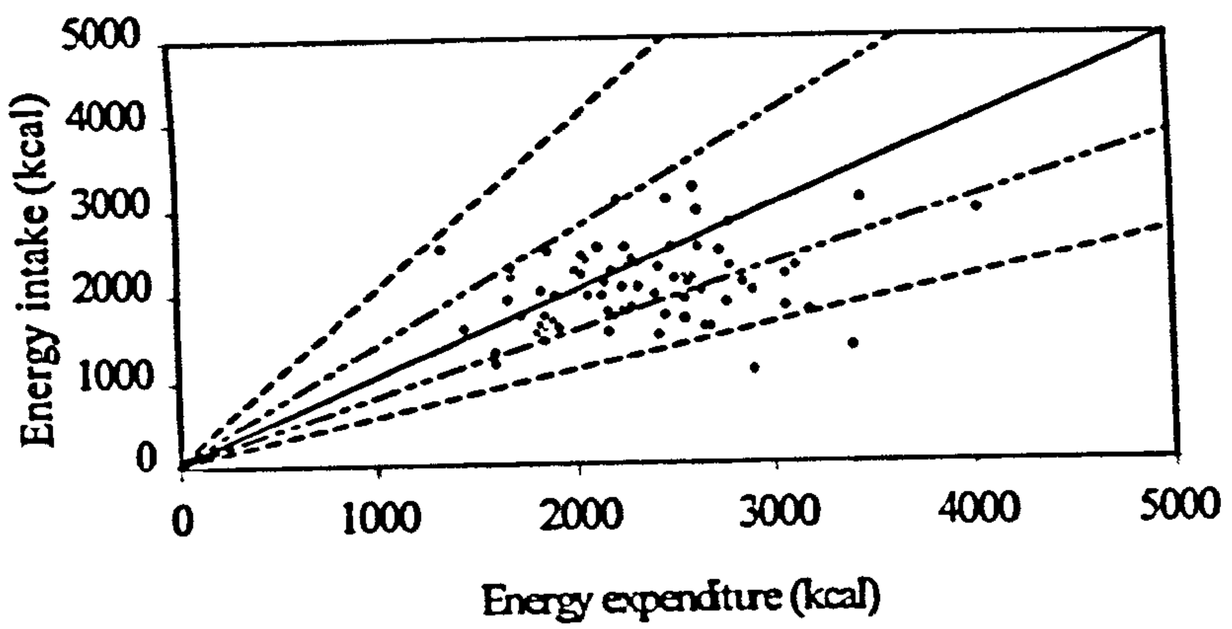


Figure 14. Energy intake vs energy expenditure
Men (65+ years)

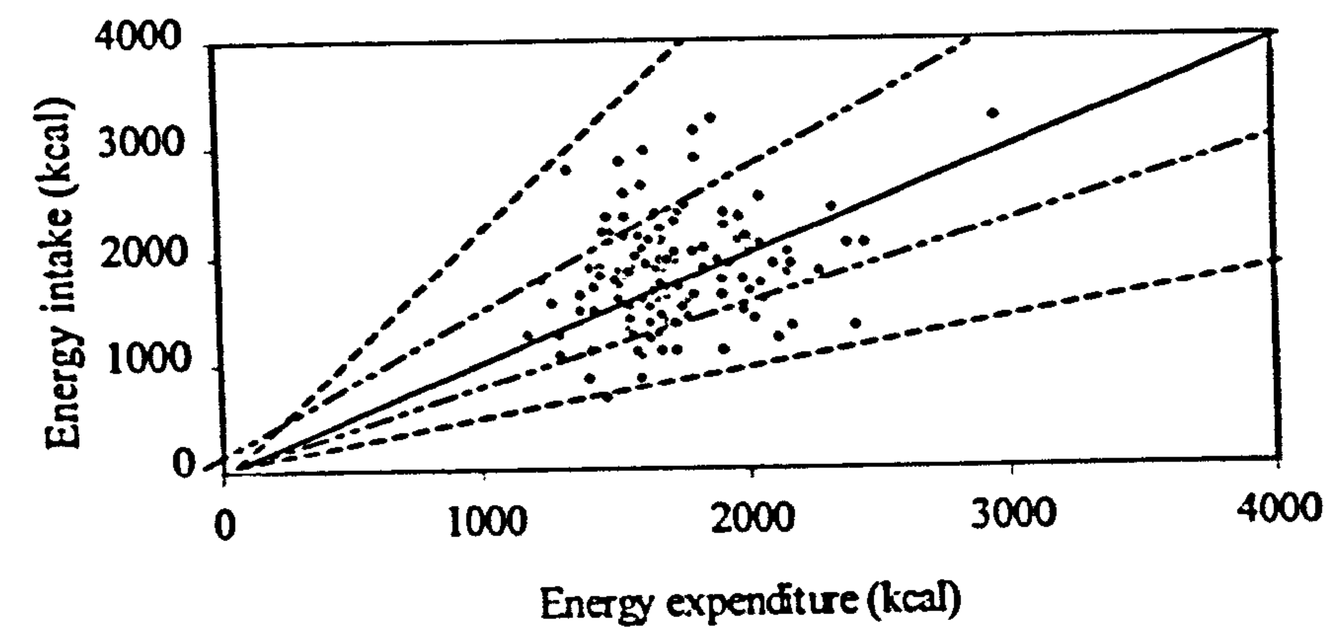


Figure 15. Energy intake vs energy expenditure
Women (25-39 years)

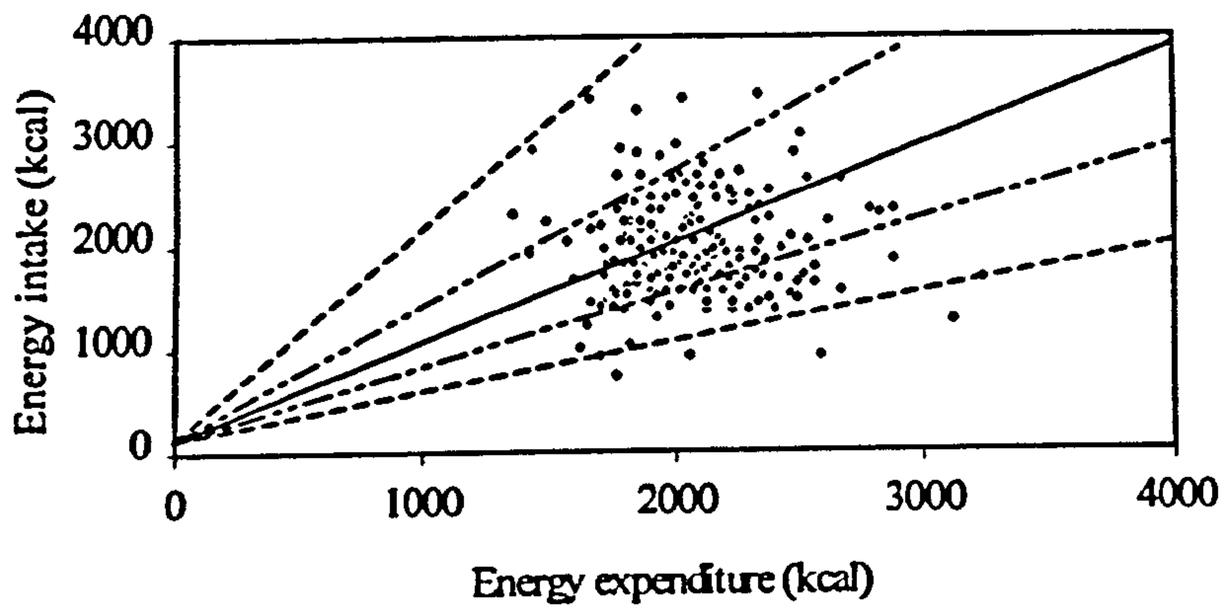


Figure 16. Energy intake vs energy expenditure
Women (40-54 years)

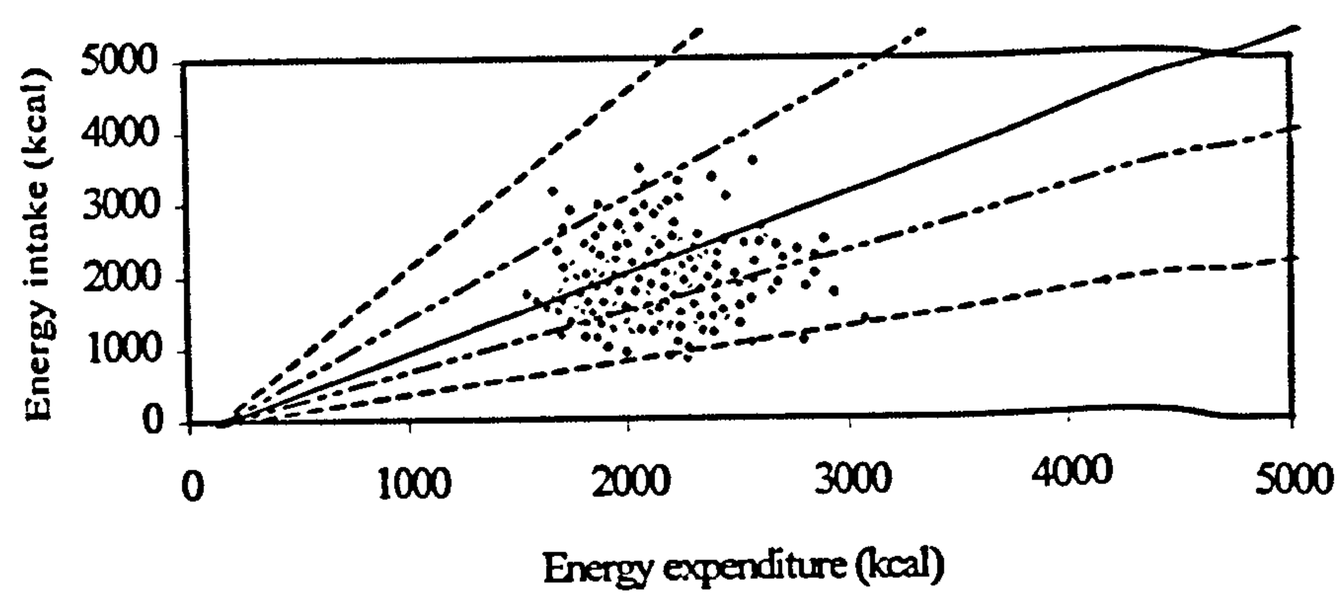


Figure 17. Energy intake vs energy expenditure
Women (55-64 years)

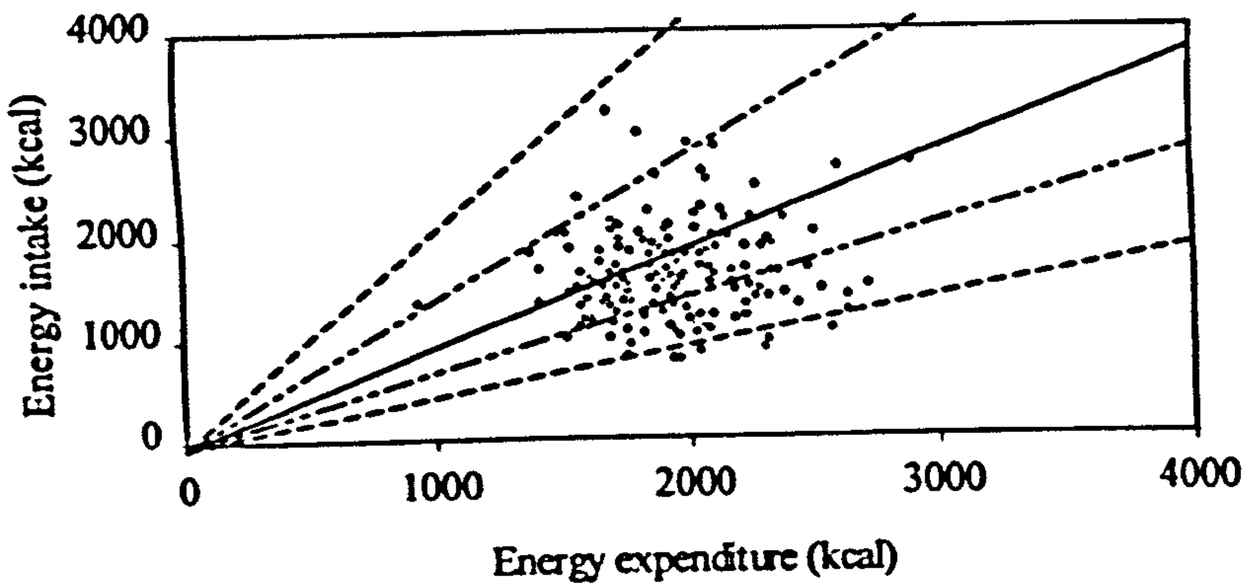
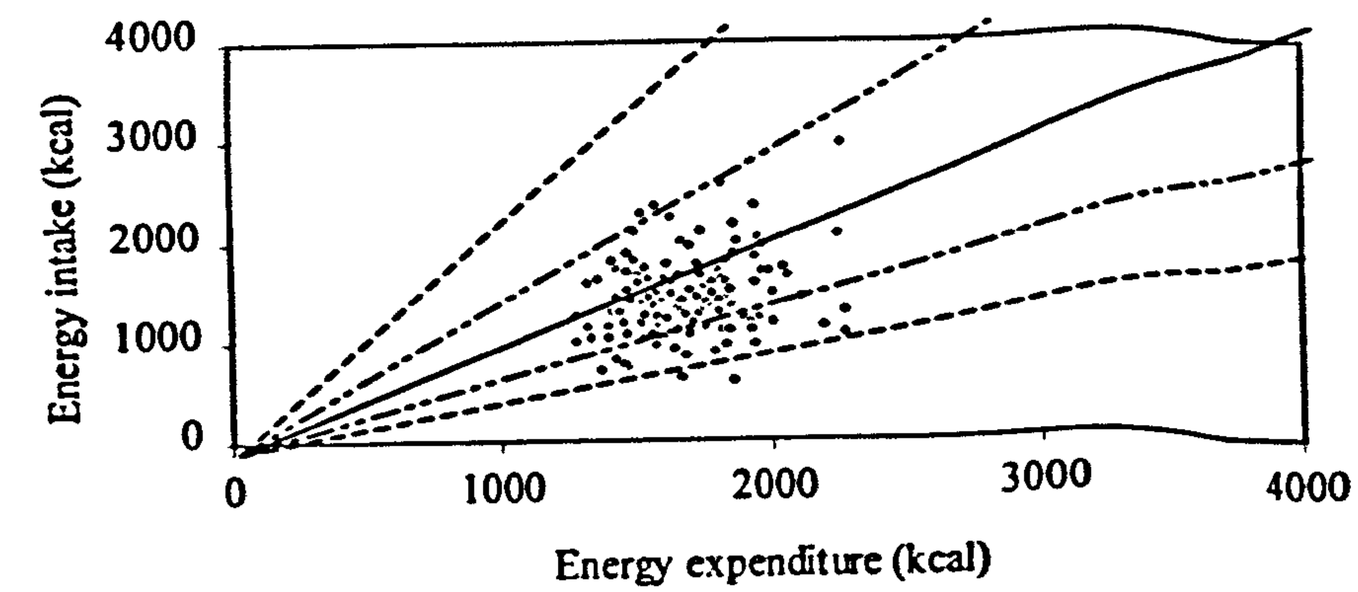


Figure 18. Energy intake vs energy expenditure
Women (65+ years)



An alternative approach to the same issue has been based on graphical techniques and simple calculations proposed by Bland and Altman (Bland & Altman, 1986). According to Bland and Altman a plot of the difference between EI and EE against their mean would be a more informative approach in assessing agreement between two methods or estimates; since we do not know the true value of the two measurements, this is the best estimate one can get.

For the EI and EE data the mean differences $\bar{d}$ in case of men and women are –231.1 and –210.8 kcal respectively. The SD of the differences are 651.2 and 511.2 kcal. We would expect most of the differences to lie between $\bar{d} \pm 2SD$. That is:

$$\bar{d} \pm 2SD = -231.1 \pm (2*651.2) = -1355.5 \text{ to } 1071.3 \text{ kcal} \quad \text{(men)}$$

$$\bar{d} \pm 2SD = -210.8 \pm (2*511.2) = -1233.5 \text{ to } 811.6 \text{ kcal} \quad \text{(women)}$$

The graphical presentation of the results is shown in Figures 19-20. The impression from these plots is practically identical to the one given by figures 9-10. Once again, only a very limited number of cases lie out the predefined range of plausible EI and EE estimates.

Figure 19. The Bland-Altman's method
Assessing agreement between two methods
Men

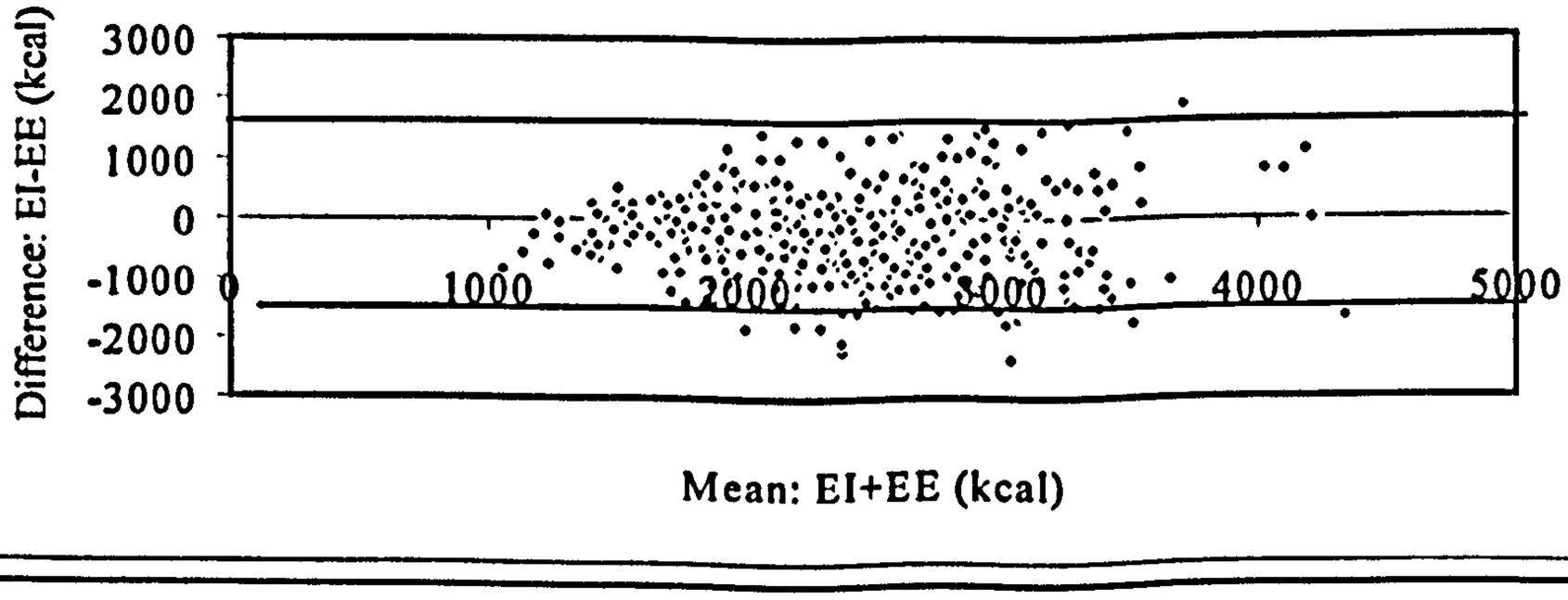
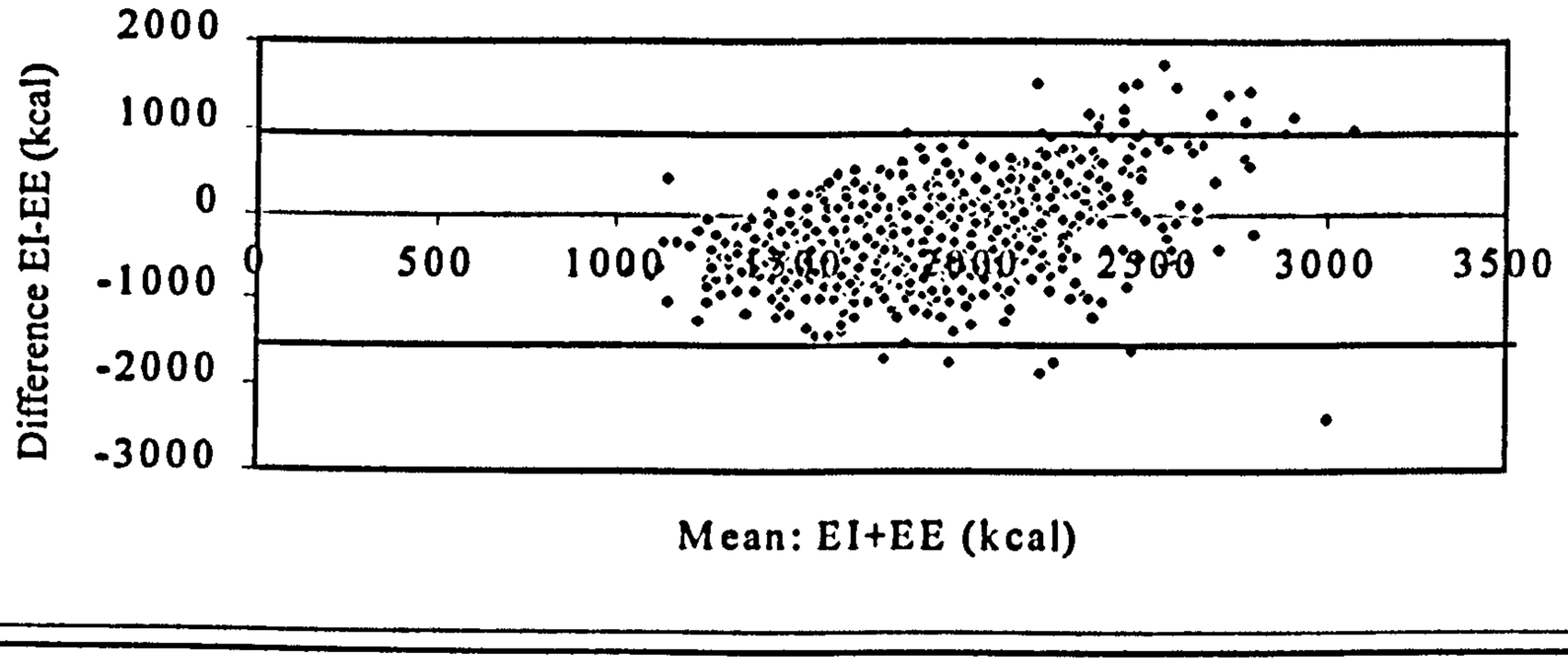


Figure 20. The Bland- Altman's method
Assessing agreement between two methods
Women



In conclusion, in every case, the vast majority of cases lies between 2SD of the line of equality supporting the notion that given the constraints of the methods used for data collection purposes, the study's EI and EE estimates seem to provide reasonably valid measures of energy intake and energy expenditure data.

7.1.2 The Goldberg cut-off point

The doubly-labeled water technique for the measurement of total energy expenditure in free living persons, enabled scientists to conduct validation studies comparing energy expenditure to energy intake (Coward, 1988, Schoeller et al, 1990). The fact however, that this method is very expensive and technically difficult to employ as a routine process, makes it impossible to use it in wide range nutritional epidemiological studies. Goldberg suggested the determination of cut-off points to represent the limits of energy needs for free living persons (Goldberg al, 1991, Black et al, 1991). In order to do that Goldberg (Goldberg et al, 1991) used data based on germane principles of energy physiology. In their approach, data from doubly-labeled water and whole body calorimetry studies on a large number of healthy

individuals were used. The cut-off point, stated as a multiple of the basic metabolic rate, was first determined as 1.35. This is defined as the lowest feasible long-term habitual intake for healthy sedentary adults. However, habitual intake is very difficult to assess, even when dietary intake is recorded over long periods of time (e.g. by food frequency questionnaires or diet history records). Thus, in order to exclude invalid energy intake records during the actual period of measurement, a second cut-off point was determined. The formula to calculate this took into consideration the daily variation of nutrition during the time period of recording, and hence the imprecision inherent in the estimation of energy intake. The latter cut-off point is more 'liberal' than the first one, and its calculation depends on the sample size and the number of days for which intake is recorded. This value results in more specific rejection of intake values deemed as biased estimates of true nutritional intake. Therefore, assuming that there is no weight loss, estimates of energy intake lower than these cut-off points could be 'suspected' of underestimating energy intake.

In the context of the current study, the measured weight and height of volunteers, were used for the estimation of their basal metabolic rate through the Schofield predictive equations (Schofield et al, 1985). The values of BMR derived from these equations were subsequently used for the calculation of EI/BMR ratio (where EI is the daily energy intake). This value is essentially the reported daily energy intake of an individual expressed in metabolic units that reflect physical activity level. The cut-off limits for the physiologically plausible energy intake of weight stable individuals were calculated from the equations developed by Goldberg (Goldberg et al, 1991). The equations are based on an expected mean total energy expenditure value (TEE/BMR) which for an average sedentary individual is estimated to be 1.55 (FAO/WHO/UNU Report, 1985). The lower cut-off limit is then defined as the lower 95% confidence limit of the expected TEE/BMR, taking into account inter-individual variation in TEE/BMR values. EI/BMR should be greater or equal to the cut-off TEE/BMR. The calculations also allow for the day-to-day variation in individual

energy intake, the error in predicting BMR, the number of days on which the intake is based and the number of individuals in the group. Based on these assumptions Goldberg (Goldberg et al, 1991) came up with the following equation to calculate the limits of a physiologically plausible EI/BMR value:

$$EI / BMR \geq 1.55 \times \exp [-2 \times (S / 100\sqrt{n})]$$

$$\text{where } S=\sqrt{ [(CV_{lw}^2 / k) + CV_B^2 + CV_P^2] }$$

where:

CV_{lw} = within-individual coefficient of variation for energy intake;
CV_B = coefficient of variation for measured or predicted BMR as estimated from Schofield equations;
CV_P = coefficient of variation for TEE/BMR as determined according to FAO/WHO/UNU;
n = number of individuals for which intake is examined (if recording is undergone on a personal basis then n=1) ;
k = number of days for which intake is recorded (for FFQ's k approaches infinity and so $CV_{lw}^2 / k = 0$).

The TEE/BMR ratio for an average sedentary individual is estimated to be 1.55 (FAO/WHO/UNU Report, 1985). The mean within individual CV for energy intake has been found in various studies to be approximately 23% (Nelson et al, 1989). The CV for estimated BMR has been determined to be 8% (Schofield, 1985) while the CV for the TEE/BMR ratio is 12.5%.

Thus in the context of our study for a physiologically plausible EI/BMR value the following assumptions apply:

$$CV_{lw}^2 = 0, \quad CV_B^2 = 8^2, \quad CV_P^2 = (12.5)^2$$

$$EI / BMR \geq 1.55 \times \exp [-2 \times (S / 100\sqrt{n})] \quad \text{where } S=\sqrt{ [8^2 + (12.5)^2] }$$

so EI / BMR $\geq$ 1.14 provided that control is conducted on an individual basis (n=1). Individuals whose energy intake was below this cut-off point are defined as under-reporters.

According to this approach 550 study participants out of 1,557 (35%) have been labelled as under-reporters of energy intake. Table 23 shows the sex and age-specific distribution of study subjects whose calculated energy intake is less than 1.14 of their basal metabolic rate. Under-reporting is slightly higher in women (35.5%) compared to men (35.1%). It increases with age; the pattern is particularly clear in women.

Table 23. Number (% of total) of under-reporters by age and sex groupings

SEX	Age groups				
	25-39	40-54	55-64	64+	TOTAL
Men	26 (19.3%)	101 (40.4%)	31 (38.8%)	51 (38.9%)	209 (35.1%))
Women	49 (21.9%)	109 (28.2%)	80 (44.4%)	103 (60.2%)	341 (35.5%)
TOTAL	75 (20.9%)	210 (33.0%)	111 (42.7%)	154 (50.9%)	550 (35.3%)

* *Percentages refer to % of the total number of subjects in the respective age and sex groups*

7.1.3 Study population sex-specific cut-off points

The Goldberg cut off has been a major tool in recent epidemiological research, since it enables examination and evaluation of data retrospectively and it does not require additional data collection. However, during the last few years, there has been a considerable amount of work exploring further the problem of under-reporting and addressing the issue of the Goldberg cut-offs' limitations. Investigators have clearly stated that the Goldberg cut-off provides a practical guide to validate energy intake against energy expenditure but draw the final conclusion that its usefulness is limited by low sensitivity and poor specificity at the individual level, unless information on physical activity is available (Black et al, 1996a, Black, 1996b, Goldberg et al, 1998).

In this context, we proceeded one step further by calculating study population sex specific cut-off points for the physiologically plausible EI/BMR values, by using sex specific mean PAL values derived from the study population; these values are 1.41 for men and 1.44 for women.

Thus, for calculating population sex-specific EI/BMR value the following assumptions apply:

$$CV_{Iw}^2 = 0, \quad CV_B^2 = 8^2, \quad CV_P^2 = (12.5)^2$$

$$EI / BMR \geq 1.41 \times \exp[-2 \times (S/100\sqrt{n})] \quad \text{where } S=\sqrt{[8^2 + (12.5)^2]} \quad \text{(men)}$$

$$EI / BMR \geq 1.44 \times \exp[-2 \times (S/100\sqrt{n})] \quad \text{where } S=\sqrt{[8^2 + (12.5)^2]} \quad \text{(women)}$$

so EI / BMR $\geq$ 1.04 for men and EI / BMR $\geq$ 1.07 for women. Individuals whose energy intake was less than 1.04 (men) and 1.07 (women) of their BMR were defined as under-reporters.

By using this approach 400 study participants out of 1557 (26%) were identified to under-report their energy intake. Table 24 shows the sex and age-specific distribution of study subjects whose EI/BMR was below 1.04 (men) or 1.07 (women). Under-reporting is more extensive in women (26.3%) compared to men (24.7%). It increases with age. The pattern is now clear in both men and women.

Table 24. Number (% of total) of under-reporters by age and sex groupings

SEX	Age groups				
	25-39	40-54	55-64	65+	TOTAL
Men	14(10.3%)	70 (28.0%)	19 (23.8%)	44 (33.6%)	147 (24.7%)
Women	27 (12.1%)	76 (19.7%)	67 (37.2%)	83 (48.5%)	253 (26.3%)
TOTAL	41 (11.4%)	146 (22.9%)	86 (33.1%)	127 (42.1%)	400 (25.7%)

* *Percentages refer to % of the total number of subjects in the respective age and sex groups*

7.1.4 Study population age- and sex-specific cut-off points

We went one step further by calculating study population sex and age specific cut-off points for the physiologically plausible EI/BMR values, by using sex and age specific mean PAL values. These are: 1.56 for men aged 25-39, 1.46 for men aged 40-54, 1.40 for men aged 55-64, 1.18 for men over 65 years, 1.48 for women aged 25-39, 1.49 for women aged 40-54, 1.43 for women aged 55-64, 1.30 for women over 65 years of age.

Thus, for calculating sex- and age- specific EI/BMR value the following assumptions apply:

$CV_{tw}^2 = 0, \quad CV_B^2 = 8^2, \quad CV_P^2 = (12.5)^2$

$EI / BMR \geq 1.56 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (men 25-39)

$EI / BMR \geq 1.46 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (men 40-54)

$EI / BMR \geq 1.40 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (men 55-64)

$EI / BMR \geq 1.18 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (men 65+)

$EI / BMR \geq 1.48 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (women 25-39)

$EI / BMR \geq 1.49 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (women 40-54)

$EI / BMR \geq 1.43 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (women 55-64)

$EI / BMR \geq 1.30 \times \exp[-2 \times (S/100\sqrt{n})]$ where $S=\sqrt{[8^2 + \overline{(12.5)^2}]}$ (women 65+)

so $EI / BMR \geq 1.16$ for men aged 25-39, 1.09 for men aged 40-54, 1.04 for men aged 55-64, 0.88 for men over 65 years, and $EI / BMR \geq 1.10$ for women aged 25-39, 1.11 for women aged 40-54, 1.06 for women aged 55-64, 0.97 for women over 65 years of age.

Thus, 390 study participants out of 1,557 (25%) whose energy intake was below these cut-off points were defined as under-reporters. Table 25 shows the sex and age-specific distribution of these subjects.

Table 25. Number (% of total) of under-reporters by age and sex groupings

	Age groups				
SEX	**25-39**	**40-54**	**55-64**	**64+**	**TOTAL**
Men	26 (19.3%)	87 (34.8%)	18 (22.5%)	14 (10.7%)	149 (25.0%)
Women	33 (14.7%)	94 (37.6)	66 (36.7%)	52 (30.4%)	245 (25.5%)
TOTAL	59 (16.4%)	181 (28.5%)	84 (32.3)	66 (21.9%)	390 (25.0%)

* *Percentages refer to the total number of subjects in the respective age and sex groups*

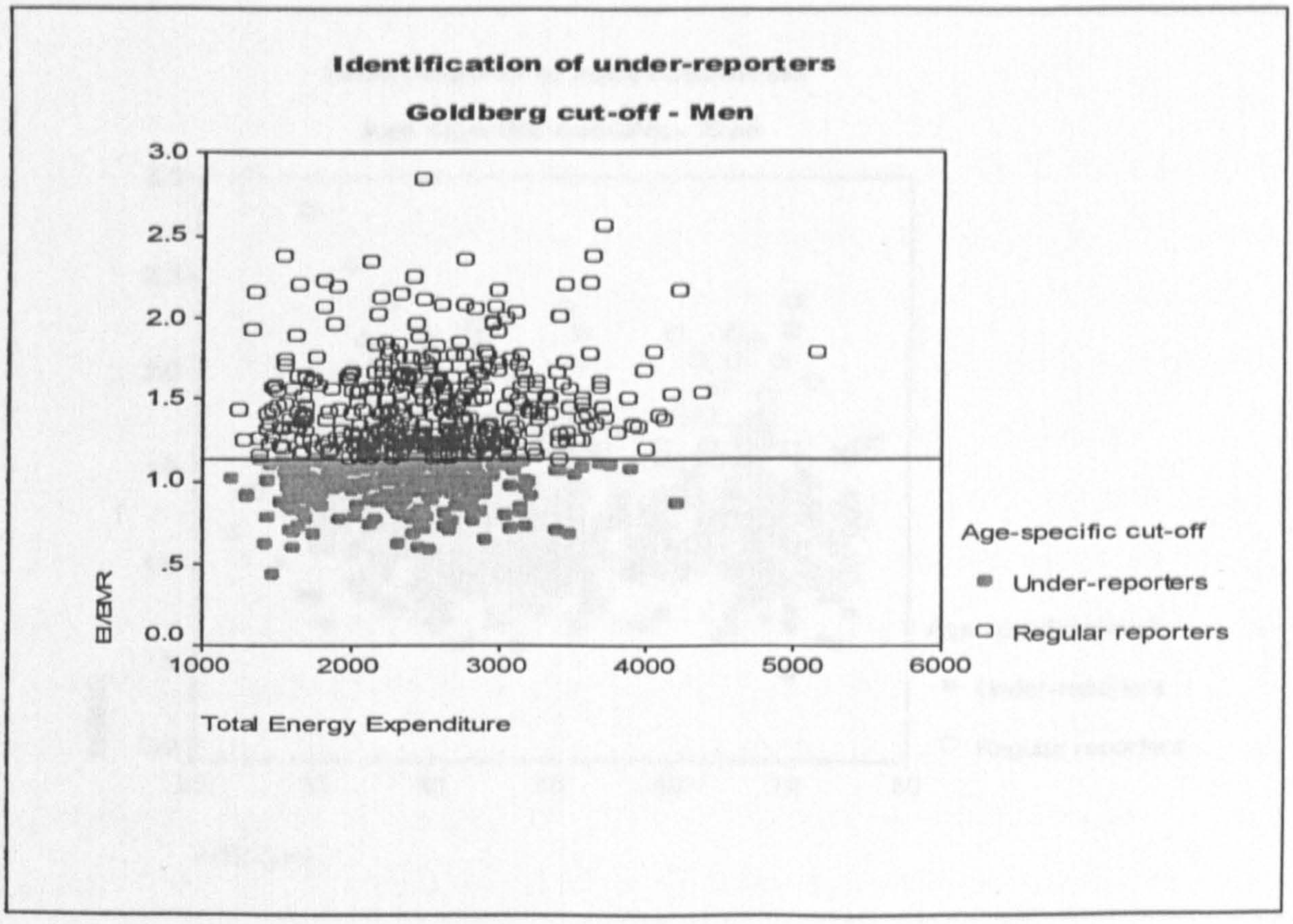
It is interesting to note that although the total number of under-reporters is practically equal to the number of under-reporters identified using the sex-specific cut-off points (the slight decrease is due to rounding errors), the age distribution of under-reporting is now greatly modified; the vast majority of under-reporters is now accumulating in

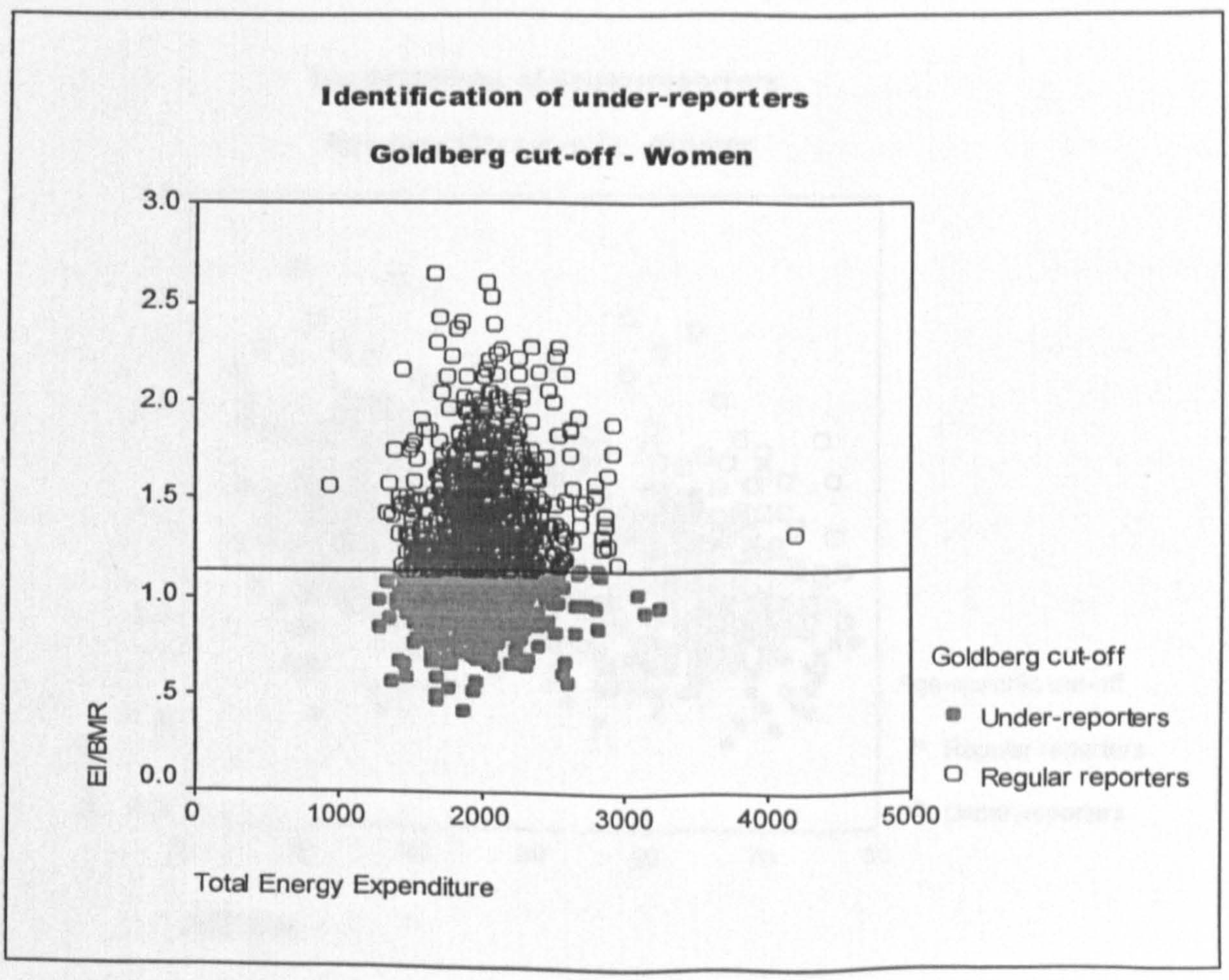
the two intermediate age groups. In fact, this observation supports the notion that has been stated previously that a single cut off point (or age-independent cut-off points) applied to all subjects of a population would inevitably find more older people among low-energy reporters. The latter has been illustratively demonstrated in the following figures. Actually, if younger people have higher energy expenditures than older persons, as was found from an analysis of Doubly Labelled Water data, then they would have higher EI/BMR for the same degree of under-reporting (Black et al, 1996a).

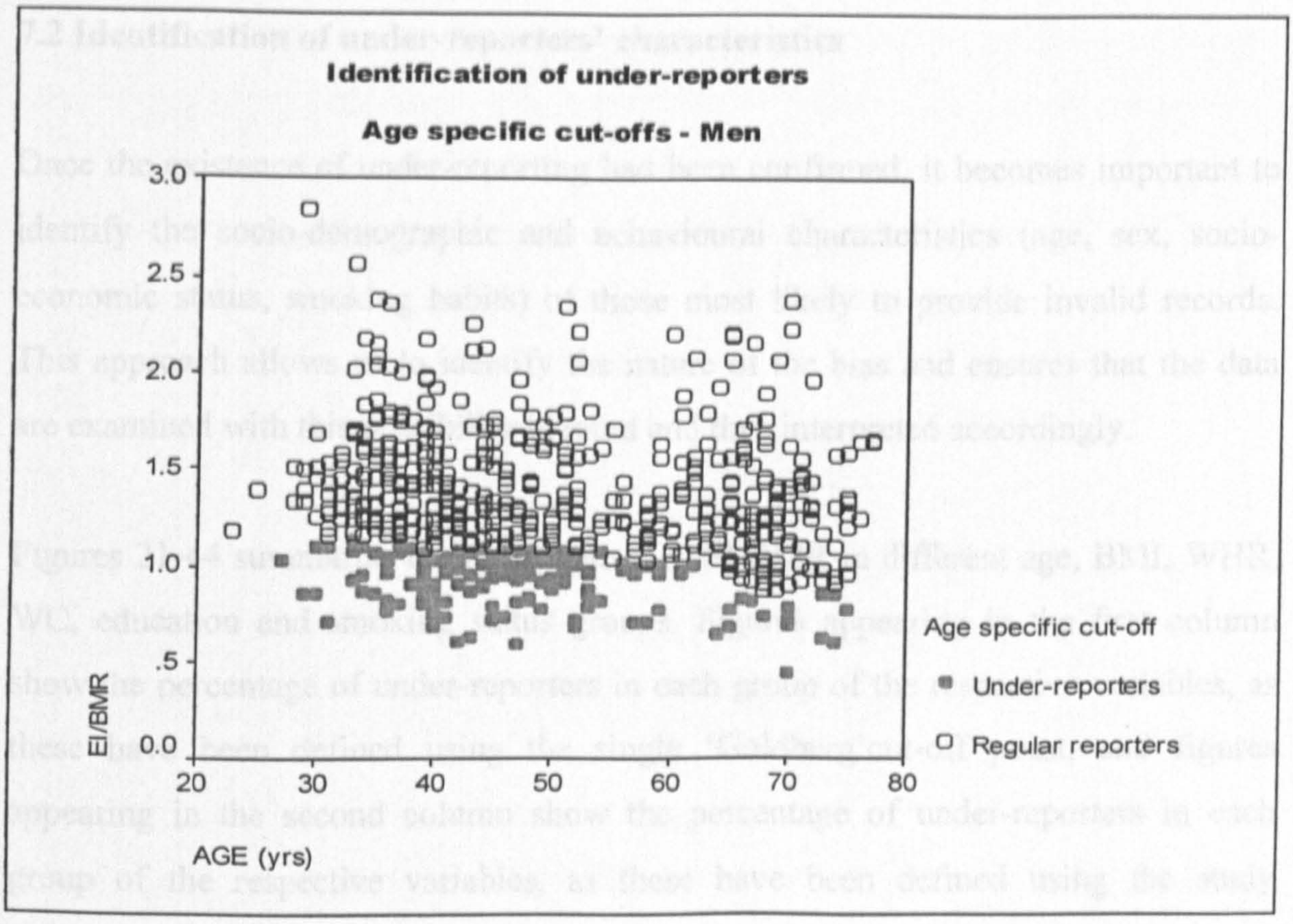
7.1.5 Conclusions

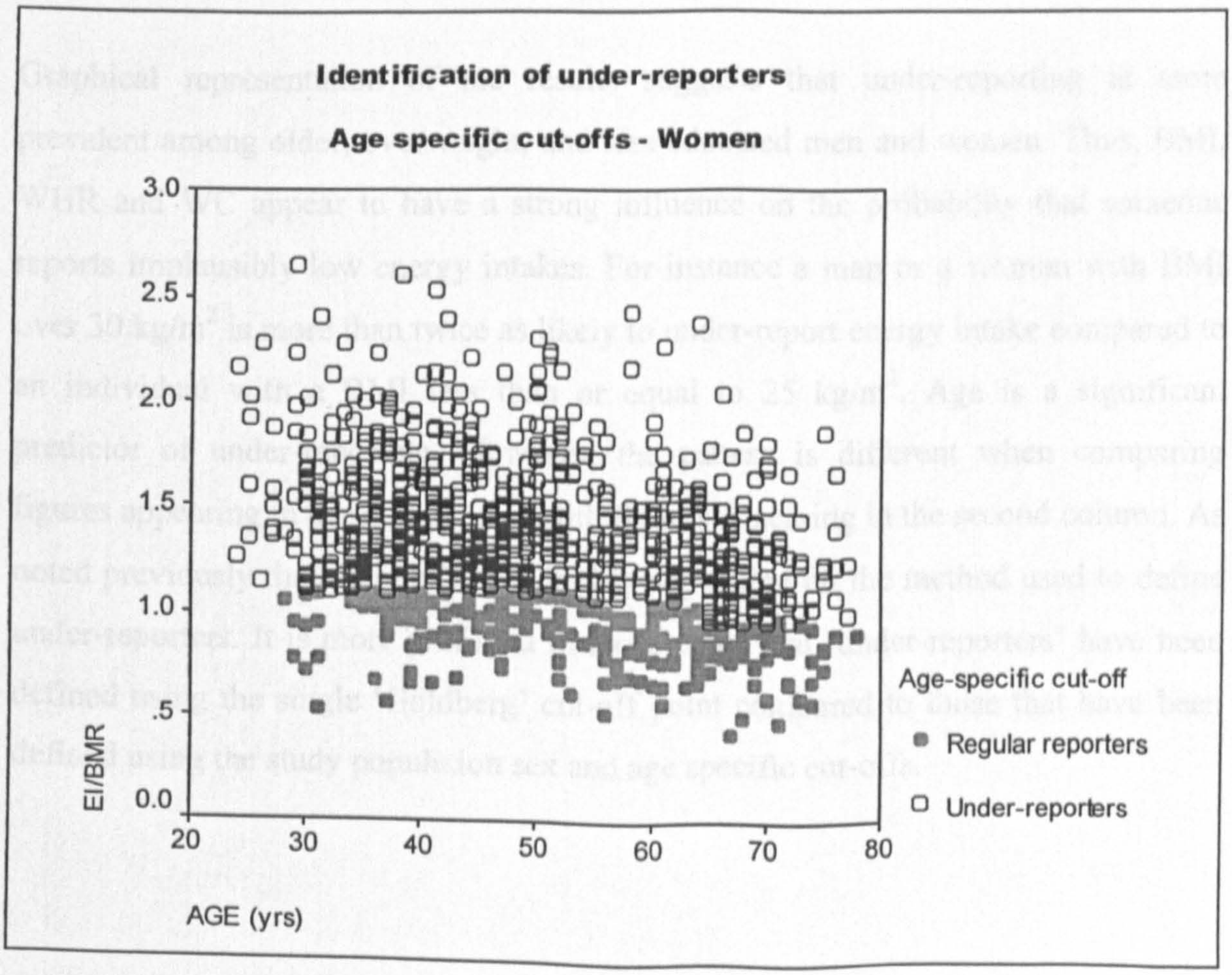
There is no doubt that under-reporting cannot be delineated with a simple cut-off point, however operationally defined. In the context of this study there has been a declining prevalence of under-reporting with decreasing values of EI/BMR as those derived from study population specific estimates of physical activity level.

However, since the problem is particularly complex, it was considered desirable and appropriate to proceed with the analysis by using more than one method for identifying under-reporters. This would allow the effect on the conclusions to be drawn from the study to be examined and interpreted accordingly. Thus, it was decided to proceed by using: a) the Goldberg single cut-off of 1.14, since this consists of the most well accepted method used from the majority of researchers in relative fields, in an effort to make appropriate adjustment to their data before analysis and; b) the study population sex and age specific cut-offs. Those have derived after applying study population specific PAL values to the respective Goldberg formula and are considered to be more representative for the current study population.


Identification of under-reporters
Goldberg cut-off - Men
3.0
2.5
2.0
1.5
1.0
.5
0.0
EI/BMR
1000
2000
3000
4000
5000
6000
Total Energy Expenditure
Age-specific cut-off
Under-reporters
Regular reporters


Identification of under-reporters
Goldberg cut-off - Women
3.0
2.5
2.0
1.5
1.0
.5
0.0
EI/BMR
0
1000
2000
3000
4000
5000
Total Energy Expenditure
Goldberg cut-off
Under-reporters
Regular reporters


Identification of under-reporters
Age specific cut-offs - Men
3.0
2.5
2.0
1.5
1.0
.5
0.0
EI/BMR
20
30
40
50
60
70
80
AGE (yrs)
Age specific cut-off
Under-reporters
Regular reporters


Identification of under-reporters
Age specific cut-offs - Women
3.0
2.5
2.0
1.5
1.0
.5
0.0
EI/BMR
20
30
40
50
60
70
80
AGE (yrs)
Age-specific cut-off
Regular reporters
Under-reporters

7.2 Identification of under-reporters' characteristics

Once the existence of under-reporting had been confirmed, it becomes important to identify the socio-demographic and behavioural characteristics (age, sex, socio-economic status, smoking habits) of those most likely to provide invalid records. This approach allows us to identify the nature of the bias and ensures that the data are examined with this possibility in mind and thus interpreted accordingly.

Figures 21-44 summarise the mean values of EI/BMR in different age, BMI, WHR, WC, education and smoking status groups. Figures appearing in the first column show the percentage of under-reporters in each group of the respective variables, as these have been defined using the single 'Goldberg'cut-off point, and figures appearing in the second column show the percentage of under-reporters in each group of the respective variables, as these have been defined using the study population sex and age specific cut-offs.

Graphical representation of the results suggests that under-reporting is more prevalent among older, overweight, and less educated men and women. Thus, BMI, WHR and WC appear to have a strong influence on the probability that someone reports implausibly low energy intakes. For instance a man or a woman with BMI over 30 kg/m^2 is more than twice as likely to under-report energy intake compared to an individual with a BMI less than or equal to 25 kg/m^2. Age is a significant predictor of under-reporting. However, the pattern is different when comparing figures appearing in the first column with figures appearing in the second column. As noted previously the effect of age is highly dependent on the method used to define under-reporters. It is more profound in those cases that 'under-reporters' have been defined using the single 'Goldberg' cut-off point compared to those that have been defined using the study population sex and age specific cut-offs.

As far as the effect of education is concerned, less educated people appear to under-report more often than others. The latter is more profound in women compared to men. Finally, concerning the effect of smoking habits in under-reporting, it seems that under-reporting is more extensive among non-smoking women but not men.

Figure 21. Mean EI/BMR and % of male under-reporters per BMI group

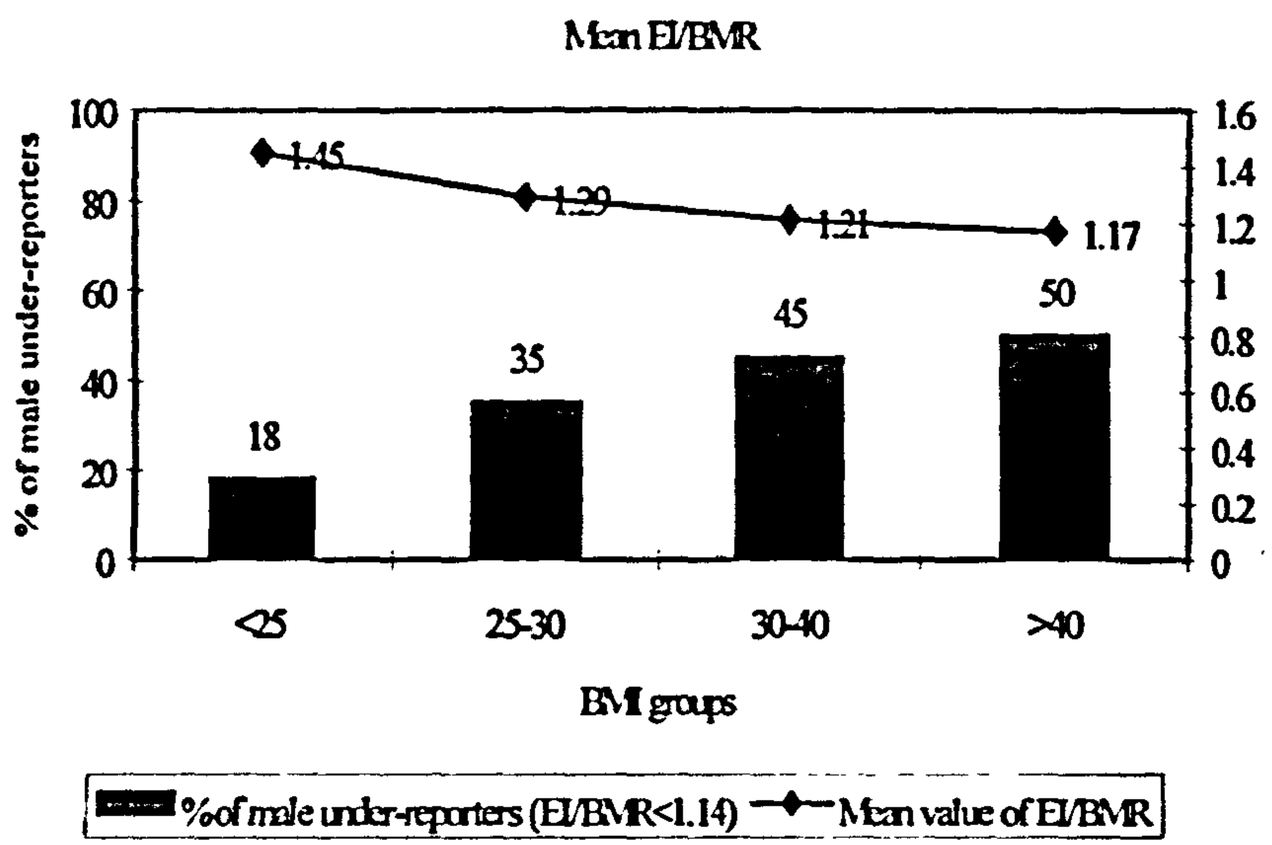


Figure 22. Mean EI/BMR and % of female undr-reporters per BMI group

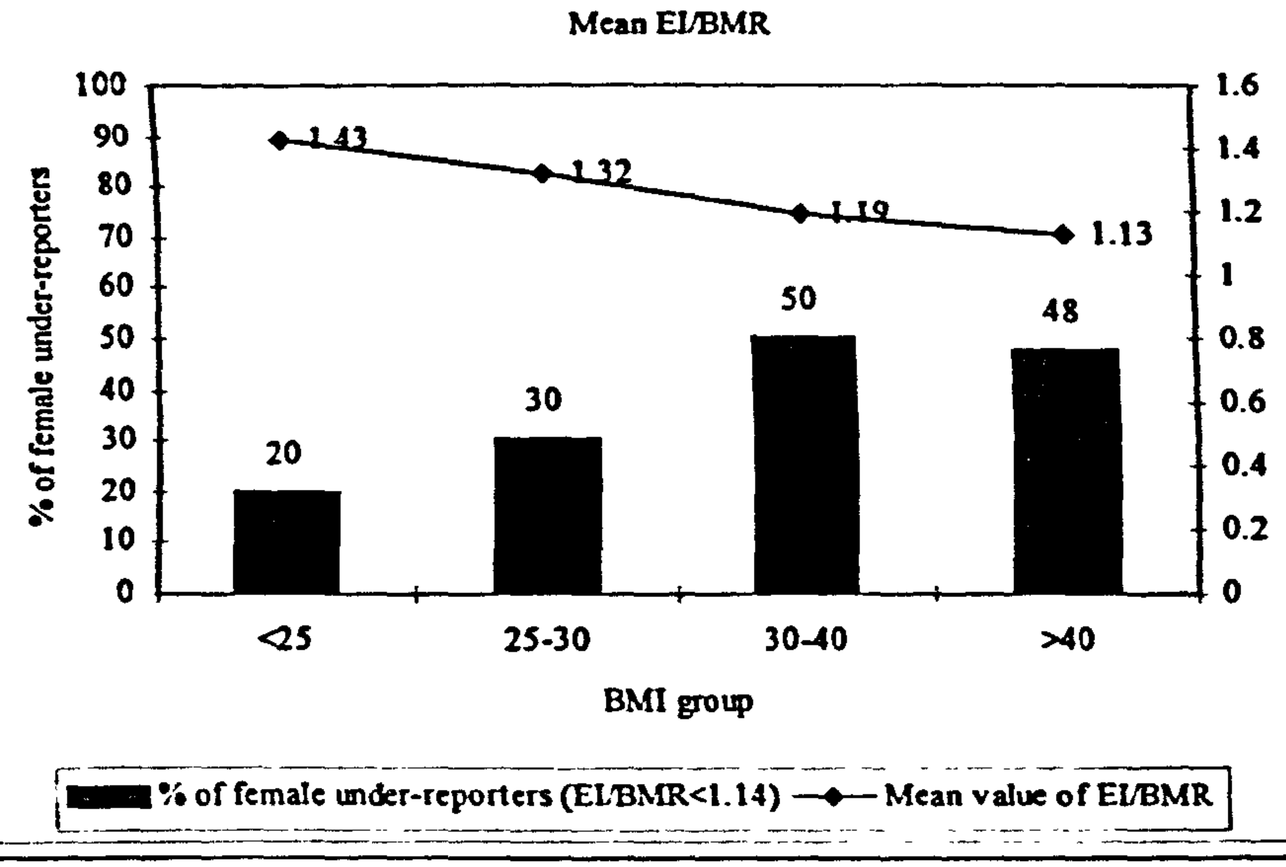


Figure 23. Mean EI/BMR and % of male under-reporters per BMI group

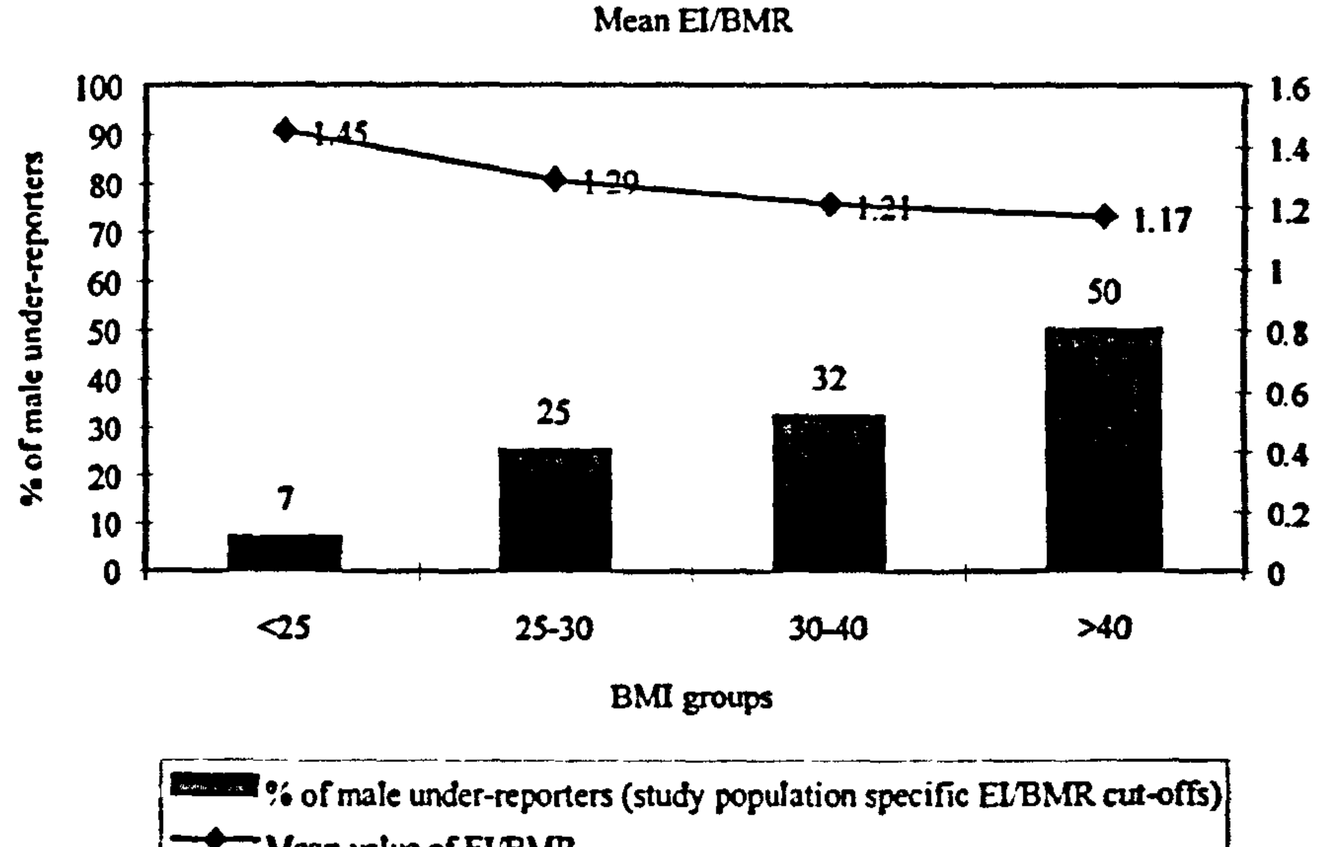


Figure 24. Mean EI/BMR and % of female under-reporters per BMI group

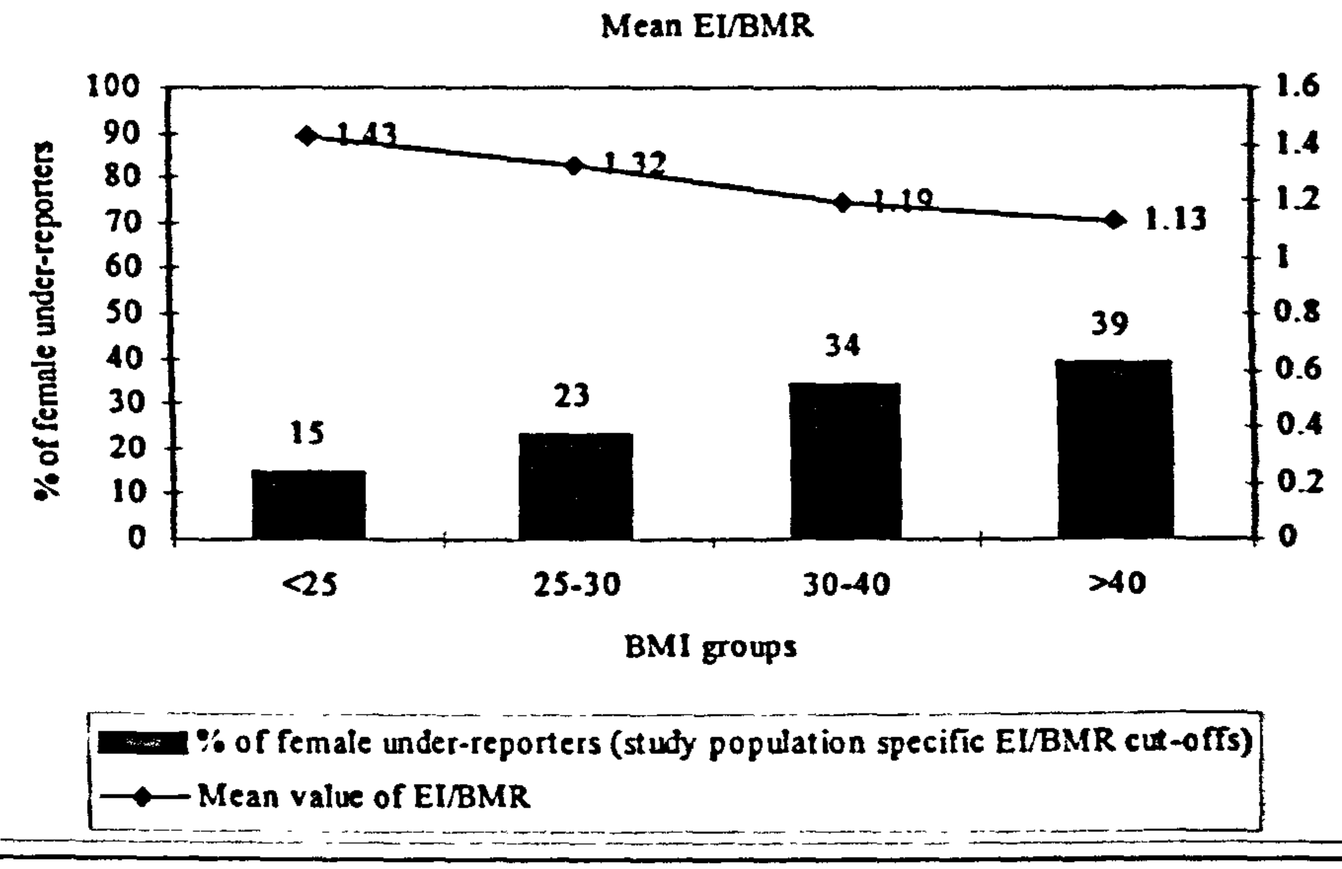


Figure 25. Mean EI/BMR and % of male under-reporters per WHR quartile

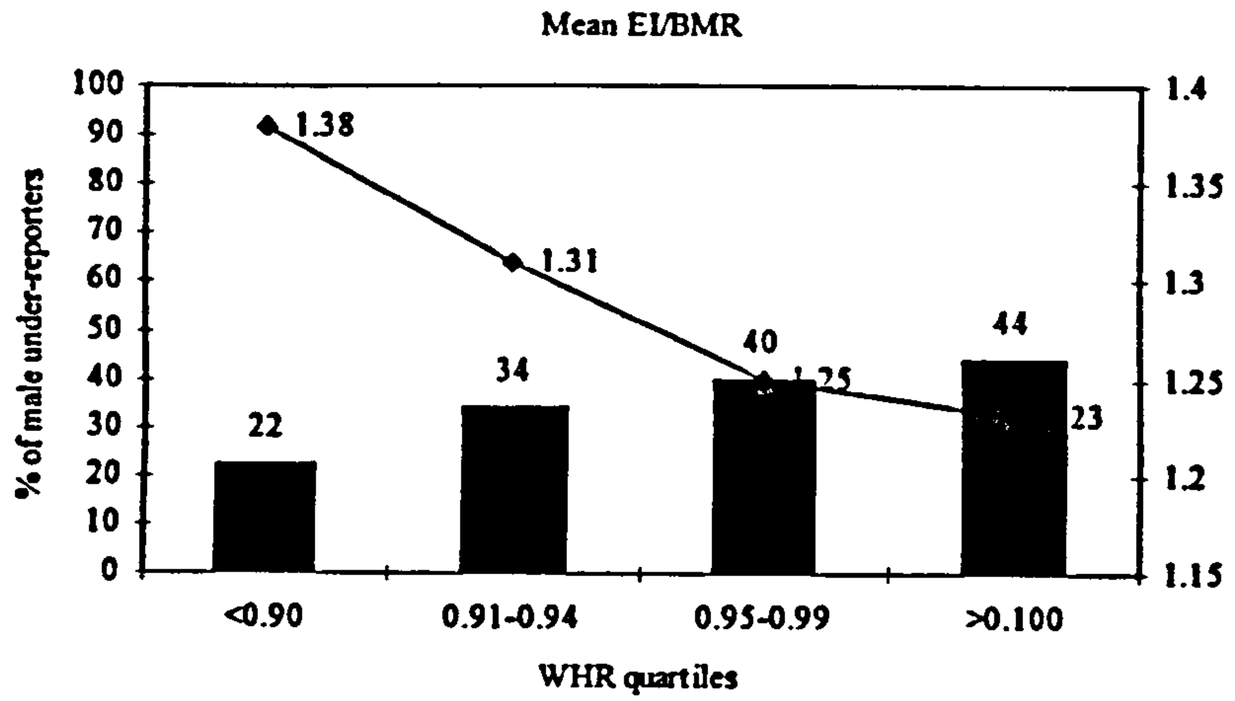


Figure 26. Mean EI/BMR and % of female under-reporters per WHR quartile

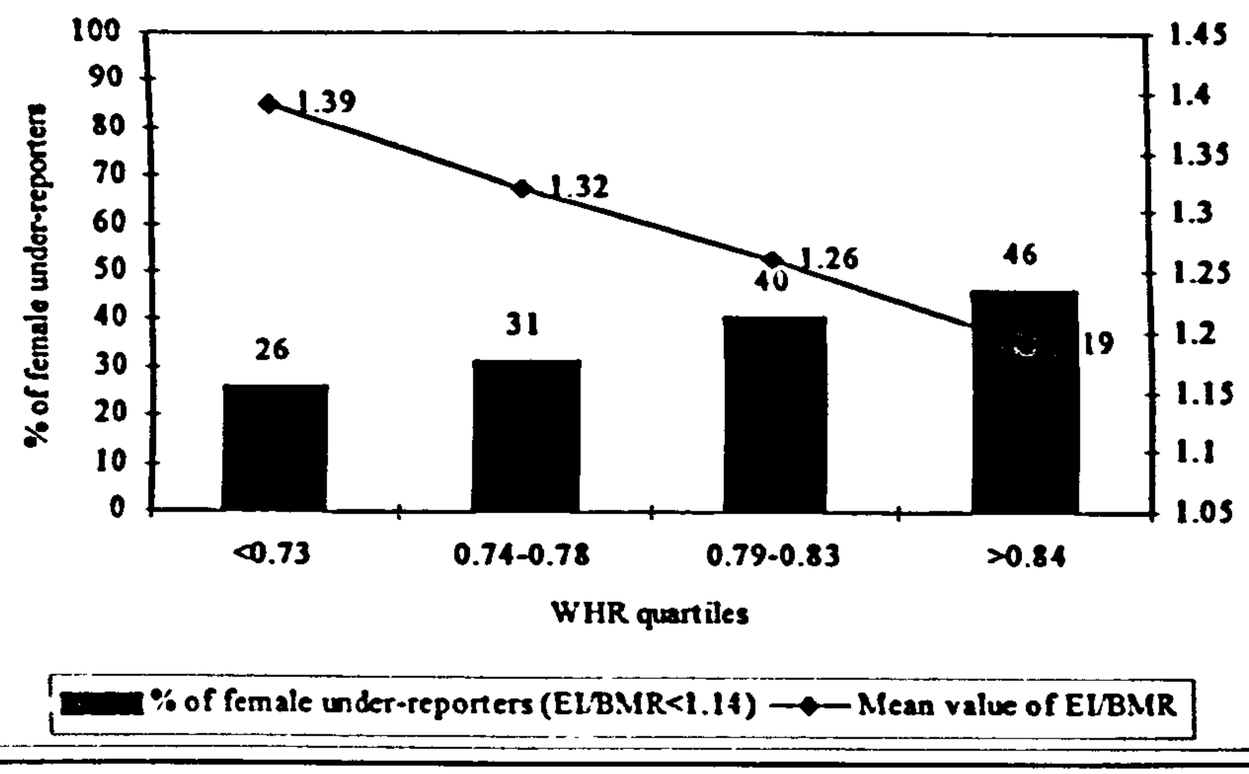


Figure 27. Mean EI/BMR and % of male under-reporters per WHR quartile

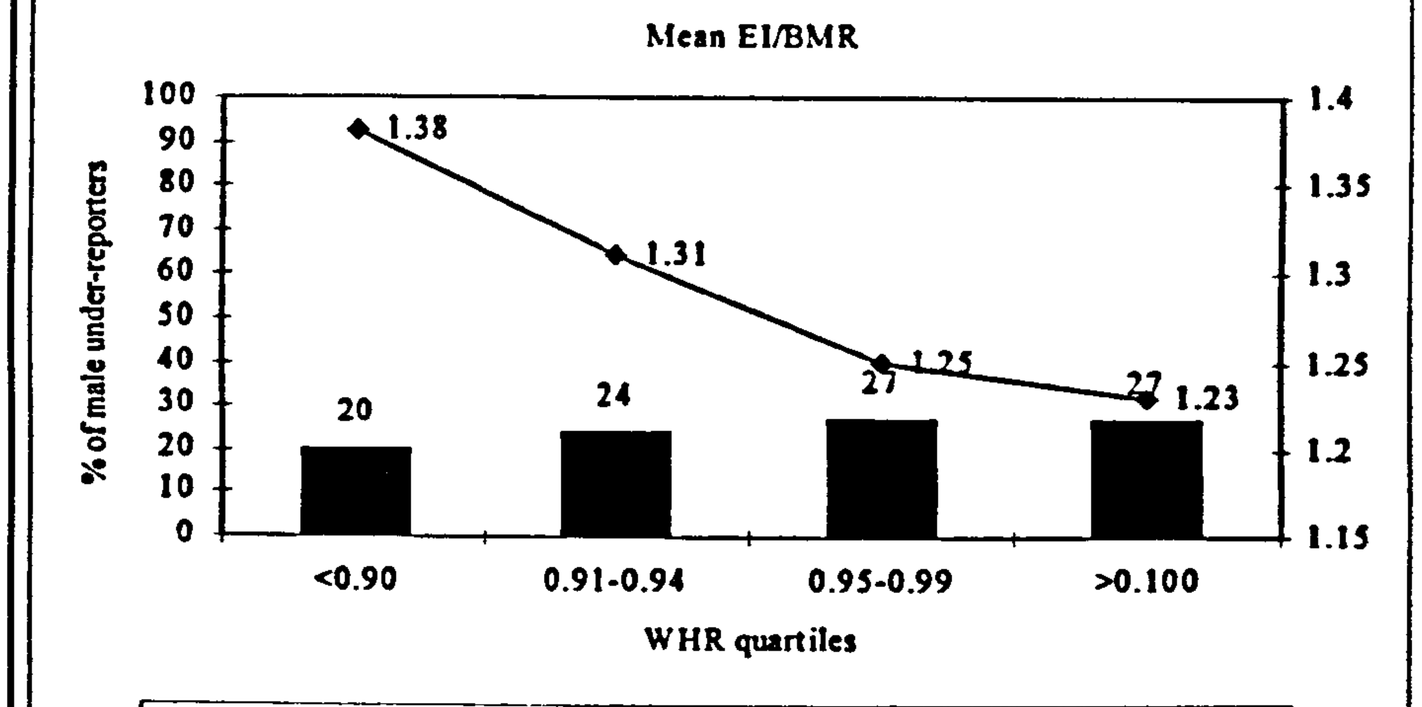


Figure 28. Mean EI/BMR and % of female under-reporters per WHR quartile

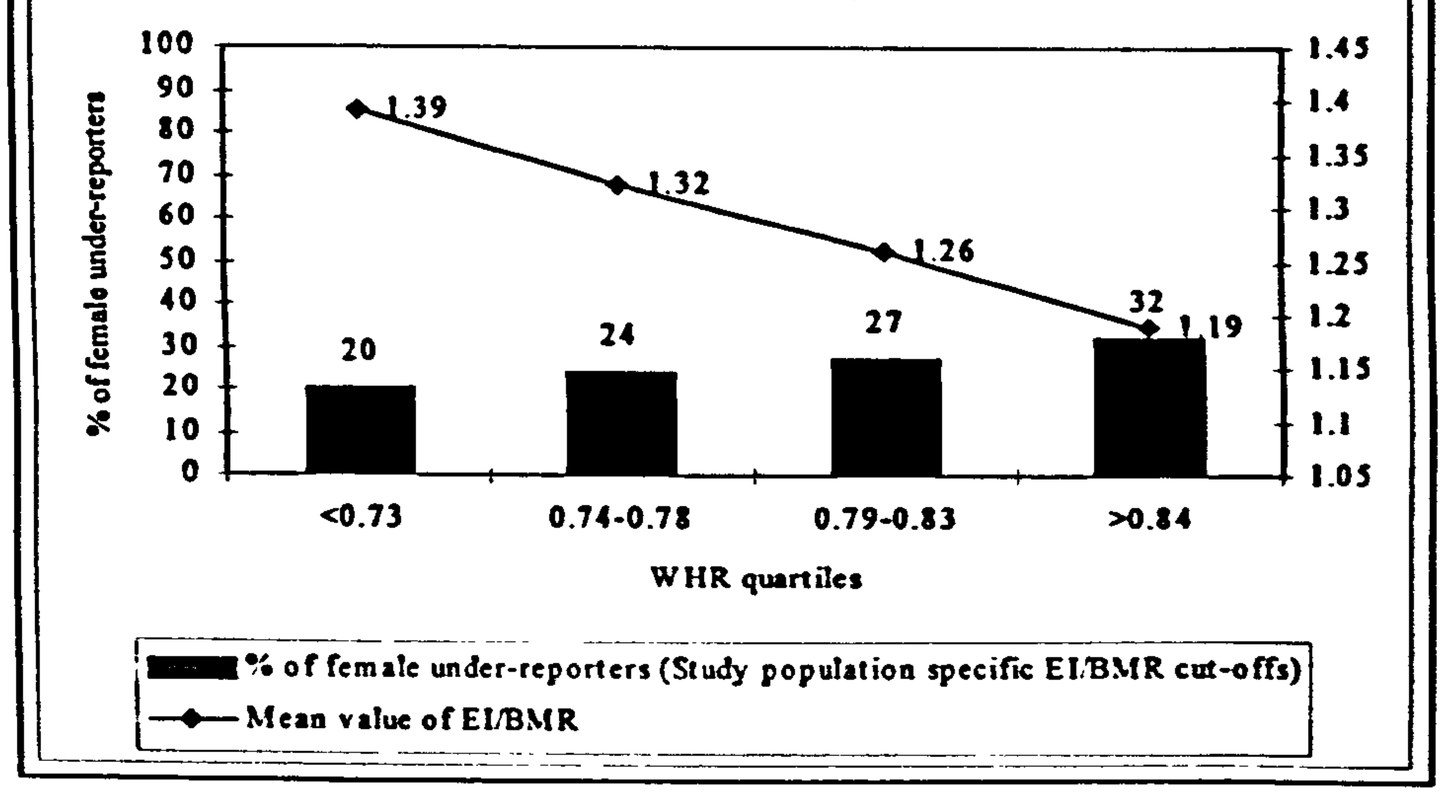


Figure 29. Mean EI/BMR and % of male under-reporters per WC quartile

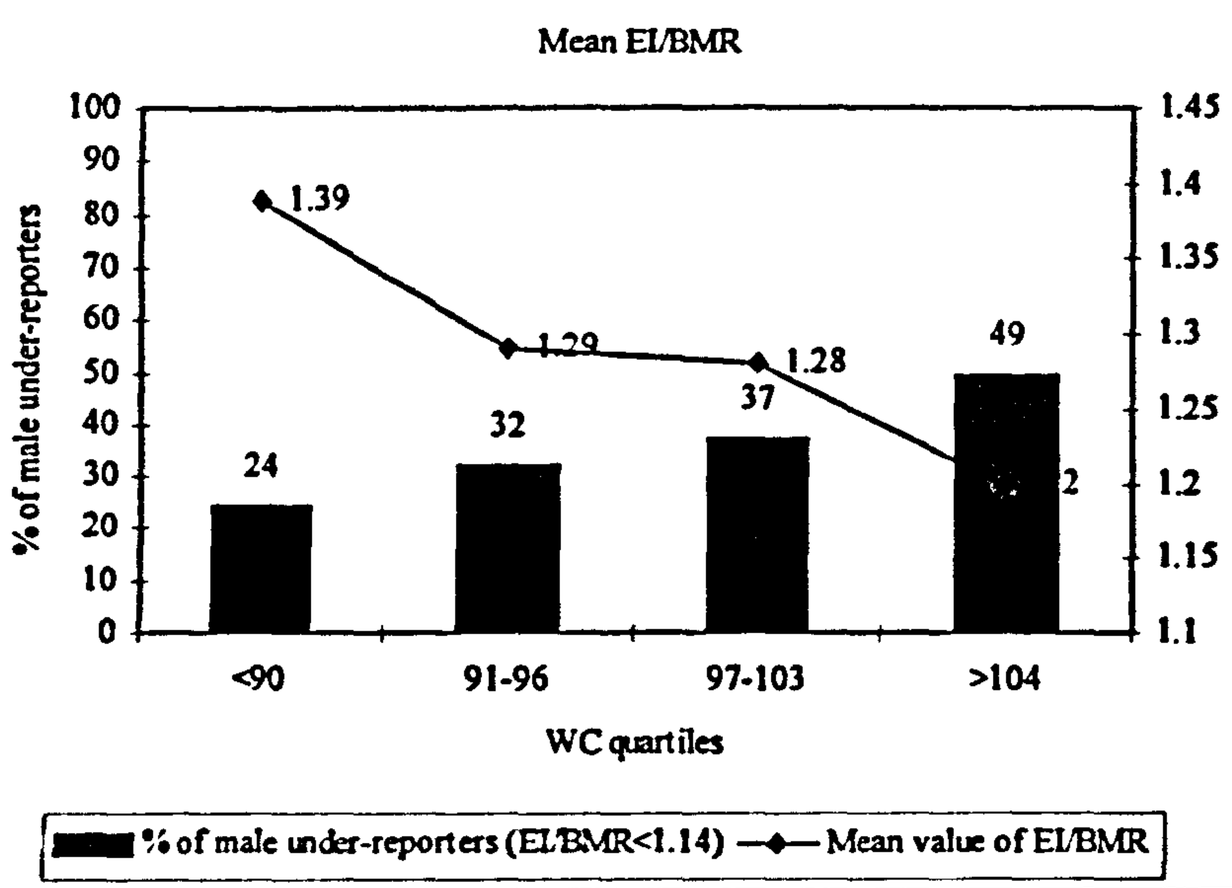


Figure 30. Mean EI/BMR and % of female under-reporters per WC quartile

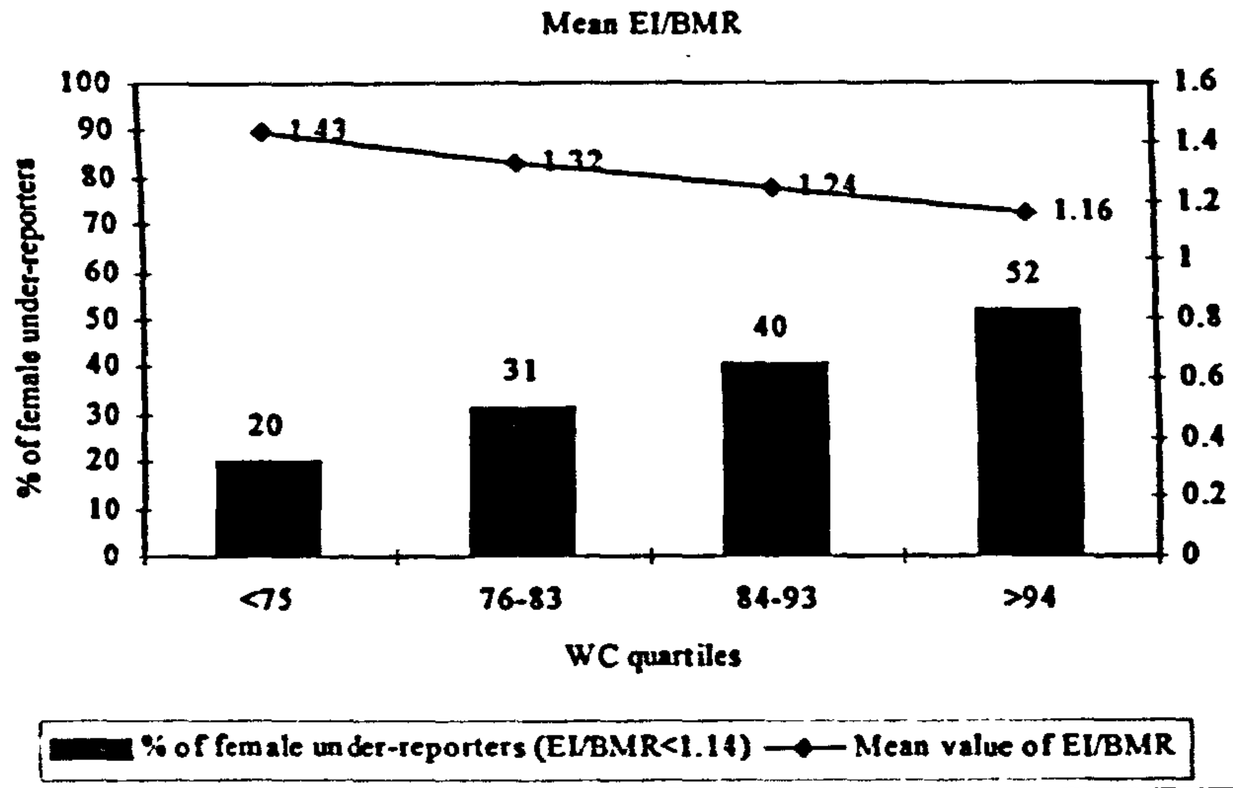


Figure 31. Mean EI/BMR and % of male under-reporters per WC quartile

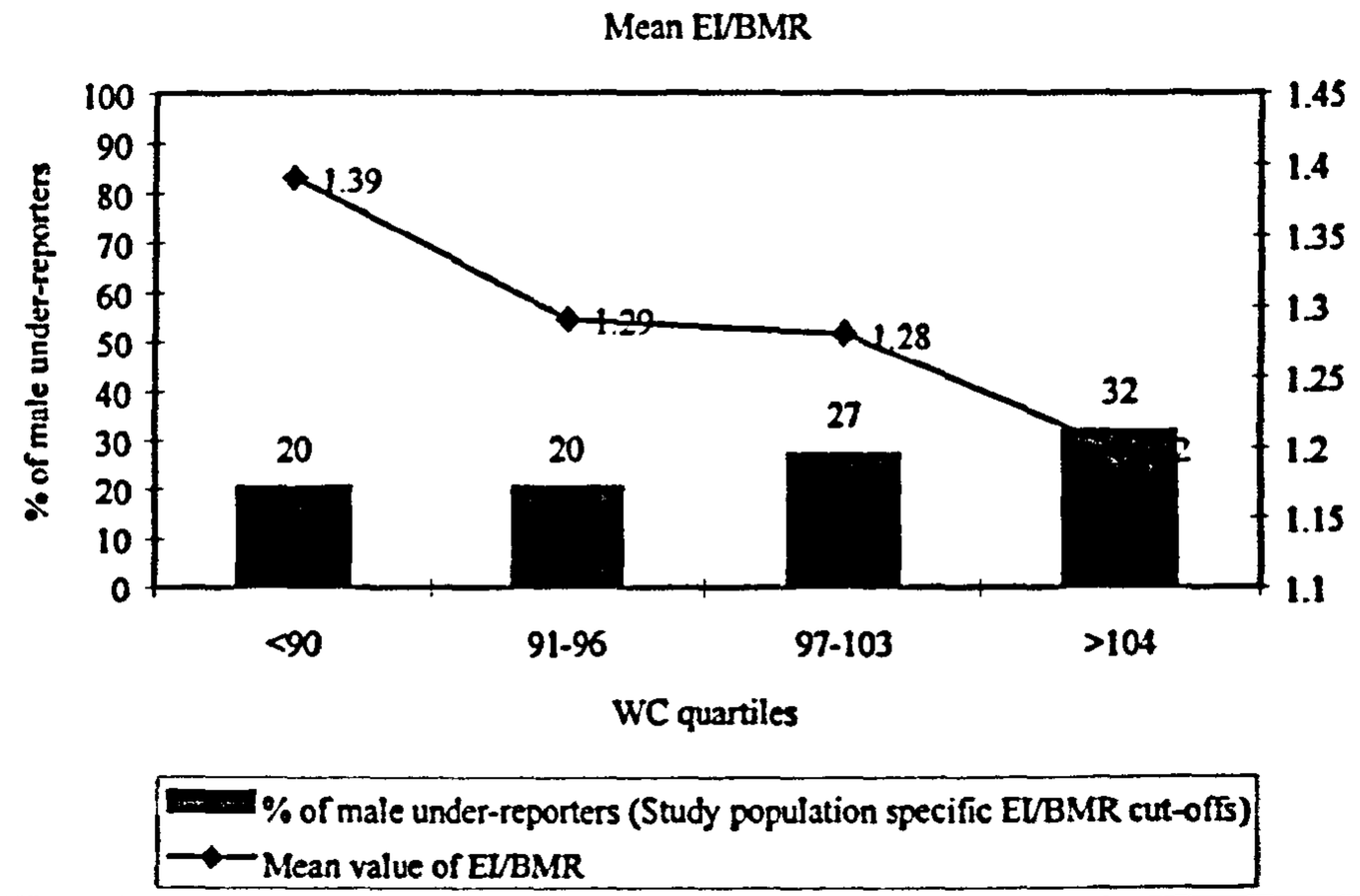


Figure 32. Mean EI/BMR and % of female under-reporters per WC quartile

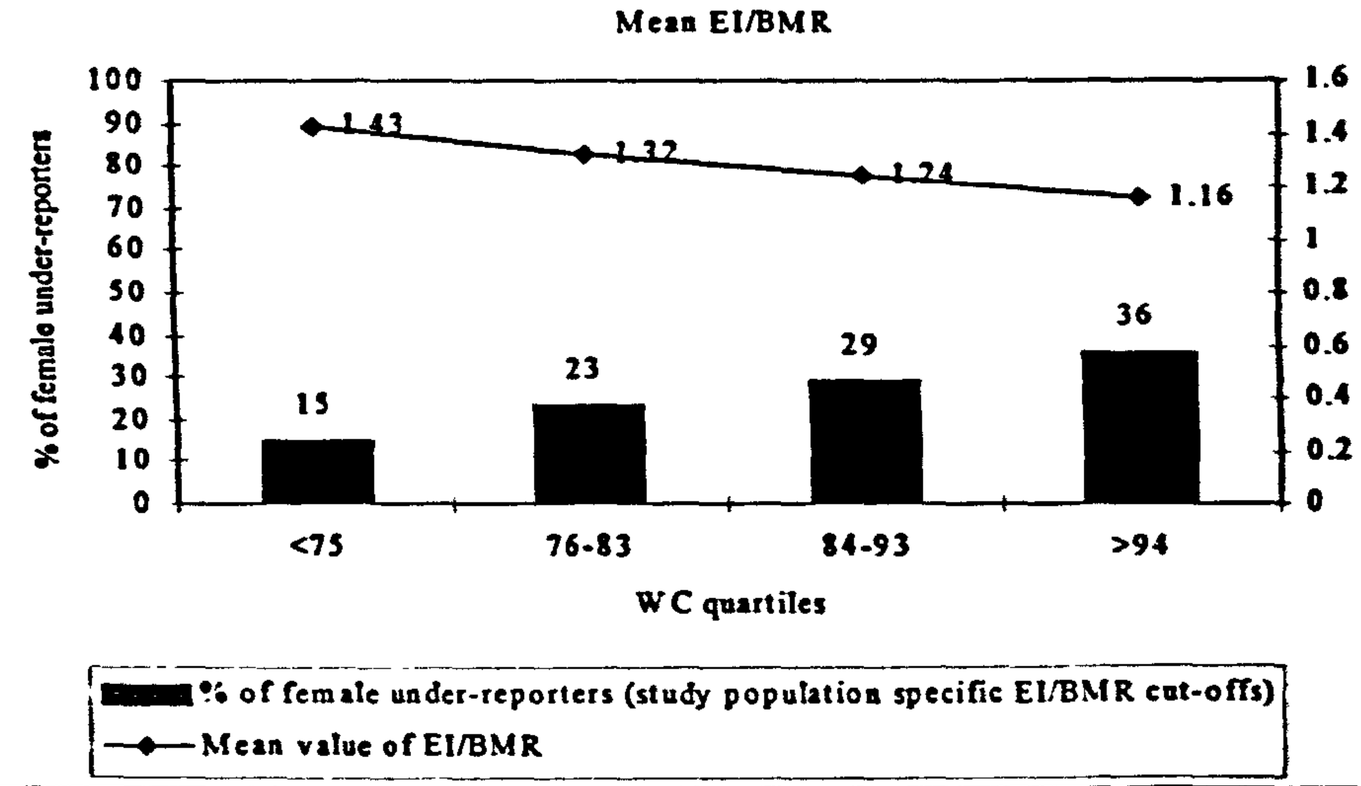


Figure 33. Mean EI/BMR and %of male under-reporters per age group

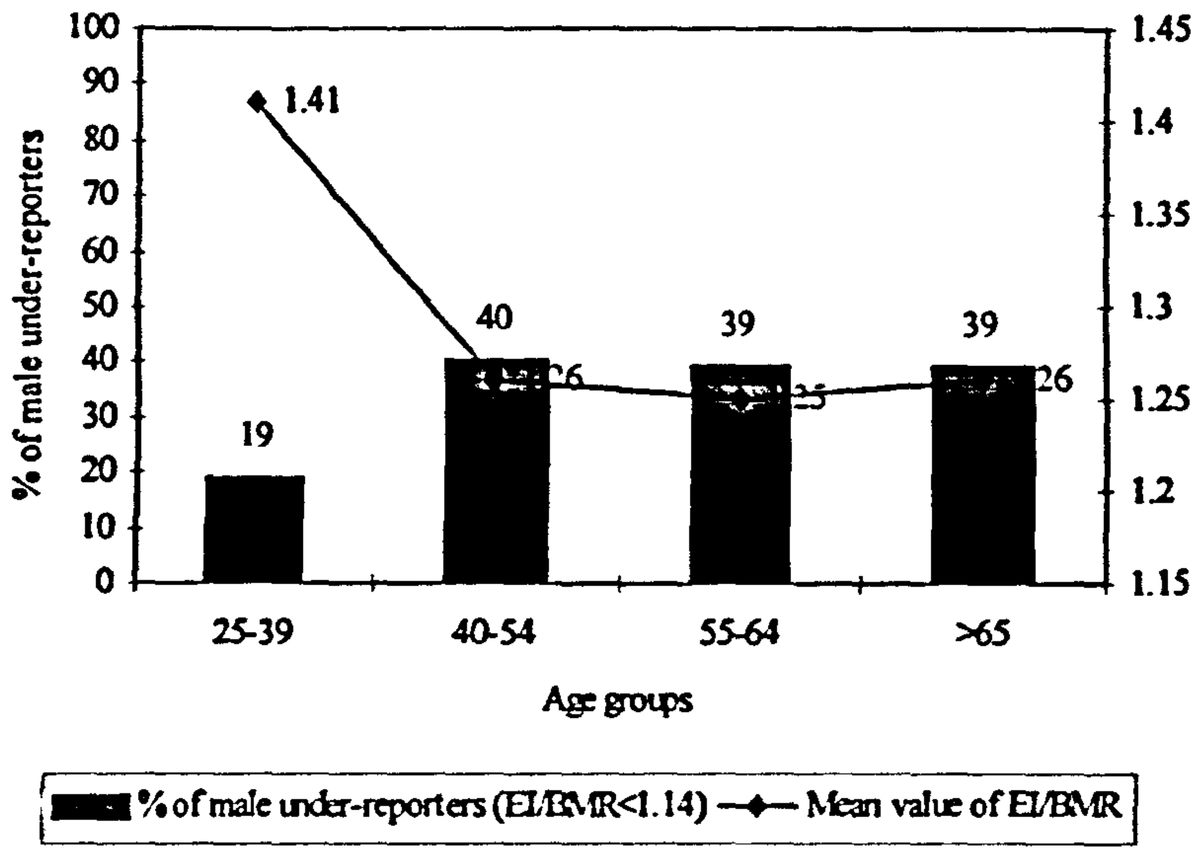


Figure 34. Mean EI/BMR and % of female under-reporters per age group

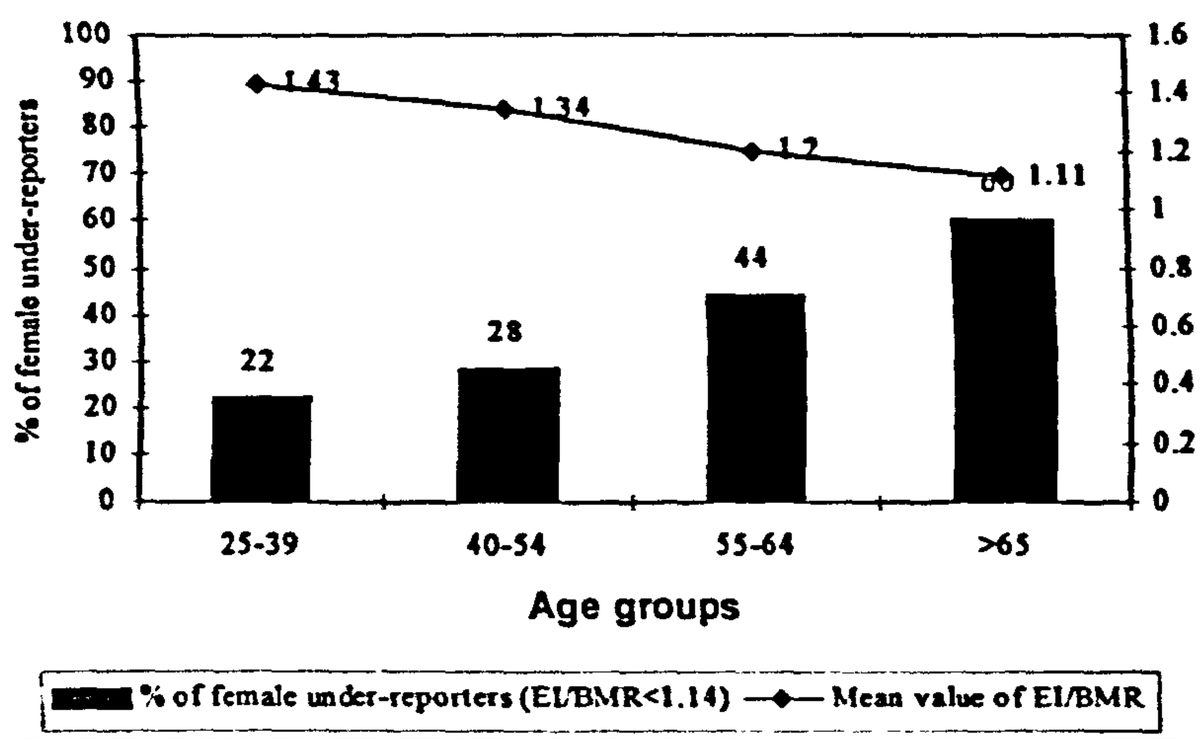


Figure 35. Mean EI/BMR and % of male under-reporters per age group

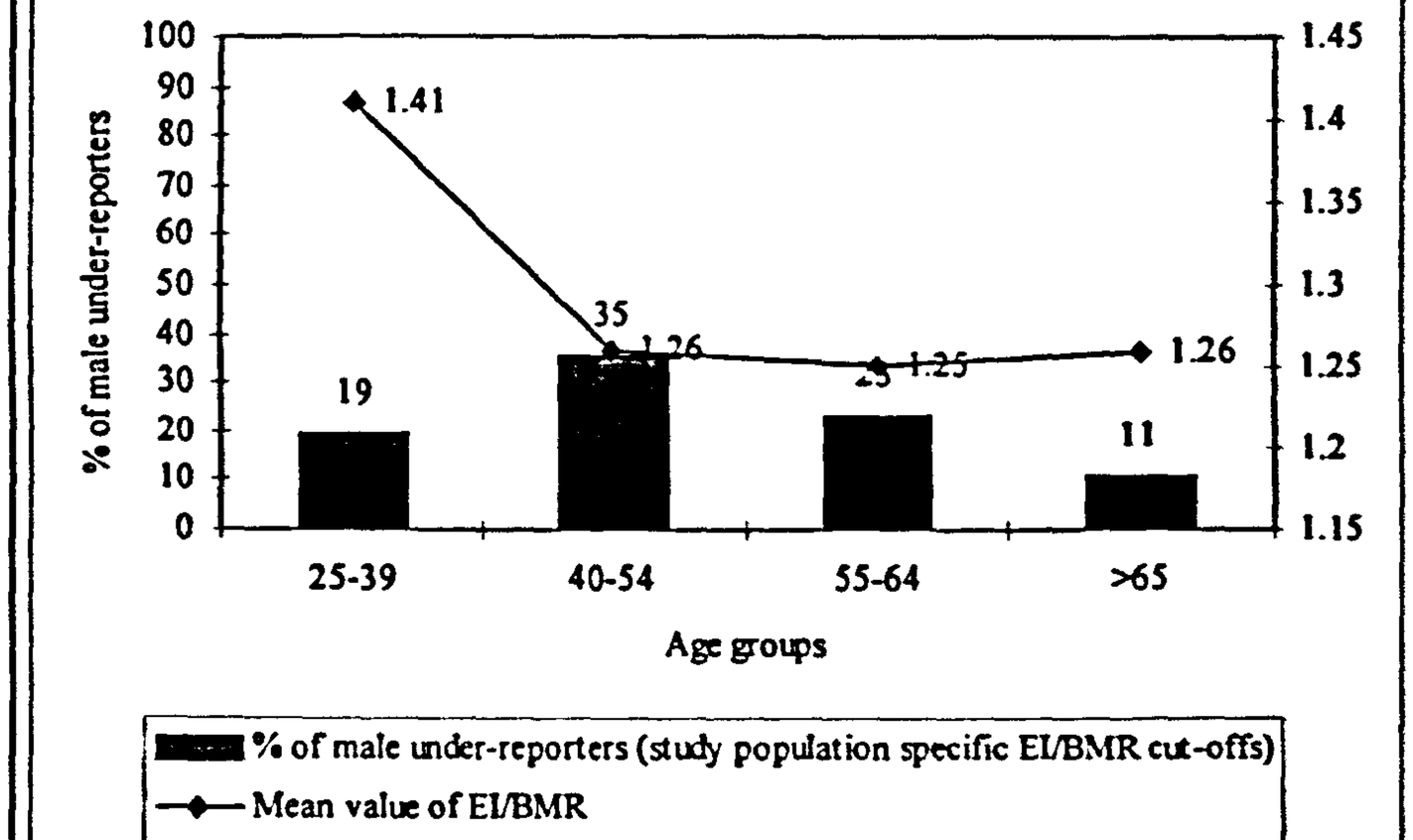
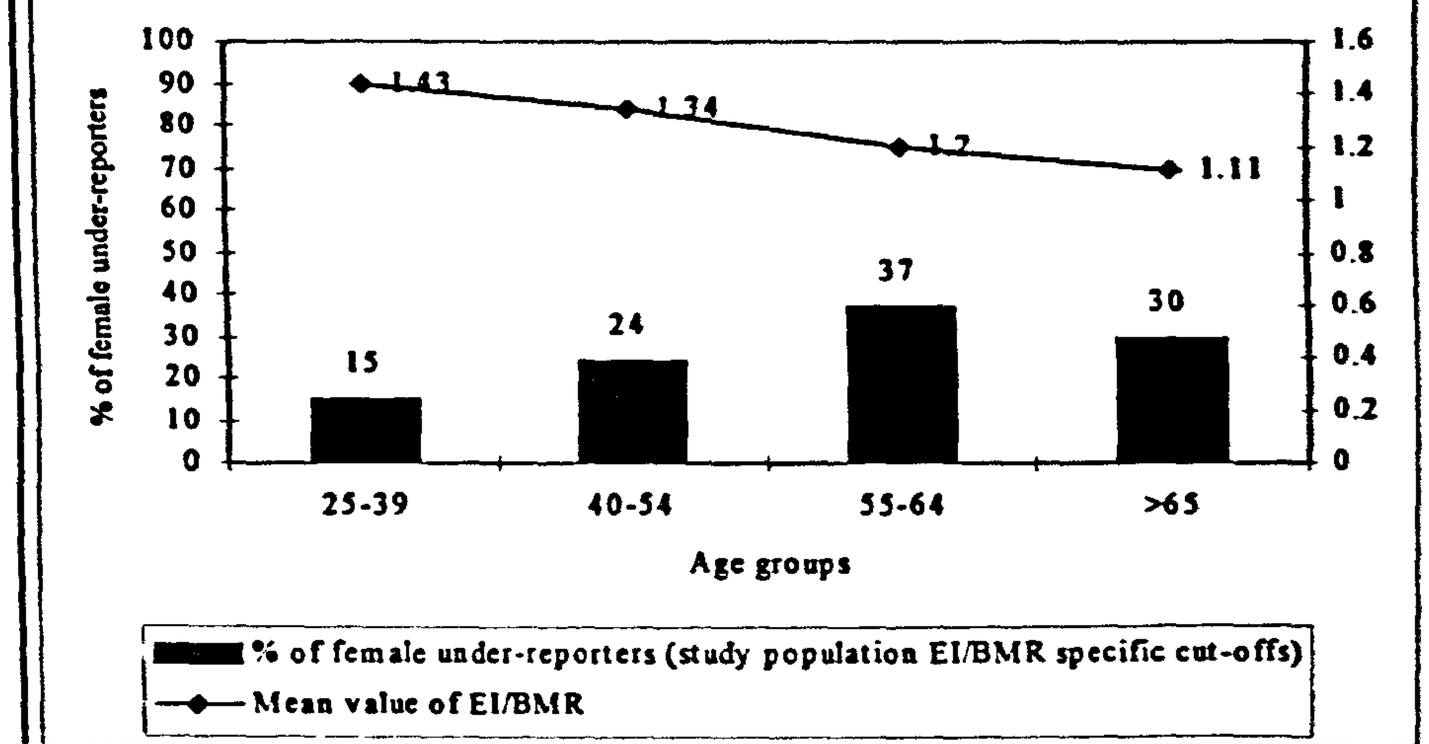


Figure 36. Mean EI/BMR and % of female under-reporters per age group



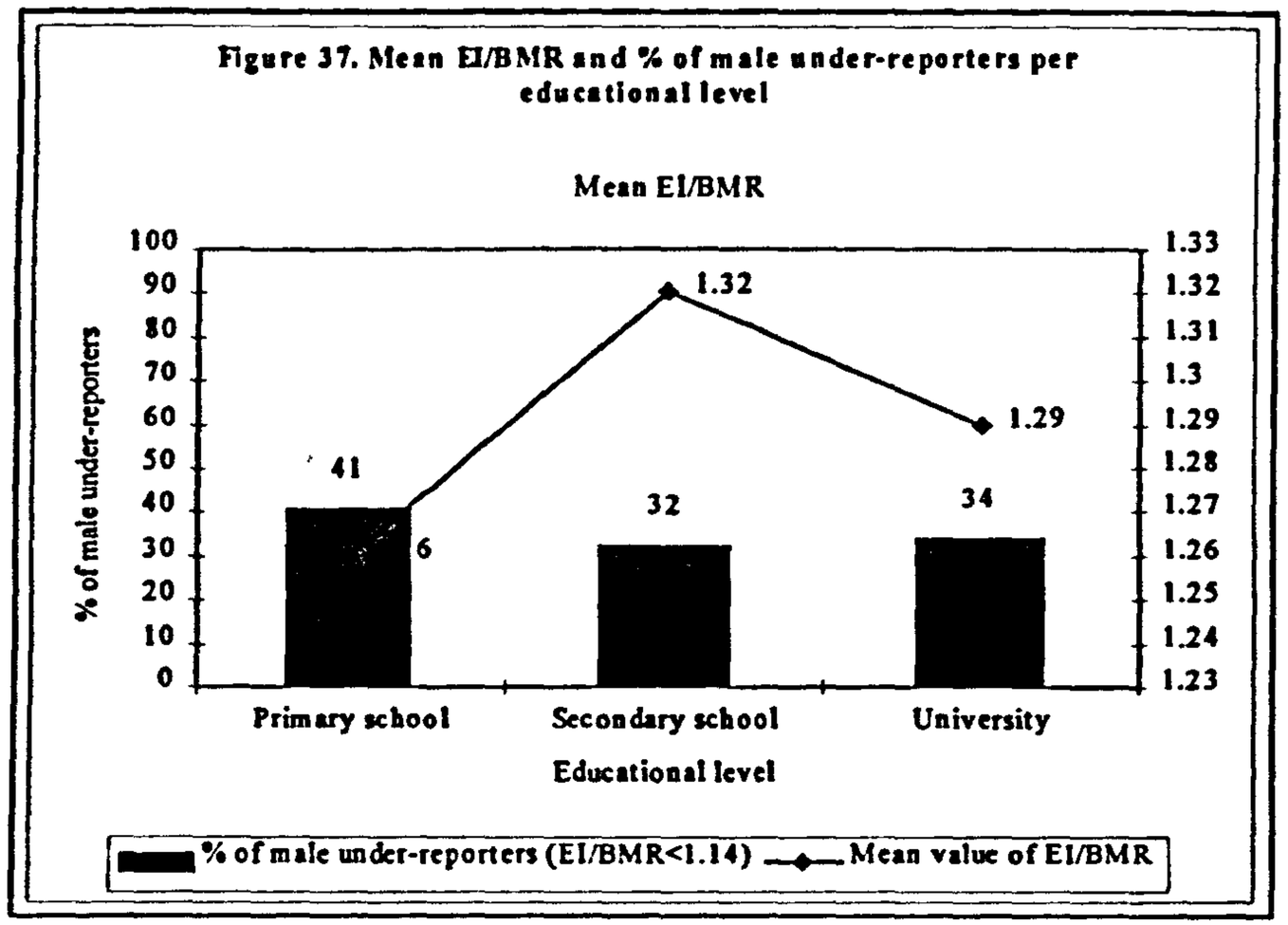


Figure 37. Mean EI/BMR and % of male under-reporters per educational level

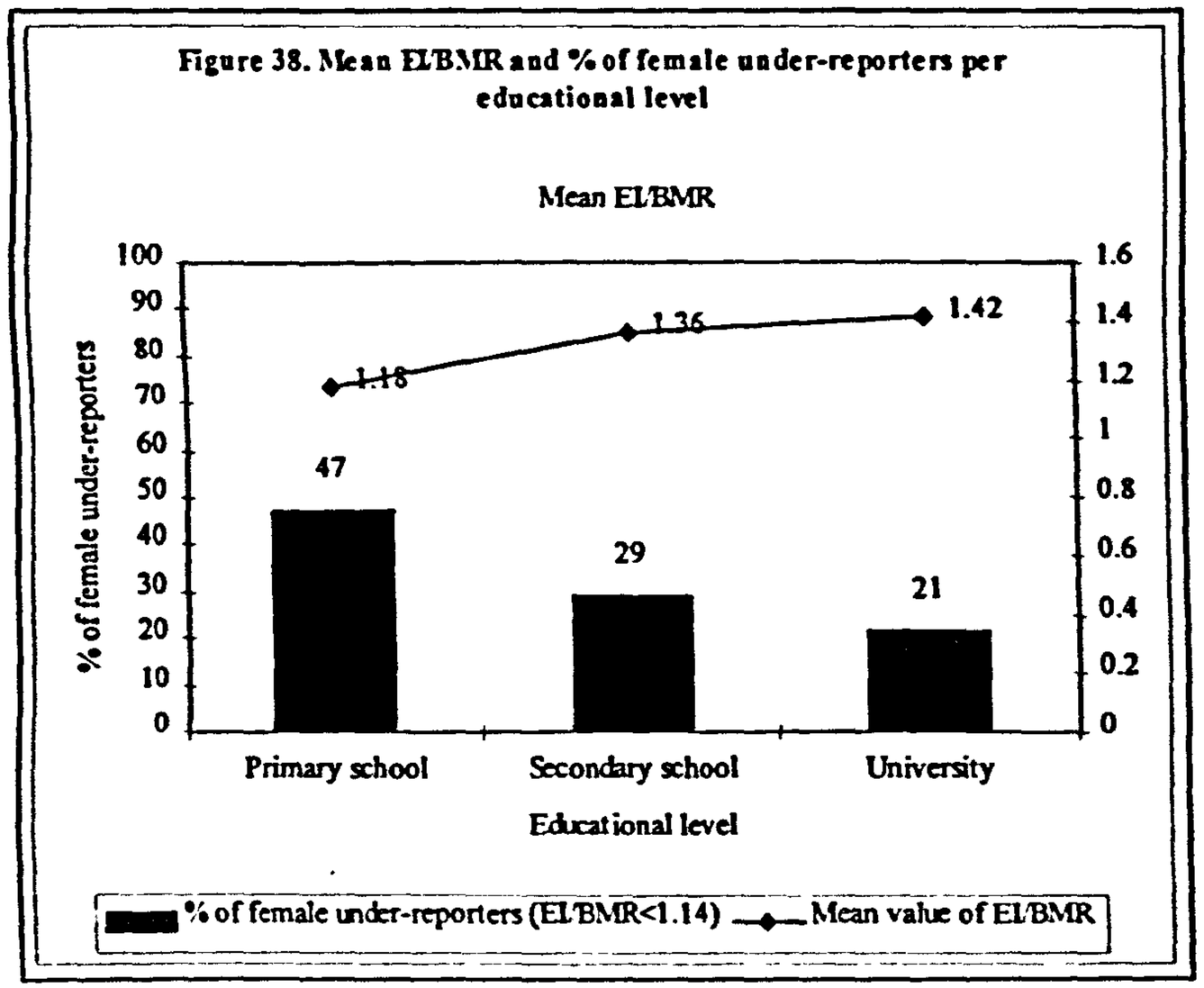


Figure 38. Mean EI/BMR and % of female under-reporters per educational level

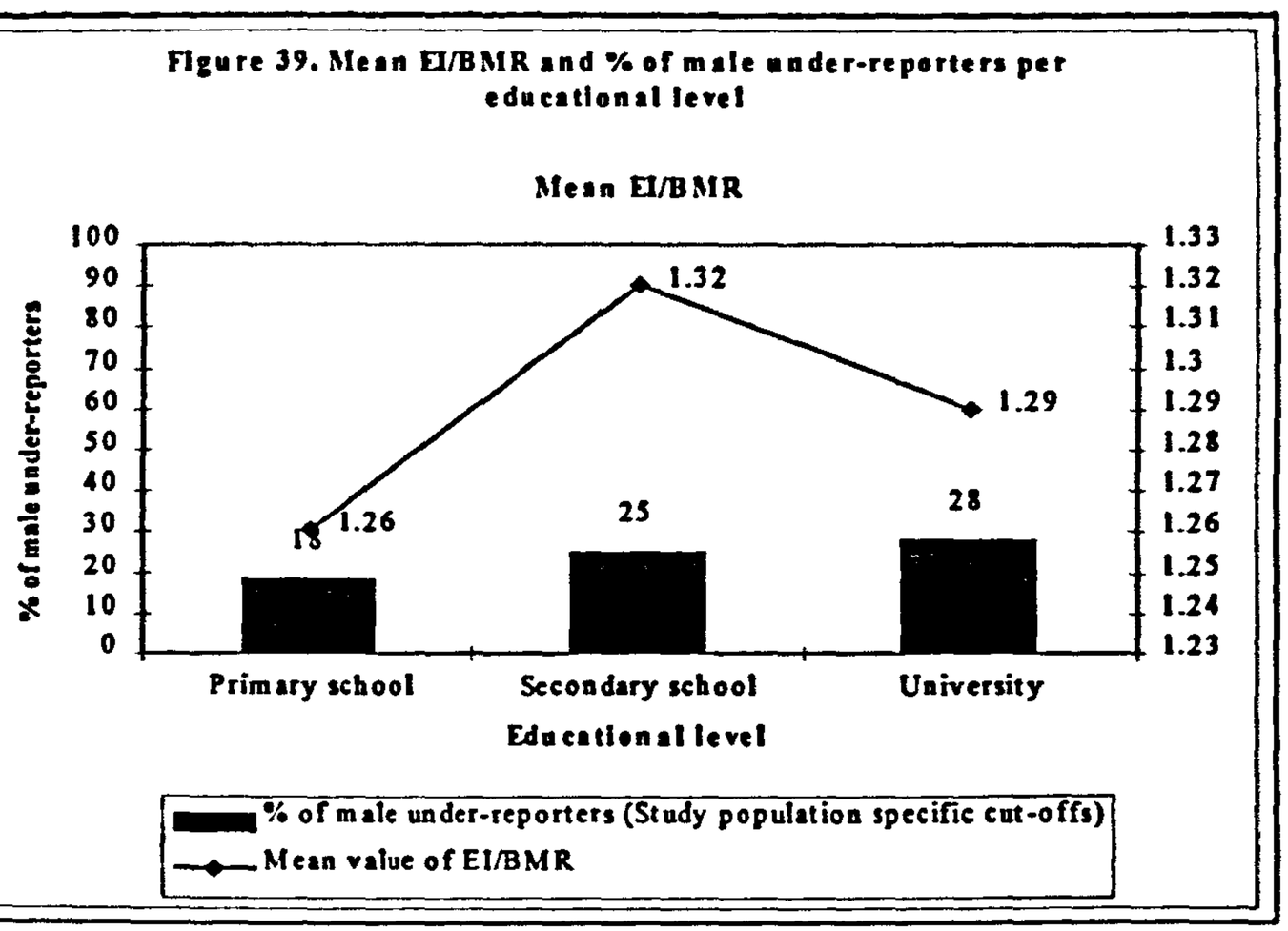


Figure 39. Mean EI/BMR and % of male under-reporters per educational level

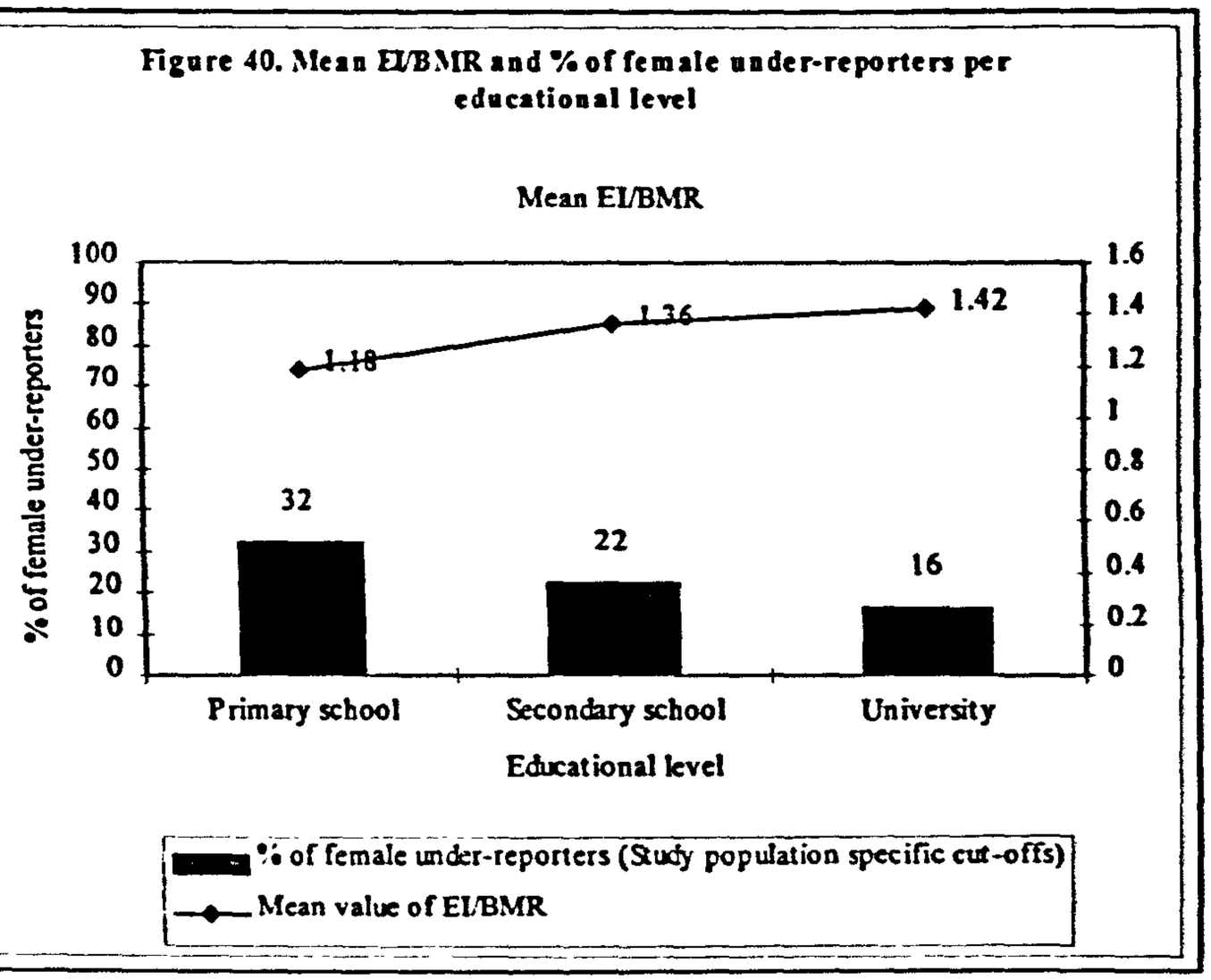


Figure 40. Mean EI/BMR and % of female under-reporters per educational level

Figure 41. Mean EI/BMR and % of male under-reporters per smoking status

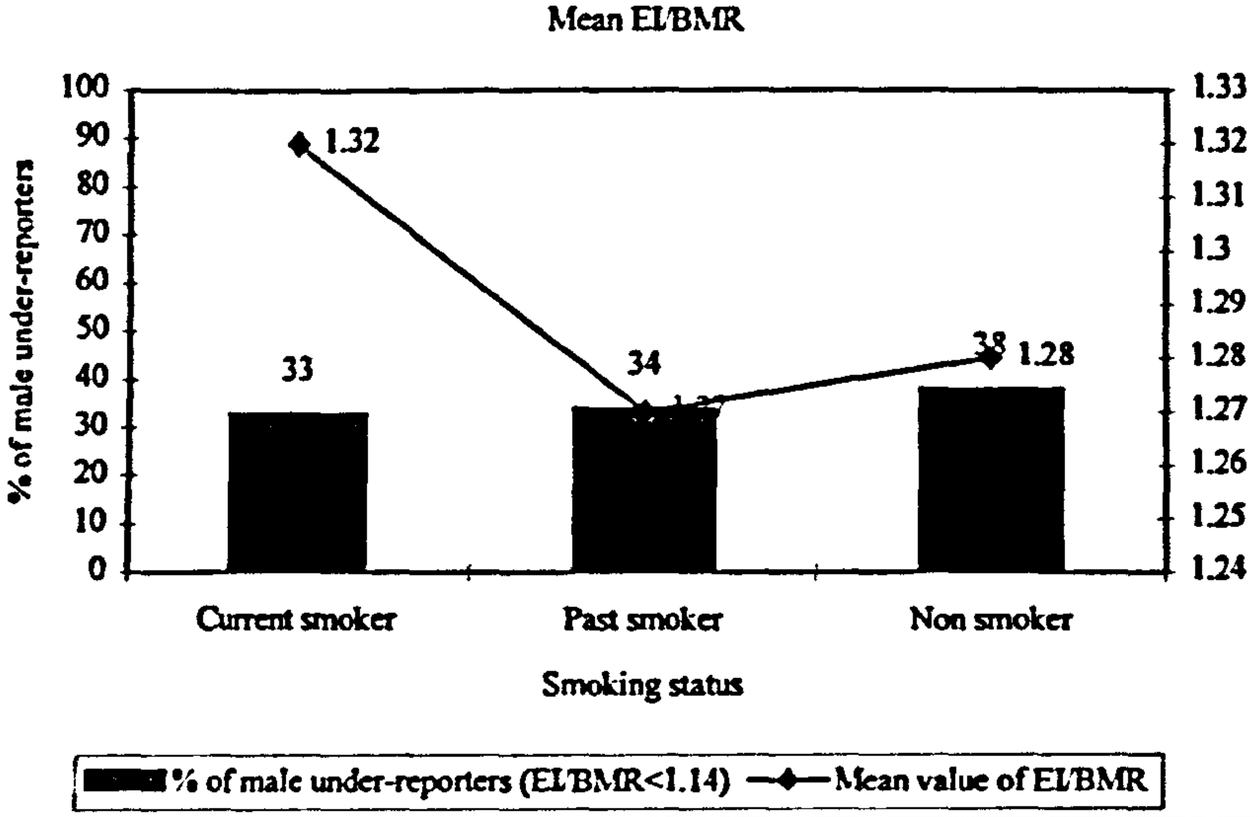


Figure 43. Mean EI/BMR and % of male under-reporters per smoking status

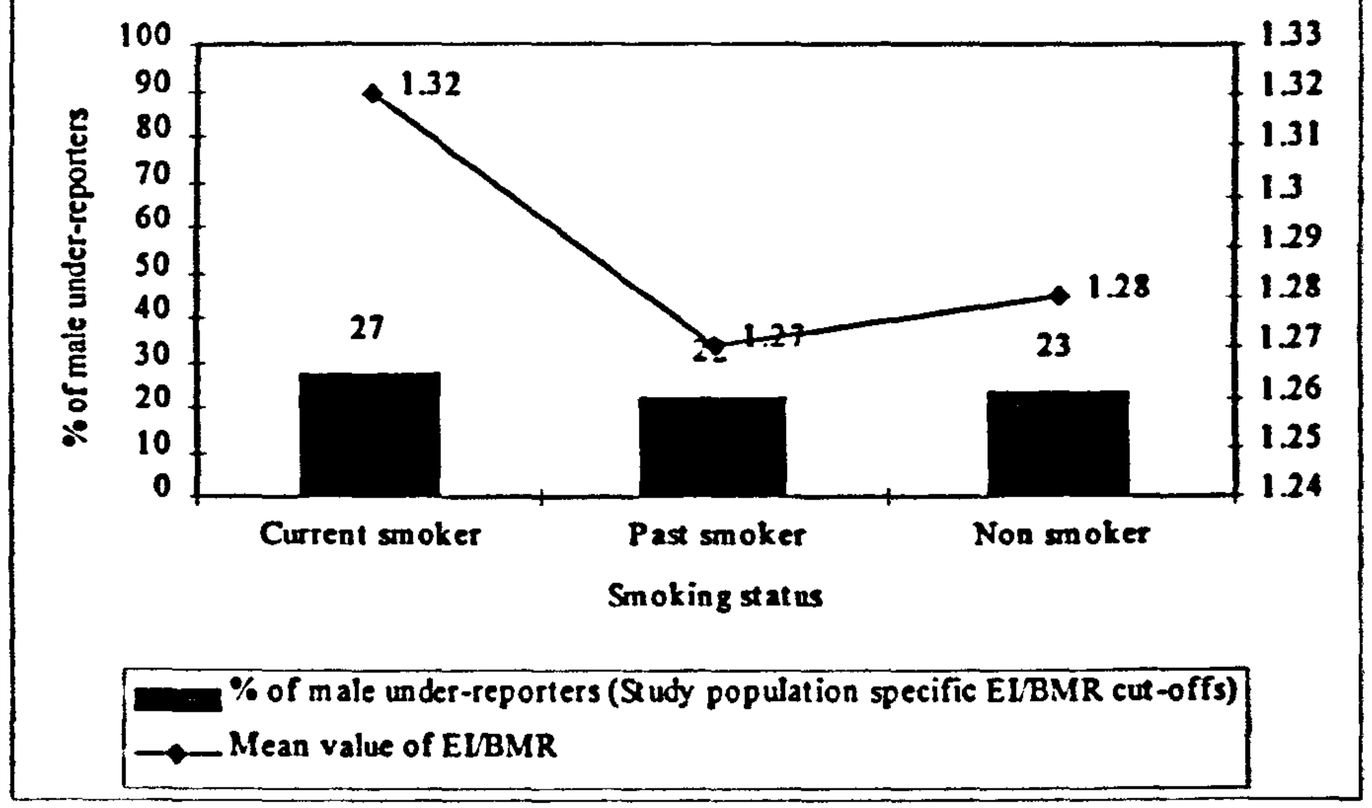


Figure 42. Mean EI/BMR and % of female under-reporters per smoking status

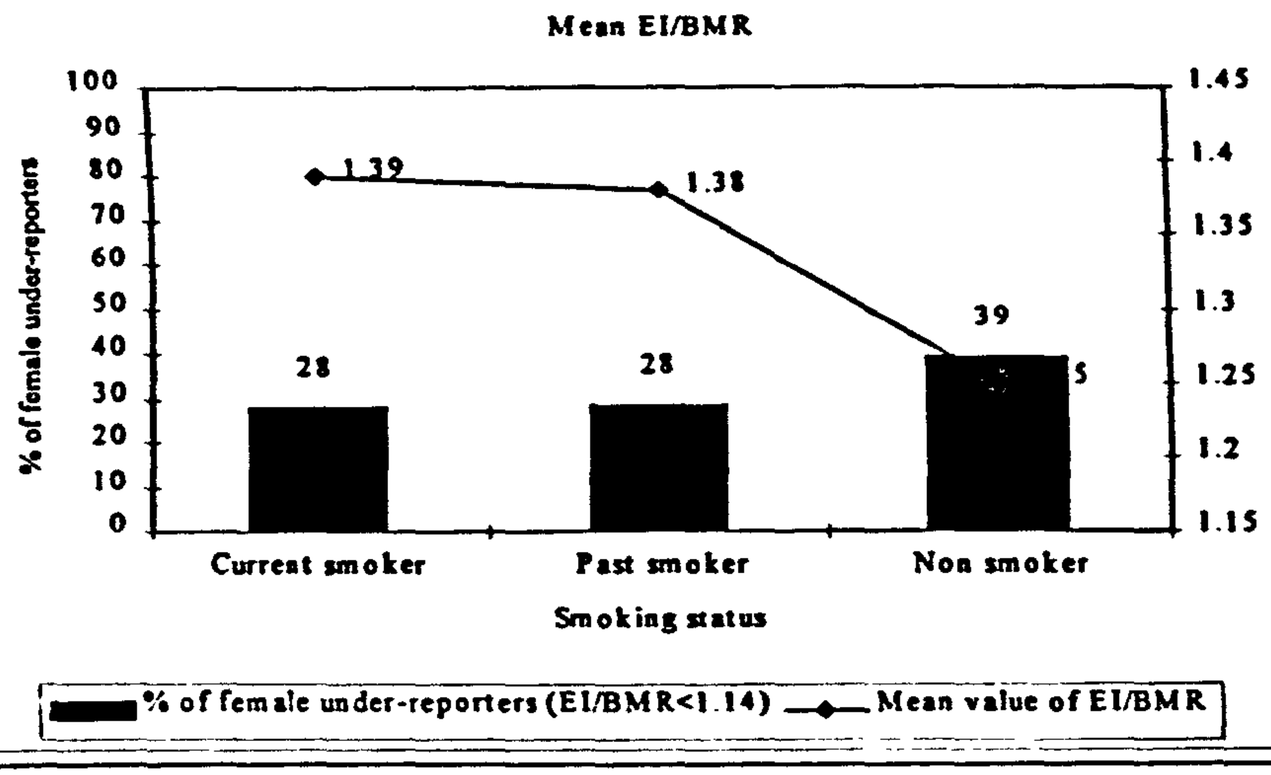
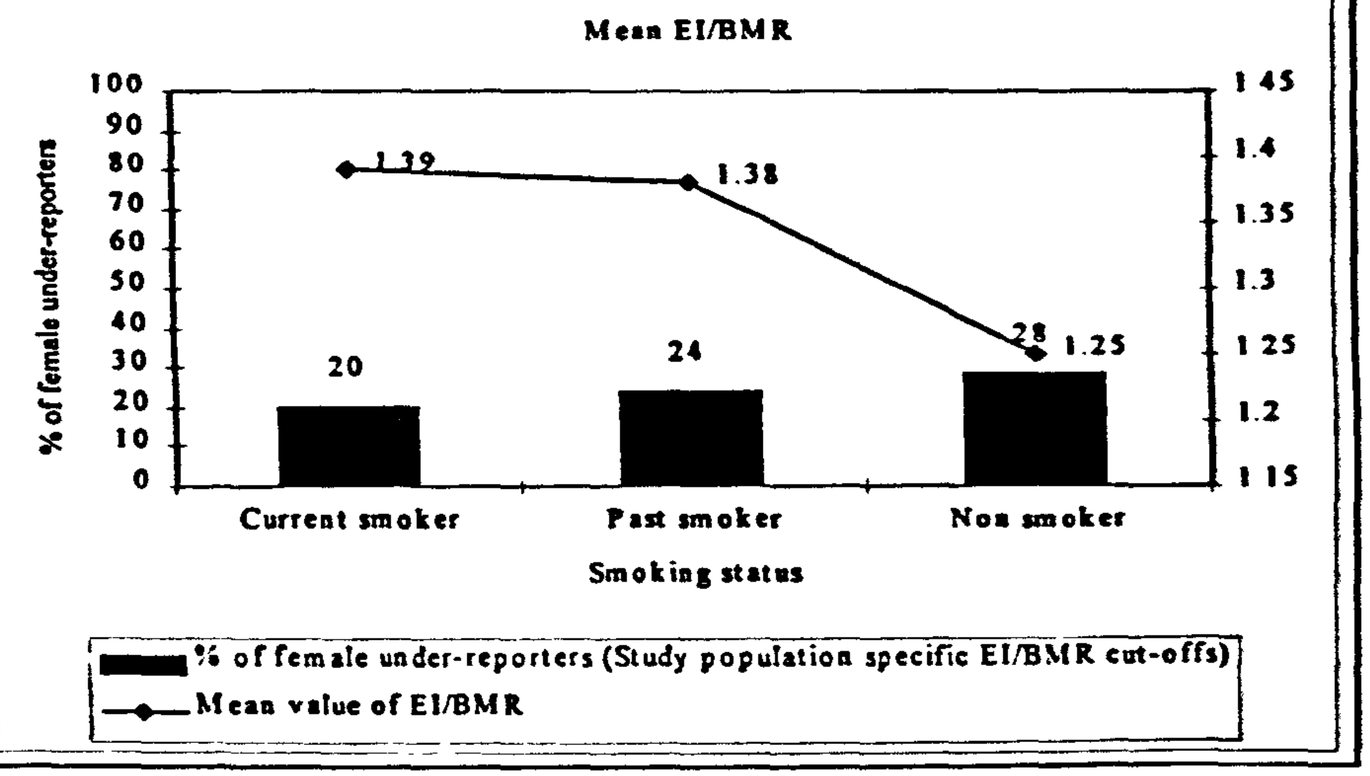


Figure 44. Mean EI/BMR and % of female under-reporters per smoking status



In Tables 26 and 27, the socio-demographic characteristics of under-reporters and regular reporters (non under-reporters) are shown separately for men and women. Table 26 refers to the 'Goldberg's' approach and Table 27 refers to the 'study population specific' approach for identifying under-reporters of energy intake. For categorical variables comparisons of percentages of under-reporters and regular reporters were made using the chi squared test. Mean values of age and physical activity among regular reporters and under-reporters were compared using the t-test.

Table 26. Socio-demographic characteristics of under-reporters & regular reporters* of energy intake

Covariates	MEN (n=596)			WOMEN (n=961)		
	Regular reporters (n=387)	Under-reporters (n=209)	P value	Regular reporters (n=620)	Under-reporters (n=341)	P value
EI/BMR (mean,se)	1.47 (0.28)	0.96 (0.13)	<0.001	1.48 (0.28)	0.95 (0.15)	<0.001
Age (mean, yrs, se)	48.98 (0.68)	52.03 (0.86)	<0.001	47.36 (0.49)	54.62 (0.69)	<0.001
Physical activity (mean, MET, se)	34.96 (0.34)	31.75 (0.36)	<0.001	34.96 (0.17)	33.87 (0.23)	<0.001
Education			<0.200			<0.001
Primary school %	25.1	31.6		38.7	62.8	
Secondary school %	36.2	31.1		30.8	22.9	
University %	38.8	37.3		30.5	14.4	
Smoking habits						
Current smoker %	39.8	36.8	<0.533	24.7	17.3	<0.001
Past smoker %	30.7	29.4		10.3	7.3	
Non smoker %	29.5	33.8		65.0	75.4	
Area of residence						
Mytilini %	40.1	49.8	<0.042	40.8	37.0	<0.024
Thessaloniki %	59.9	50.2		59.2	63.0	

* Under-reporters were identified based on EI/BMR < 1.14

Table 27. Socio-demographic characteristics of under-reporters & regular reporters* of energy intake

	MEN (n=596)			WOMEN (n=961)		
	Regular reporters (n=451)	Under-reporters (n=145)	P value	Regular reporters (n=716)	Under-reporters (n=245)	P value
EI/BMR (mean,se)	1.41 (0.30)	0.92 (0.14)	<0.001	1.43 (0.29)	0.90 (0.14)	<0.001
Age (mean, yrs, se)	50.85 (0.65)	47.57 (0.89)	0.003	48.79 (0.48)	53.28 (0.78)	<0.001
Physical activity (mean, MET,se)	34.26 (0.32)	32.54 (0.43)	0.001	34.68 (0.16)	34.28 (0.28)	0.212
Education			0.060			<0.001
Primary school %	29.7	20.0		42.9	60.0	
Secondary school %	33.9	35.9		29.2	24.5	
University %	36.4	44.1		27.9	15.5	
Smoking habits			0.504			0.071
Current smoker %	37.5	42.8		23.7	17.1	
Past smoker %	31.3	27.6		9.5	8.6	
Non smoker %	31.3	29.7		66.8	74.3	
Area of residence			0.849			0.192
Mytilini %	43.2	44.1		40.6	35.9	
Thessaloniki %	56.8	55.9		59.4	64.1	

* Under-reporters were identified based on study population specific EI/BMR cut-offs

Formal statistical tests revealed that older, less physically active, less educated men and women are more prone to under-reporting. The effect of education is most profound in women. However, this does not necessarily imply that less educated men are better in dietary reporting compared to less educated women, but most probably, that more educated men are not any better in dietary reporting compared to less educated men, since in traditional Greek households men are not the persons who are expected to take care of the preparation of meals and subsequently are less likely to accurately report their habitual dietary intake. This statement was further supported by assessing the percentage of under-reporters in each level of education.

Furthermore, significant association has been found between under-reporting and not smoking in women (as in Pryer et al, 1997). The latter was not the case in men. This finding gives a hint of how health consciousness may act and have implications in dietary reporting, since tobacco smoking is broadly considered to be an inverse indicator of healthy lifestyle.

Similar results were obtained when the analysis was conducted the other way round, that is when the mean EI/BMR was examined and compared in pre-defined categories of age, education and smoking status, as well as appropriate sex specific quartiles for physical activity. The mean EI/BMR intakes were compared either by t-test or F-test (ANOVA). Results are shown in Table 28. Once again under-reporting is more prevalent among older, less active, less educated men and women. Concerning the effect of smoking habits in under-reporting, it seems that under-reporting is more extensive among non-smokers women but not men.

Table 28. Mean values & standard errors of EI/BMR in categories of socio-demographic characteristics

Covariates	MEN (n=596)		WOMEN (n=961)	
	Mean EI/BMR (SE)	P value	Mean EI/BMR (SE)	P value
Age groups		<0.001		<0.001
<39	1.41 (0.03)		1.43 (0.02)	
40-54	1.26 (0.02)		1.34 (0.02)	
55-64	1.25 (0.03)		1.20 (0.02)	
65+	1.26 (0.03)		1.11 (0.02)	
Physical activity (quartiles)		<0.001		<0.001
< 31.55 * OR 29.24 **	1.15 (0.02)		1.16 (0.02)	
31.56-34.42* OR 29.25-32.90**	1.25 (0.02)		1.22 (0.03)	
34.43-37.06* OR 32.91-37.29**	1.31 (0.02)		1.30 (0.03)	
>37.07* OR >37.30**	1.33 (0.02)		1.41 (0.03)	
Education		<0.001		<0.001
Primary school	1.26 (0.03)		1.18 (0.01)	
Secondary school	1.32 (0.02)		1.36 (0.02)	
University %	1.29 (0.01)		1.42 (0.01)	
Smoking habits		0.291		<0.001
Current smoker %	1.32 (0.02)		1.39 (0.02)	
Past smoker %	1.27 (0.02)		1.38 (0.02)	
Non smoker %	1.28 (0.01)		1.25 (0.02)	
Area of residence		0.027		0.038
Mytilini %	1.26 (0.02)		1.32 (0.02)	
Thessaloniki %	1.32 (0.01)		1.27 (0.01)	

*Cut-off point for men ** Cut-off point for women

In order to evaluate whether a) under-reporting is linked to the degree of obesity and b) whether the diet is under-reported as a whole or whether there is a selective under-reporting in specific macro-nutrients, as has been suggested by several authors (Poppitt et al, 1995, Goldberg & Black, 1998), we proceeded to comparisons of mean values of obesity indices as well as mean percent of energy derived from macronutrients between under-reporters and regular reporters. The mean values were compared by using the t-test. Results are shown in Tables 29 and 30. Table 29 refers to the 'Goldberg's' approach and Table 30 refers to the 'study population specific' approach for identifying under-reporters of energy intake.

Table 29. Mean values & SD of obesity indices & dietary data in under-reporters* & regular reporters

Covariates	MEN (n=596)					WOMEN (n=961)				
	Regular reporters (n=387)		Under-reporters (n=204)		P value	Regular reporters (n=620)		Under-reporters (n=341)		P value
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
Obesity indices										
BMI	27.8	0.18	29.2	0.25	<0.001	28.04	0.19	30.8	0.28	<0.001
WHR	0.94	0.004	0.96	0.01	<0.001	0.77	0.003	0.80	0.004	<0.001
WC	95.8	0.53	99.6	0.68	<0.001	82.2	0.47	88.9	0.68	<0.001
Dietary data										
Energy intake, kcal	2531	27.3	168	22.1	<0.001	2032	16.2	1319	13.1	<0.001
Fat, g	124	1.66	81.7	1.21	<0.001	104.6	0.98	65.9	0.82	<0.001
MUF, g	61.7	0.84	41.6	0.65	<0.001	51.9	0.47	33.8	0.47	<0.001
PUF, g	15.5	0.29	10.5	0.25	<0.001	12.7	0.17	8.59	0.17	<0.001
SAT, g	35.8	0.59	22.0	0.46	<0.001	30.8	0.40	17.5	0.29	<0.001
Carbohydrate, g	248	2.97	170	2.62	<0.001	204	1.79	138	1.54	<0.001
Protein mean, g	89.1	1.00	60.1	0.93	<0.001	73.9	0.63	48.8	0.60	<0.001
Alcohol mean, g	17.3	1.08	9.85	0.94	<0.001	3.39	0.24	1.46	0.17	<0.001
Fat, (%) *	43.9	0.26	43.7	0.36	<0.001	46.2	0.17	44.8	0.28	<0.001
MUF, % *	21.9	0.16	22.3	0.24	0.140	23.0	0.11	22.9	0.20	<0.001
PUF, % *	5.51	0.01	5.61	0.11	0.462	5.62	0.06	5.87	0.11	0.066
SAT, % *	13.5	0.01	11.8	0.14	<0.001	13.5	0.01	11.8	0.14	<0.001
Carbohydrate, % *	40.5	0.20	42.2	0.34	<0.001	40.5	0.20	42.2	0.34	<0.001
Protein, % *	14.5	0.06	14.8	0.10	0.078	14.5	0.06	14.8	0.10	0.078
Alcohol, % *	1.14	0.08	0.75	0.08	0.001	1.14	0.08	0.75	0.08	0.001

* Under-reporters were identified based on EI/BMR< 1.14

Table 30. Mean values & SD of obesity indices & dietary data in under-reporters* & regular reporters

Covariates	MEN (n=596)					WOMEN (n=961)				
	Regular reporters (n=451)		Under-reporters (n=145)			Regular reporters (n=716)		Under-reporters (n=245)		
	Mean	SE	Mean	SE	P value	Mean	SE	Mean	SE	P value
Obesity indices										
BMI	27.95	0.17	29.5	0.31	<0.001	28.4	0.18	30.9	0.34	<0.001
WHR	0.94	0.003	0.95	0.01	0.108	0.78	0.003	0.80	0.01	0.006
WC	96.5	0.48	99.3	0.85	0.004	83.3	0.44	88.6	0.83	<0.001
Dietary data										
Energy intake, kcal	2412	27.7	1684	27.5	<0.001	1957	16.0	1261	15.2	<0.001
Fat, g	118	1.62	81.8	1.48	<0.001	100	0.97	63.4	0.98	<0.001
MUF, g	58.8	0.82	41.9	0.82	<0.001	49.9	0.47	32.7	0.56	<0.001
PUF, g	14.9	0.28	10.3	0.26	<0.001	12.3	0.16	8.18	0.21	<0.001
SAT, g	33.9	0.57	22.0	0.56	<0.001	29.3	0.39	16.7	0.33	<0.001
Carbohydrate, g	238	2.91	170	3.28	<0.001	198	1.71	131	1.77	<0.001
Protein mean, g	85.1	1.01	59.9	1.12	<0.001	71.1	0.62	47.0	0.72	<0.001
Alcohol mean, g	16.2	0.96	10.1	1.21	0.001	3.21	0.22	1.23	0.11	<0.001
Fat, (%) *	43.9	0.25	43.8	0.43	0.907	45.9	0.16	45.0	0.36	0.017
MUF, % *	21.9	0.15	22.5	0.30	0.097	23.0	0.11	23.2	0.25	0.420
PUF, % *	5.55	0.08	5.56	0.12	0.935	5.67	0.06	5.84	0.14	0.282
SAT, % *	12.5	0.12	11.7	0.22	0.003	13.3	0.10	11.9	0.17	<0.001
Carbohydrate, % *	39.7	0.26	40.4	0.49	0.192	40.8	0.20	42.0	0.42	0.013
Protein, % *	14.1	0.07	14.3	0.16	0.521	14.5	0.06	14.9	0.13	0.028
Alcohol, % *	4.60	0.25	3.92	0.43	0.175	1.12	0.07	0.68	0.06	<0.001

* Under-reporters were identified based on study population specific EI/BMR cut-offs

The most robust finding is an association between a high BMI (as well as a high WC and WHR) and low energy reporting. This finding clearly has implications for the specific study investigating the association between energy intake and energy yielding nutrients with obesity and will be discussed later. As expected, mean daily energy and macronutrient intake, in absolute values, are significantly lower in under-reporters compared to regular reporters, both for men and women. When it comes to the percent contribution of different macronutrients to total energy intake, it must be noted that whereas percent of fat and alcohol is less in under-reporters than in regular reporters (especially in women), percent of carbohydrate is higher in under-reporters than in non-under-reporters, whilst percent of protein remains relatively stable (slightly higher in under-reporters) among the two comparison groups. The latter might have something to do with the effect of health consciousness on dietary reporting, since under-reporters might tend to under-report foods and subsequently nutrients which they believe to be less healthy dietary choices. However differences between under-reporters and regular reporters in the proportion of energy intake from macronutrients, are rather small even when they are statistically significant.

Similar results were obtained when under-reporting was assessed by examining the mean EI/BMR in different sub-groups (lean-normal-overweight-obese) as well as in appropriate quartiles of nutrient intakes. Mean EI/BMR values were compared by using the F-test. Results are summarised in Tables 31-34.

Table 31. Mean values & standard errors of obesity indices per EI/BMR quartile - Men

EI/BMR	BMI	WHR	WC
<1.04	29.30 (0.32)	0.96 (0.006)	99.98 (0.84)
1.04-1.25	28.42 (0.27)	0.95 (0.006)	97.78 (0.85)
1.26-1.50	28.36 (0.29)	0.94 (0.006)	96.98 (0.87)
>1.51	27.19 (0.30)	0.93 (0.006)	93.83 (0.76)
P value	<0.001	<0.001	<0.001

Table 32. Mean values and standard errors of obesity indices per EI/BMR quartile - Women

EI/BMR	BMI	WHR	WC
<1.06	31.13 (0.33)	0.81 (0.005)	89.67 (0.82)
1.06-1.26	29.45 (0.33)	0.80 (0.005)	85.88 (0.75)
1.26-1.49	28.43 (0.32)	0.78 (0.005)	83.12 (0.75)
>1.50	27.15 (0.28)	0.76 (0.005)	80.01 (0.71)
P value	<0.001	<0.001	<0.001

Table 33. Mean values & standard errors of macronutrient data per EI/BMR quartile - Men

EI/BMR	% Fat	% MUF	%PUF	%SAT	% CHO	% Protein	% Alcohol
<1.04	44.1 (0.43)	22.7 (0.31)	5.66 (0.13)	11.7 (0.22)	40.7 (0.46)	14.29 (0.16)	3.43 (0.38)
1.04-1.25	43.4 (0.40)	22.0 (0.23)	5.39 (0.11)	11.9 (0.21)	40.1 (0.43)	14.32 (0.13)	4.60 (0.42)
1.26-1.50	43.9 (0.43)	21.6 (0.28)	5.77 (0.17)	12.5 (0.22)	39.8 (0.44)	14.12 (0.13)	4.51 (0.41)
>1.51	44.2 (0.46)	21.9 (0.27)	5.38 (0.10)	12.9 (0.21)	38.9 (0.50)	13.96(0.12)	5.21 (0.50)
P value	0.527	0.062	0.084	<0.001	0.064	0.183	0.031

Table 34. Mean values & standard errors of macronutrient data per EI/BMR quartile– Women

EI/BMR	% Fat	% MUF	%PUF	%SAT	% CHO	% Protein	% Alcohol
<1.06	44.6 (0.36)	22.9 (0.25)	5.99 (0.15)	11.6 (0.16)	42.5 (0.43)	14.7 (0.13)	0.67 (0.06)
1.06-1.26	45.8 (0.29)	23.3 (0.19)	5.69 (0.10)	12.6 (0.16)	40.9 (0.33)	14.7 (0.10)	1.06 (0.13)
1.26-1.49	45.7 (0.27)	23.1 (0.18)	5.51 (0.09)	13.0 (0.16)	41.2 (0.34)	14.7 (0.09)	0.88 (0.11)
>1.50	46.8 (0.28)	22.7 (0.19)	5.67 (0.09)	14.4 (0.17)	39.7 (0.32)	14.4 (0.10)	1.43 ((0.15)
P value	<0.001	0.247	0.025	<0.001	<0.001	0.048	<0.001

The graphical presentation of mean values of obesity indices and macronutrient intakes according to EI/BMR sex specific quartiles are shown in Figures 45-64.

Finally Tables 35 and 36 refer to mean daily intake values of energy and macro-nutrients (percent and absolute values) before and after exclusion of under-reporters as those have been identified by the two different methods. Tables have been prepared separately for men and women. As expected all values increase when data from under-reporters are excluded.

Table 35.Effect of under-reporting on behavioural & dietary data – Men

Covariates	MEN					
	All subjects (n=596)		*After excluding under-reporters*			
			(n=345)*		(n=451)**	
	Mean	SD	Mean	SD	Mean	SD
Energy intake	2235	621	2531	538	2412	589
Fat intake, g	109	34.8	124	32.7	118	34.4
MUF, g	54.7	17.4	61.7	16.6	58.8	17.3
PUF, g	13.8	5.66	15.5	5.76	14.9	5.85
SAT, g	31.0	12.2	35.8	11.8	33.9	12.2
Carbohydrate intake, g	221	64.2	249	58.5	237.9	61.8
Protein intake, g	78.9	22.5	89.1	19.7	85.1	21.4
Alcohol intake, g	14.7	19.3	17.3	21.3	16.2	20.4
Fat intake, %	43.9	5.23	44.0	5.24	43.9	5.24
MUF, %	22.1	3.35	21.9	3.20	21.9	3.27
PUF, %	5.55	1.59	5.51	1.56	5.55	1.65
SAT, %	12.3	2.65	12.6	2.58	125	2.62
Carbohydrate intake %	39.9	5.61	39.5	5.59	39.7	5.53
Protein intake, %	14.2	1.61	14.1	1.44	14.1	1.52
Alcohol %	4.43	5.25	4.73	5.29	4.60	5.28
Physical activity level –METs	33.8	6.45	35.0	6.75	34.3	6.77
Age	50.1	13.2	49.0	13.4	50.9	13.8

* Under-reporters were identified based on EI/BMR< 1.14

** Under-reporters were identified based on study population specific EI/BMR cut-offs

Table 36. Effect of under-reporting on behavioural & dietary data – Women

Covariates	WOMEN					
	All subjects (n=961)		*After excluding under-reporters*			
			(n=620)*		(n=716)**	
	Mean	SD	Mean	SD	Mean	SD
Energy intake	1780	492	2033	404	1957	427
Fat intake, g	90.9	29.6	105	24.6	100	25.8
MUF, g	45.5	13.8	51.9	11.7	50.0	12.5
PUF, g	11.3	4.49	12.7	4.37	12.3	4.38
SAT, g	26.1	10.8	30.8	10.1	29.3	10.4
Carbohydrate intake, g	181	51.0	205	44.8	199	45.6
Protein intake, g	64.9	18.7	73.8	15.9	71.1	16.7
Alcohol intake, g	2.70	5.24	3.39	5.99	3.21	5.91
Fat intake, %	45.7	4.70	46.2	4.25	45.9	4.33
MUF, %	23.0	3.16	23.01	2.78	22.9	2.88
PUF, %	5.71	1.74	5.62	1.44	5.67	1.59
SAT, %	12.9	2.70	13.5	2.58	13.3	2.66
Carbohydrate intake %	41.1	5.63	40.5	5.11	40.8	5.23
Protein intake, %	14.6	1.64	14.5	1.49	14.5	1.51
Alcohol %	1.00	1.79	1.14	1.93	1.12	1.99
Physical activity level –METs	34.6	4.35	35.0	4.29	34.7	4.31
Age	49.9	12.9	47.4	12.2	48.8	12.9

* Under-reporters were identified based on EI/BMR< 1.14

** Under-reporters were identified based on study population specific EI/BMR cut-offs

Figure 45. Mean BMI per quartile of EI/BMR among men

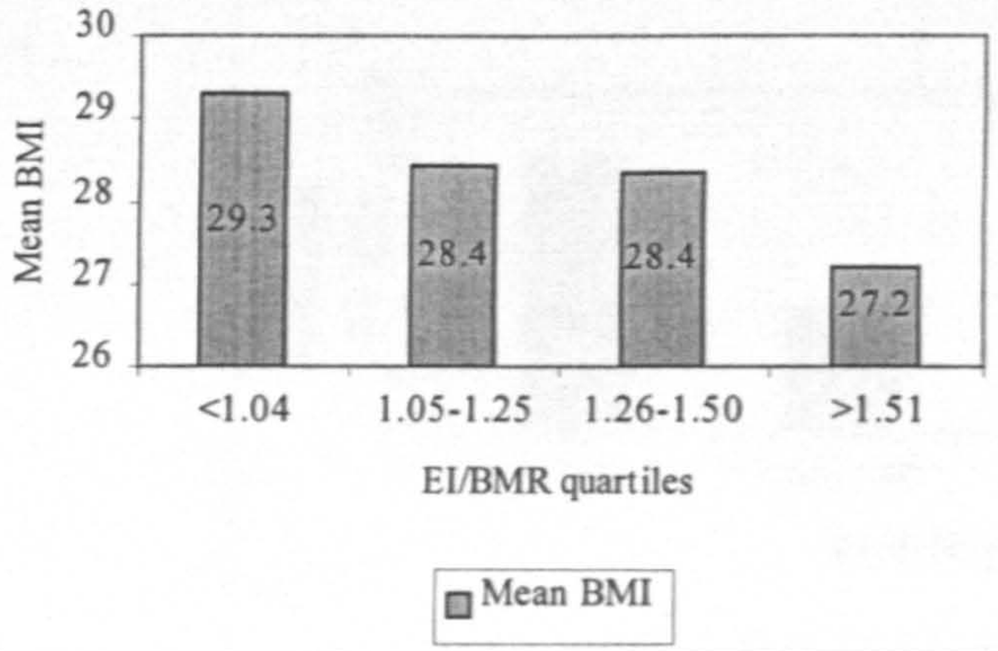


Figure 47. Mean WHR per quartile of EI/BMR among men

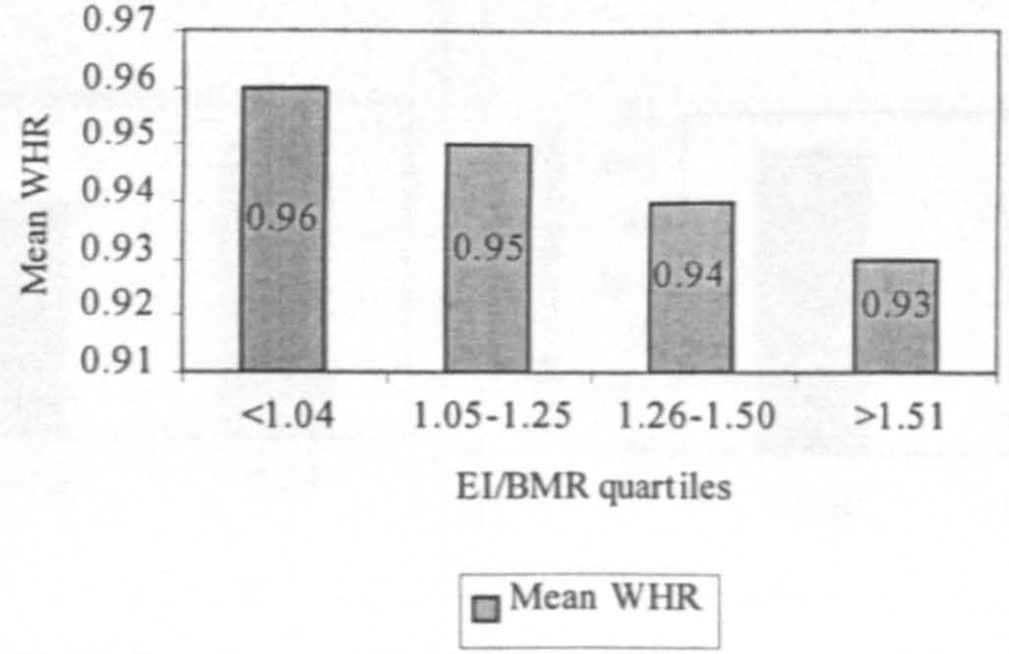


Figure 49. Mean WC per quartile of EI/BMR among men

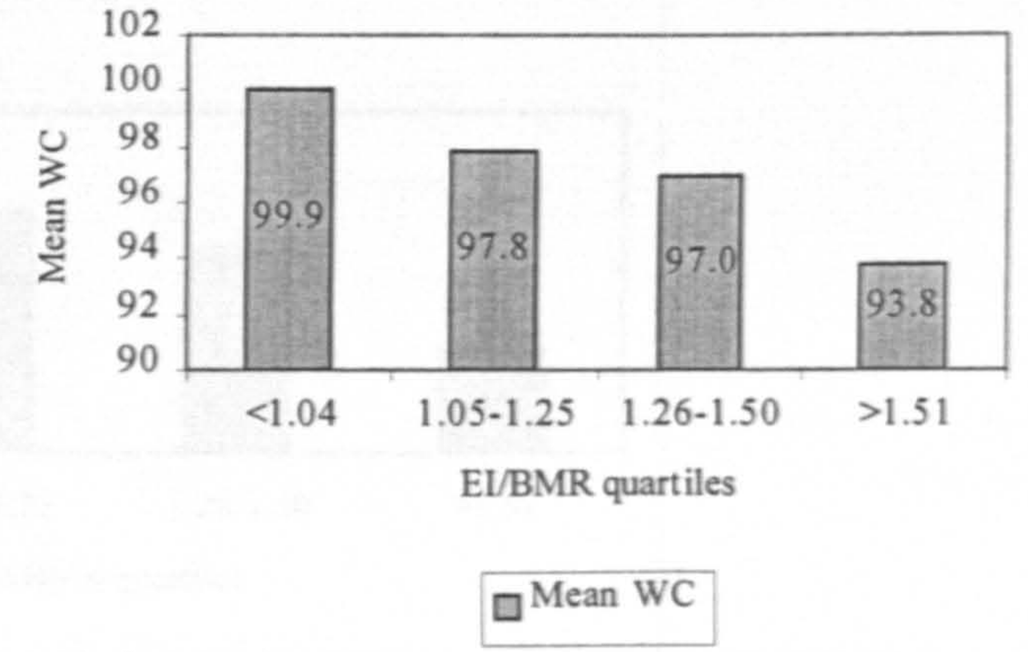


Figure 46. Mean BMI per quartile of EI/BMR among women

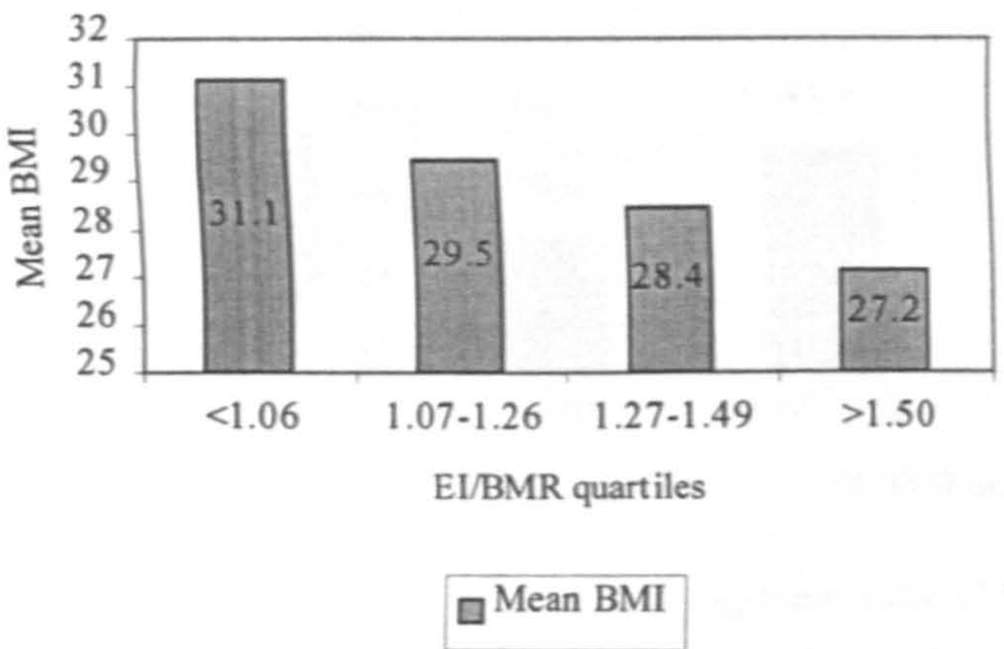


Figure 48. Mean WHR per quartile of EI/BMR among women

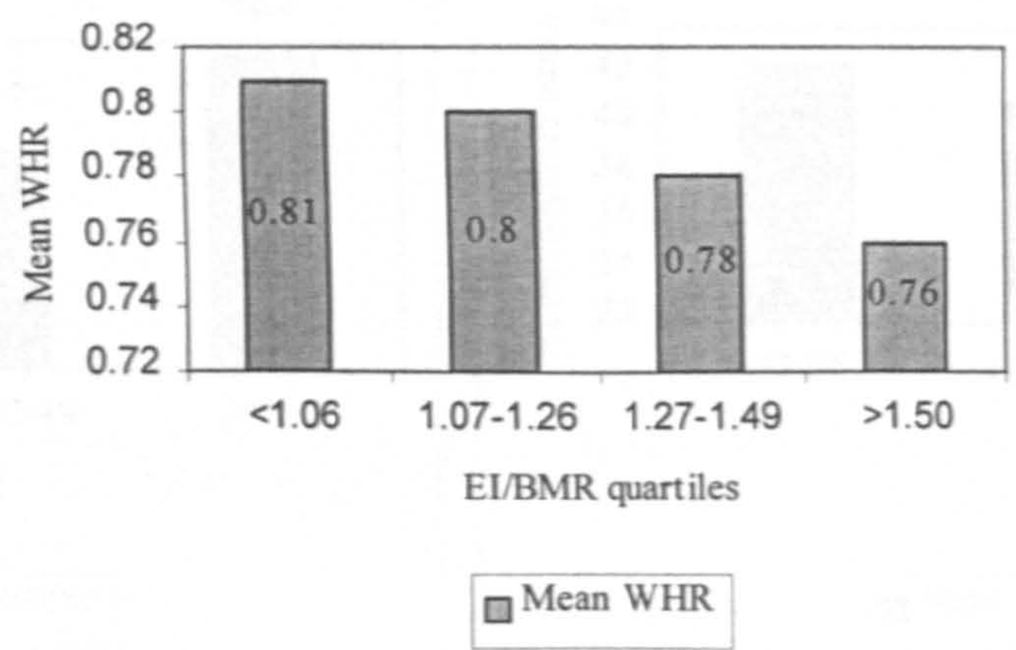


Figure 50. Mean WC per quartile of EI/BMR among women

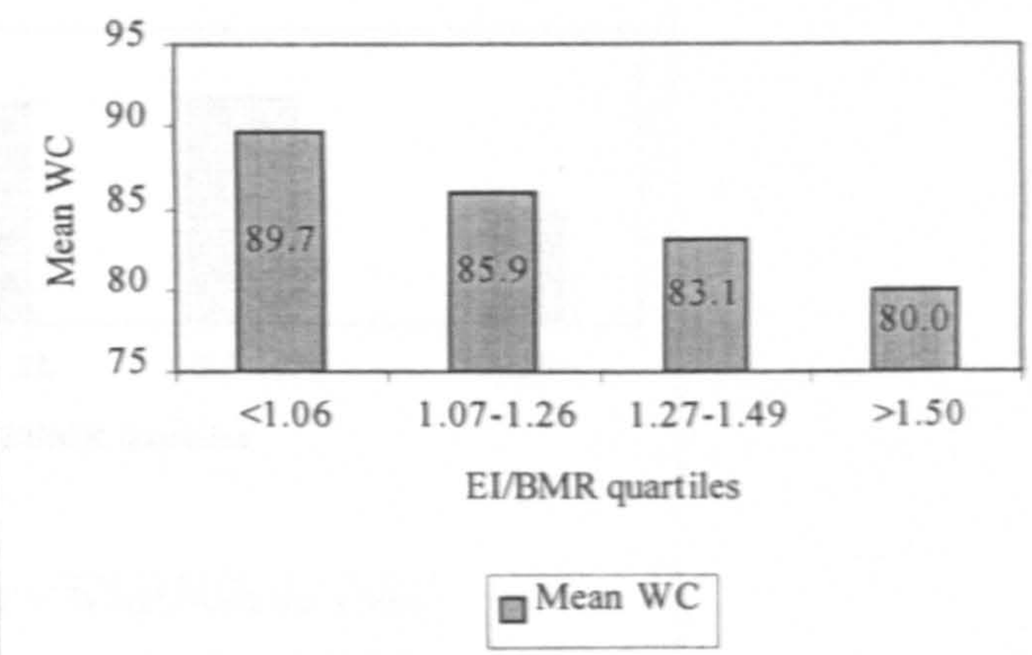


Figure 51. Mean value of % fat intake per quartile of EI/BMR among men

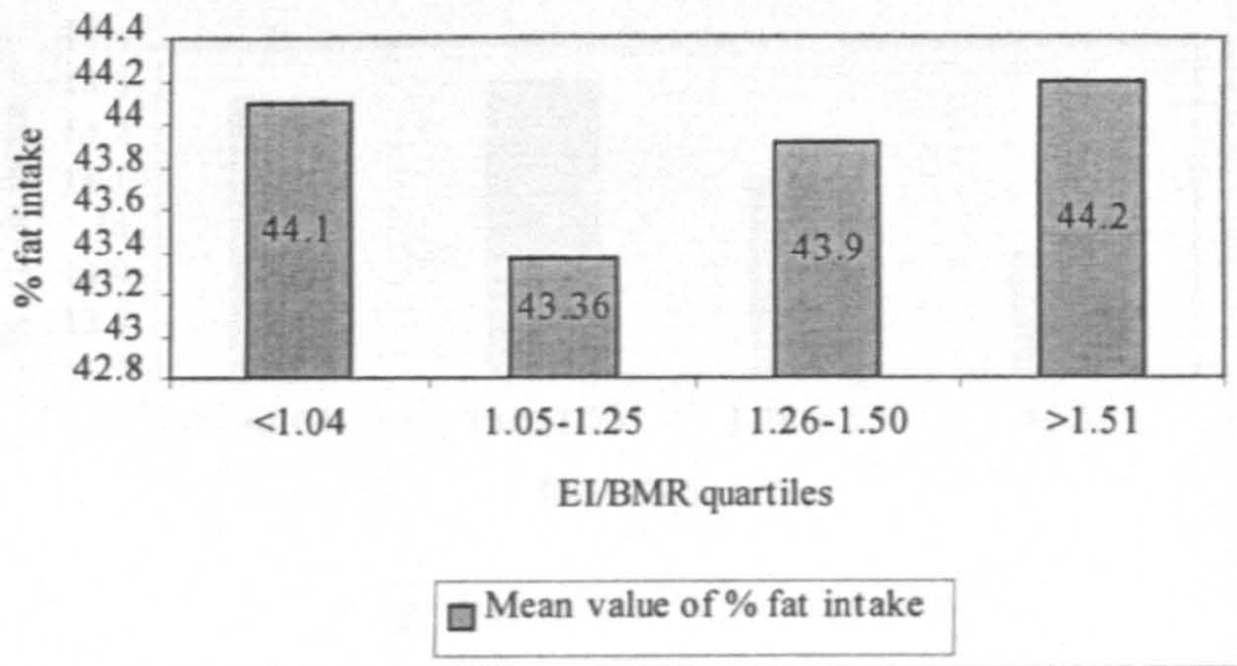


Figure 52. Mean value of % fat intake per quartile of EI/BMR among women

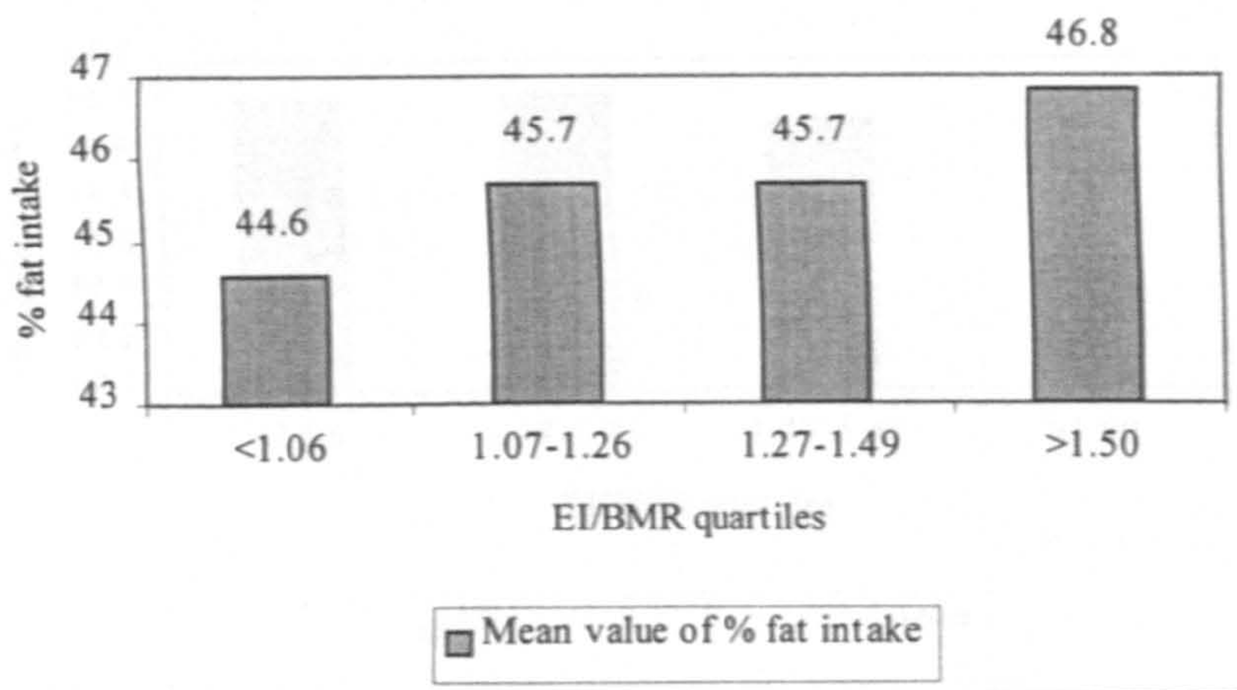


Figure 53. Mean value of % carbohydrate intake per quartile of EI/BMR among men

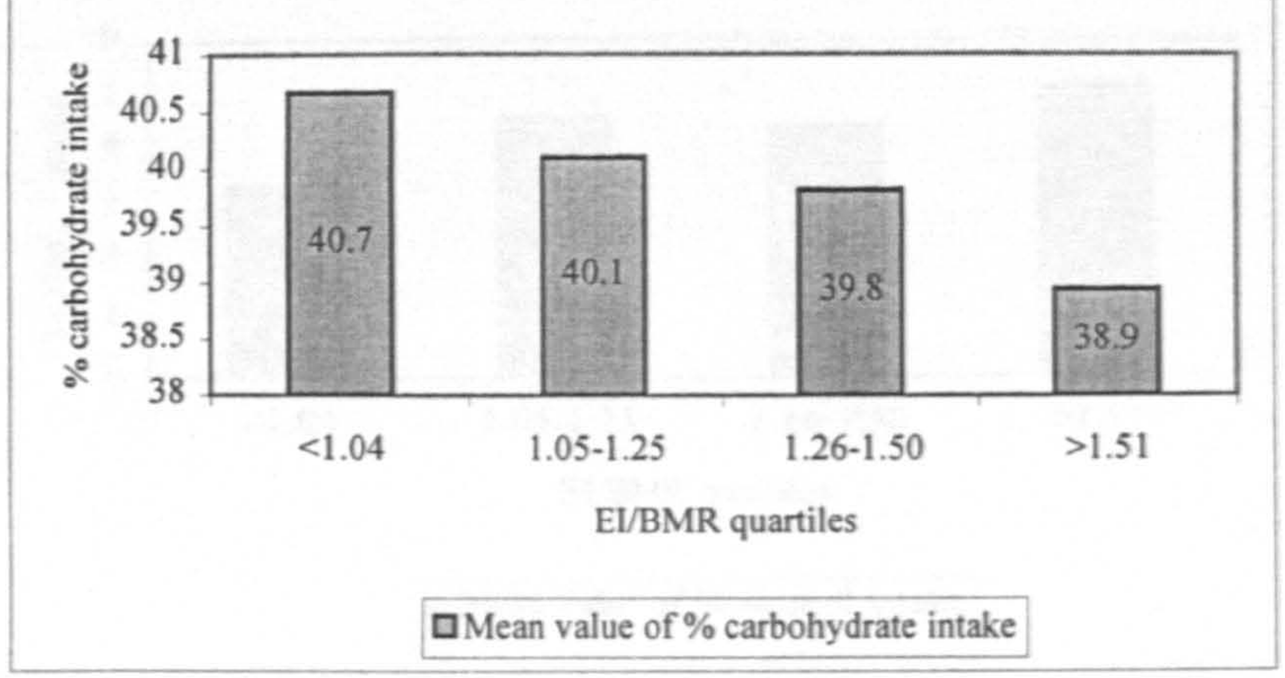


Figure 54. Mean value of % carbohydrate intake per quartile of EI/BMR among women

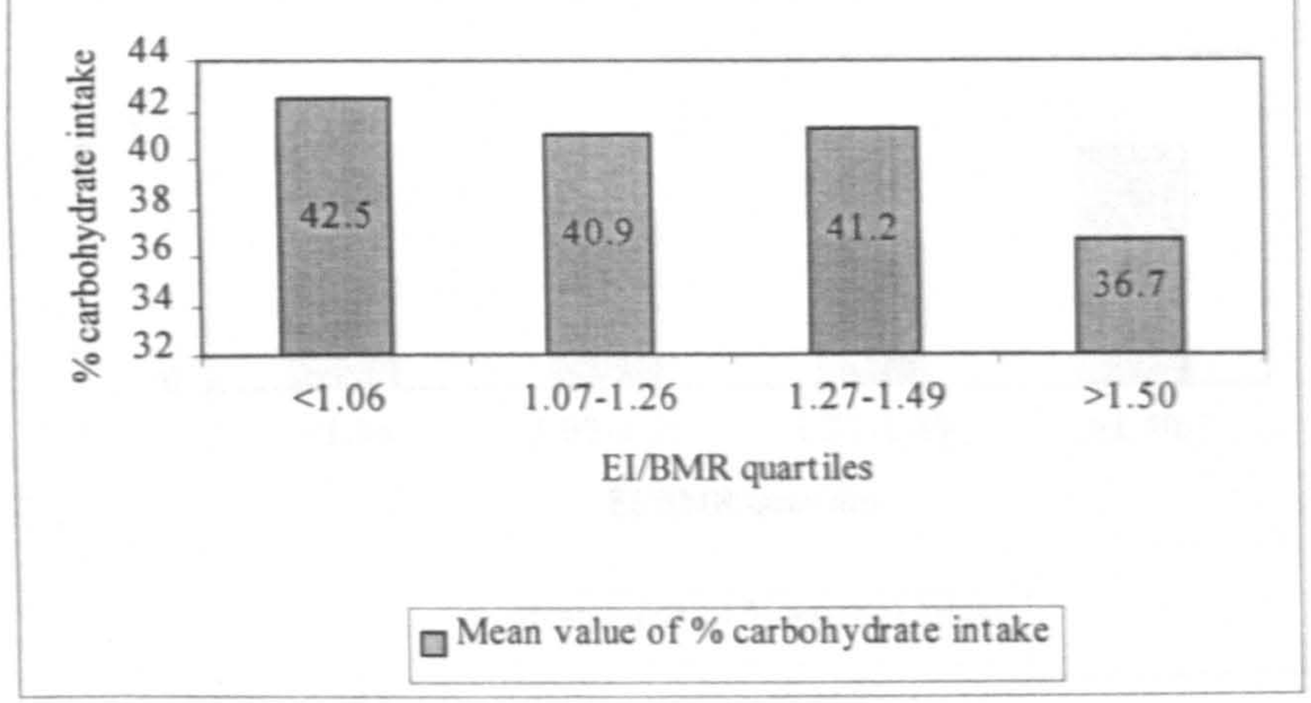


Figure 55. Mean value of % protein intake per quartile of EI/BMR among men

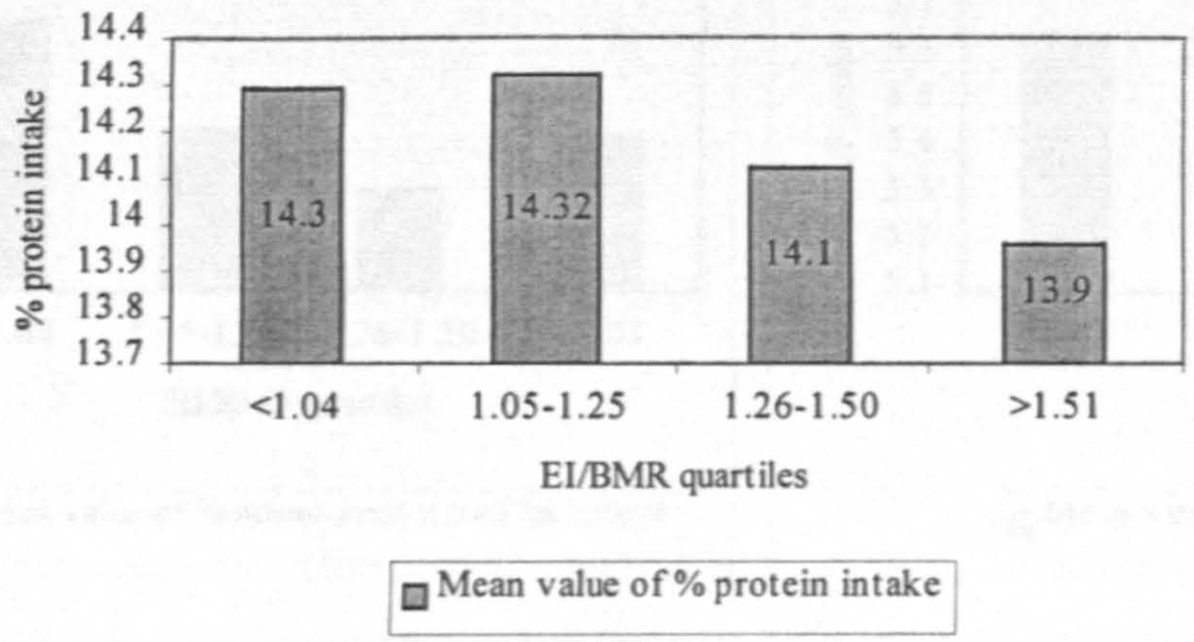


Figure 57. Mean value of % alcohol intake per quartile of EI/BMR among men

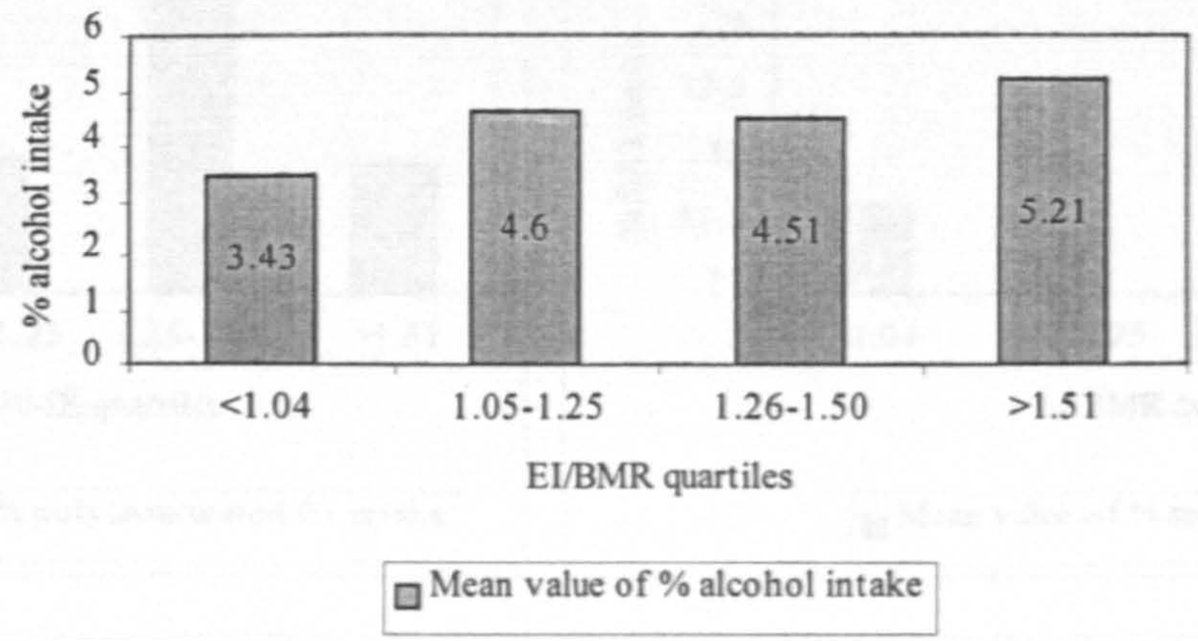


Figure 56. Mean value of % protein intake per quartile of EI/BMR among women

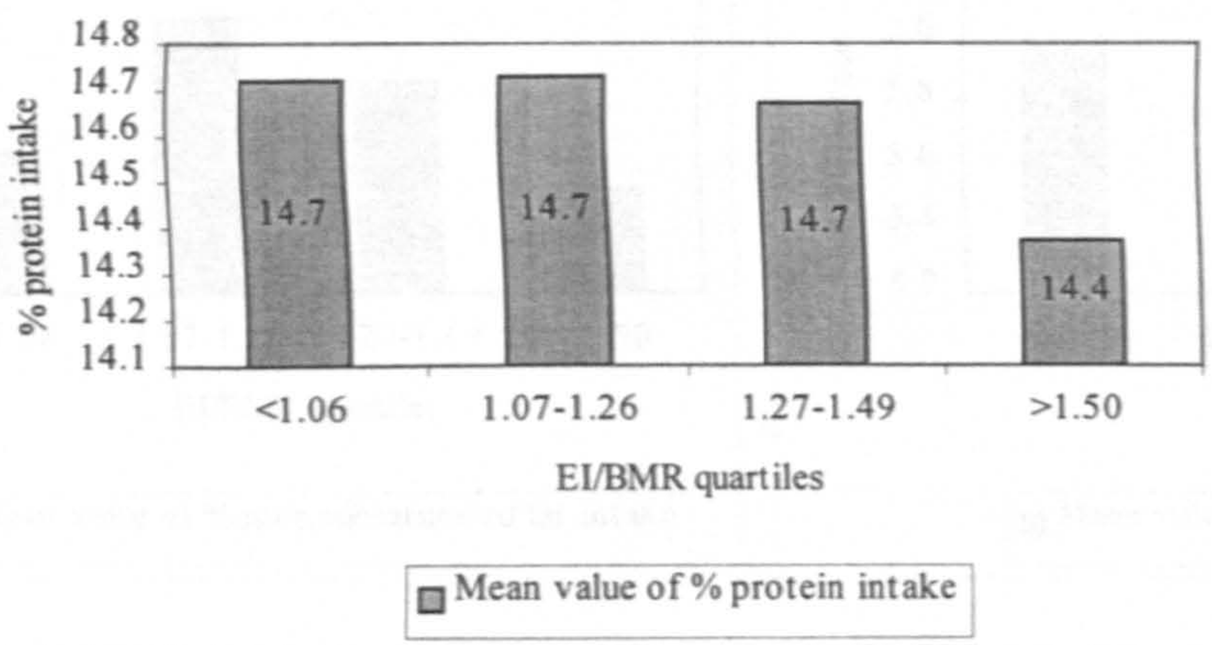


Figure 64. Mean value of % alcohol intake per quartile of EI/BMR among women

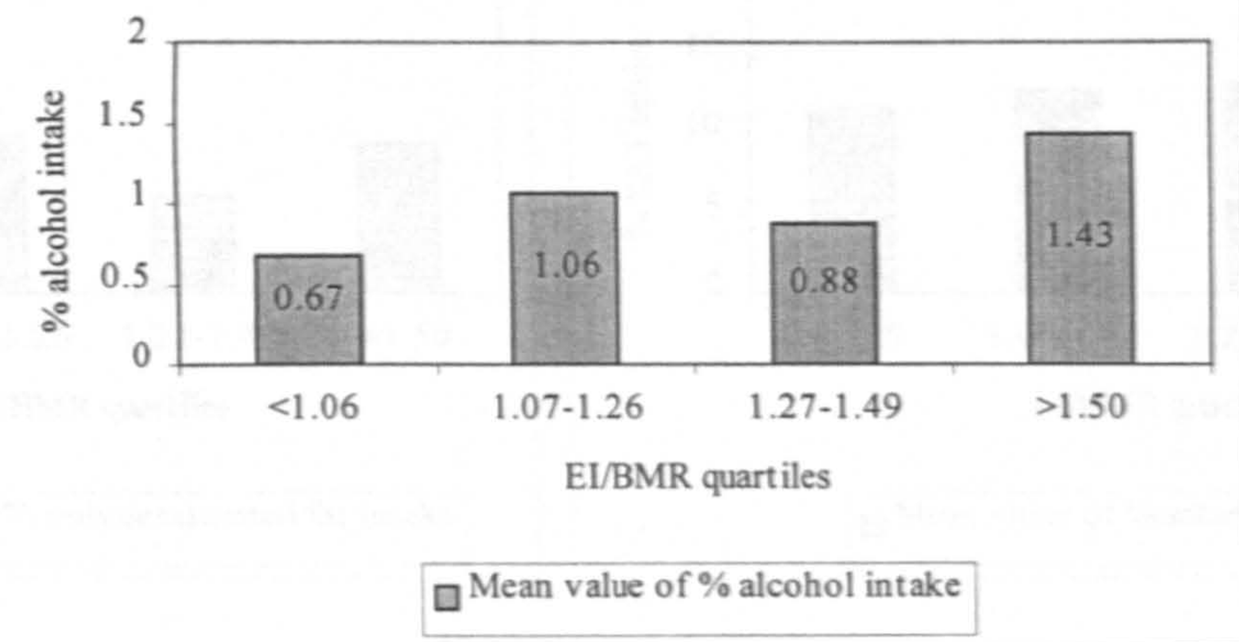


Figure 59. Mean value of % MUF intake per quartile of EI/BMR among men

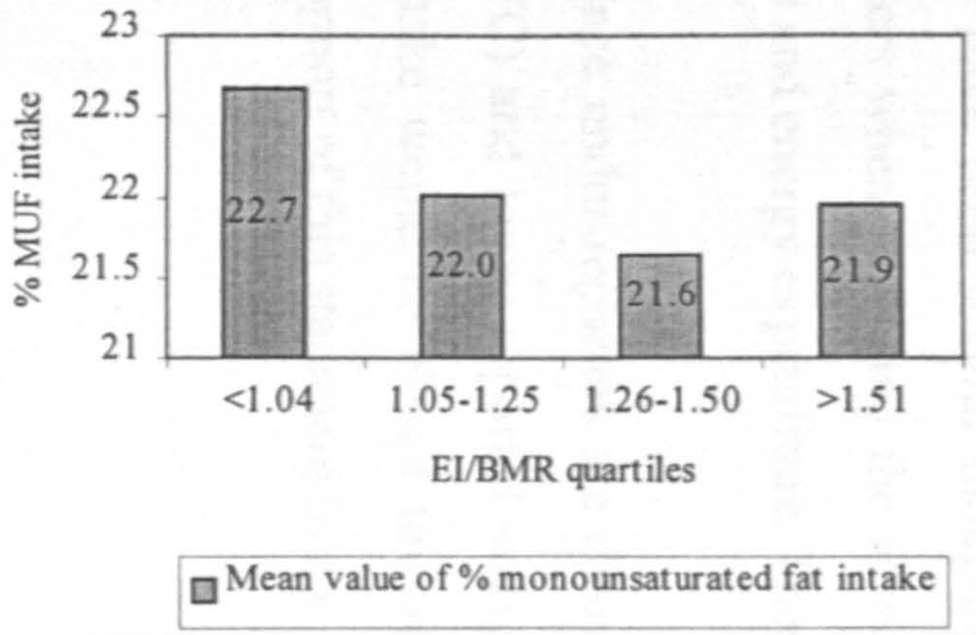


Figure 60. Mean value of % MUF intake per quartile of EI/BMR among women

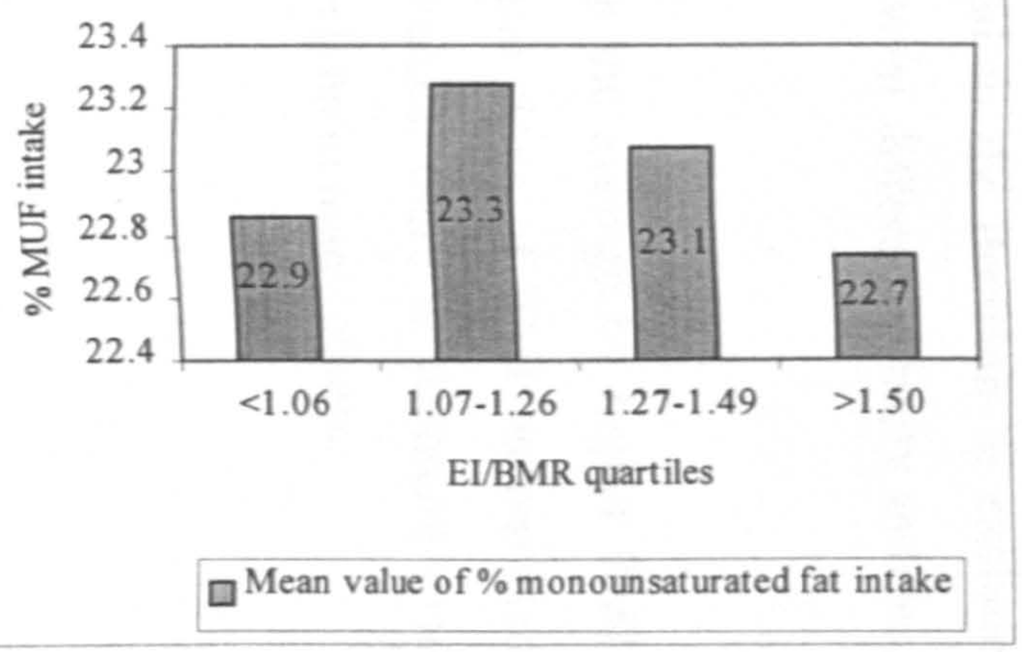


Figure 61. Mean value of % PUF per quartile of EI/BMR among men

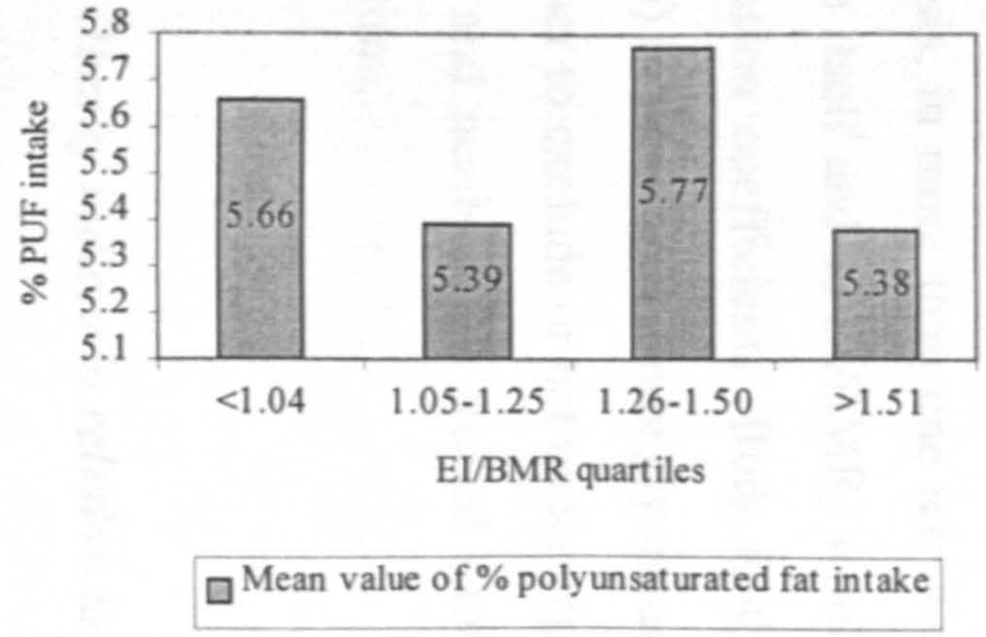


Figure 62. Mean value of % PUF per quartile of EI/BMR among women

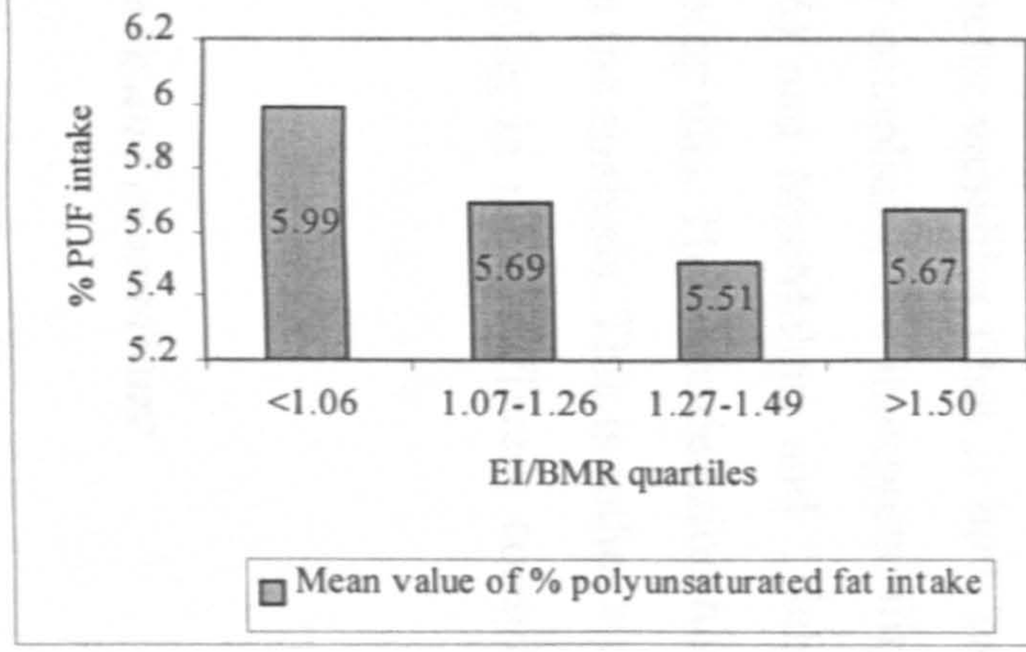


Figure 62. Mean value of % SAT intake per quartile of EI/BMR among men

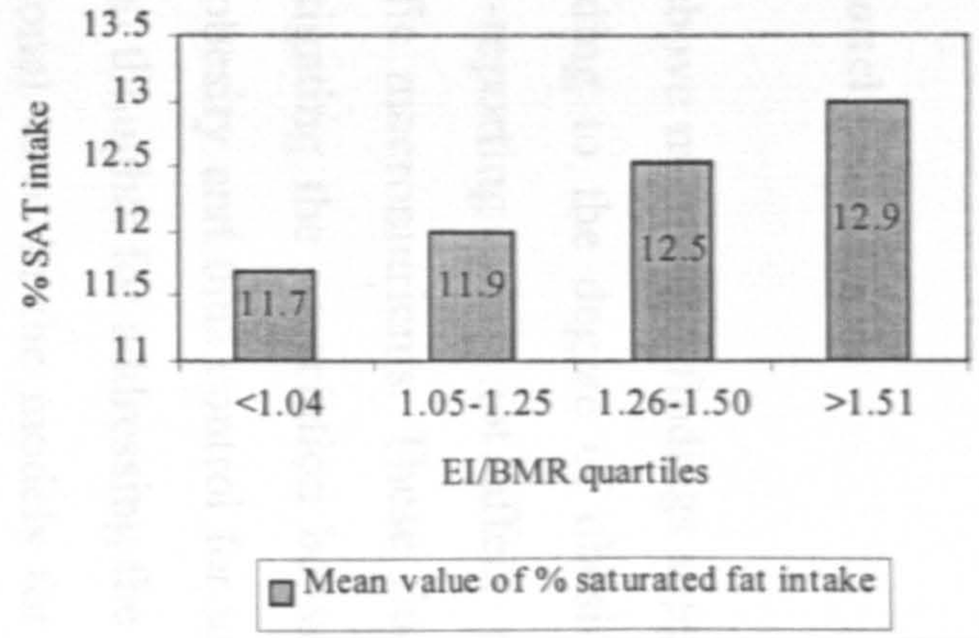
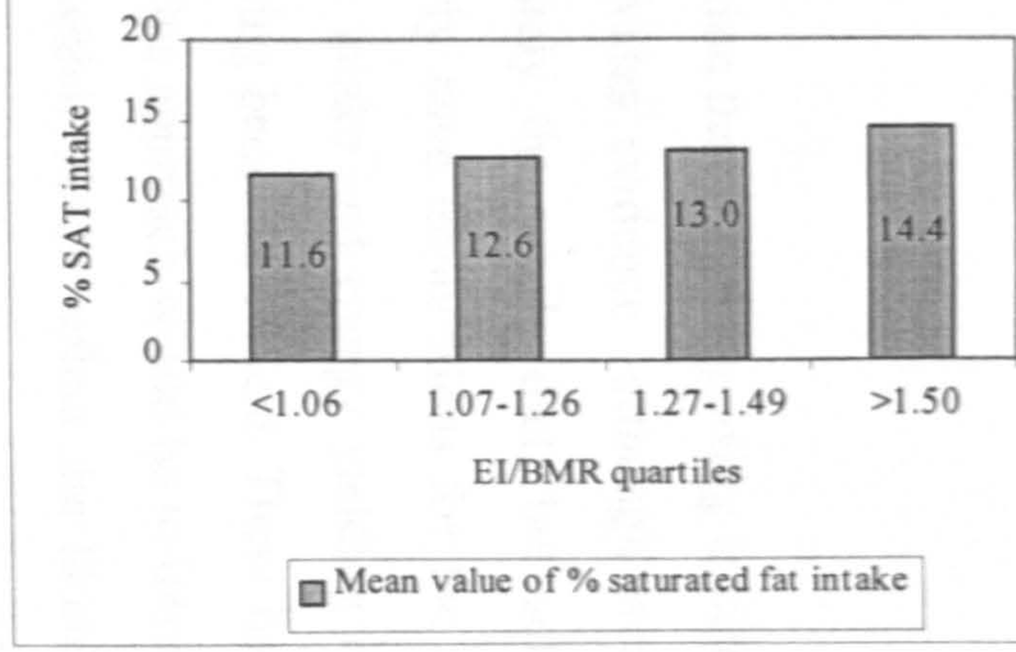


Figure 52. Mean value of % SAT intake per quartile of EI/BMR among women



7.3 Conclusions

The above mentioned findings support the notion that under-reporting is differential according to the degree of obesity and provides evidence –although weak- that under-reporting does not affect proportionately the whole diet, but selectively specific macronutrients. These findings clearly have implications for the analysis investigating the association between energy intake and energy yielding nutrients with obesity and thus control for under-reporting becomes critical. There have been several thoughts for addressing the problem. One approach would be to introduce an additional term in the models for EI/BMR which would reflect the likelihood of under-reporting. However, the existence of energy intake, a crucial variable in the analysis, in more than one terms of the predictor variables (that is both as energy intake itself and as EI/BMR) would seriously complicate the interpretability of the regression coefficients. Both Rothman (1986) and MacMahon and Trichopoulos (1996) in their books strongly argue against doing this. Thus the decision turns to be whether to exclude or not under-reporters from the analysis. This is rather a complex issue and needs to be treated cautiously keeping in mind different considerations each time.

7.3.1 Obesity indices in relation to energy intake and expenditure

There have been several thoughts which lead to the decision to exclude under-reporters when exploring the association between different obesity indices, energy intake and energy expenditure. These are:

- Since under-reporters are characterised on the average by higher BMI (WHR, WC) and lower reported energy intake, the slope of BMI on reported energy intake would be biased towards higher values; there is abundant evidence in support of this statement in our study, as well as in many others.

- Under-reporting, however operationally defined is a strong negative confounder of the association between BMI and energy intake, because under-reporting is positively associated with BMI and inversely with energy intake. Thus, in crude cross-sectional data, one may be unable to detect, as one should, a positive association between BMI and reported energy intake.

- Since among under-reporters, the slope of BMI on energy is biased high, there would appear to be an apparent interaction of the relation between BMI and energy intake by level of the ratio of energy intake to BMR (EI/BMR). There appears to be no way to adequately accommodate this bias. It would not be right to control for level of under-reporting for at least two reasons: a) because of the existence of interaction; and b) because the high values of the slope of BMI on energy intake among under-reporters is simply wrong and thus cannot be incorporated in the calculation of a weighted coefficient. The only solution, only partially satisfactory, is to restrict the analysis concerning BMI as a function of energy intake among regular reporters (that is excluding under-reporters). The bias that tends to increase the slope of BMI on energy intake among mild under-reporters, will hopefully balance the negative confounding introduced by under-reporting being positively associated with BMI and inversely with energy intake.

7.3.2 Obesity indices in relation to nutrient intake

The fact that there has been some evidence –although weak- that under-reporting does not affect proportionately the whole diet but selectively specific macronutrients supported our notion not to simply exclude under-reporters from the dataset. There has been concern that by simply excluding under-reporters there would be danger to introduce unknown bias into the dataset and may also eliminate those subjects of greatest interest to the scientific questions posed. In our case we considered more desirable to repeat the analyses with and without including subjects defined as under-reporters and examine the effect on the conclusions to be drawn form the study. It is

important that the possibility of (systematic as well as random) bias is acknowledged and that the data are examined with this possibility in mind and interpreted accordingly.

8. BMI IN RELATION TO ENERGY INTAKE AND EXPENDITURE

Before addressing the problem of whether different types of energy-generating nutrients, and particularly fat intake, have different effects on BMI, it was considered important to clarify the nature of the association between the basic elements of the energy balance equation, that is energy intake and energy expenditure, with BMI.

The considerations mentioned in chapter 7.3.1 lead to the decision of excluding all those subjects who were defined as under–reporters and thus restrict the analysis on data from regular reporters. According to the Goldberg single cut-off 1,007 participants, that is 387 men and 620 women are eligible to be included in the analysis. According to the study population specific EI/BMR cut-off points 1,167 participants, that is 451 men and 716 women are eligible to be included in the analysis. In Tables 26, 27, 28 and 29 (Chapter 7.2), mean values and standard deviations of BMI (in kg/m^2), daily energy intake (in kcals) and daily energy expenditure (in MET hours per day) are shown separately for men and women.

Variables which, according to univariate analysis, might confound the association between BMI, energy intake and expenditure are age, sex, tobacco smoking and education. Thus, for this particular analysis body mass index was regressed on age (25-39, 40-54, 55-64, 65+ categorically) total energy intake (in five hundred calories increments, continuously) and energy expenditure (in 5 MET-hours per day increments, continuously); tobacco smoking (current smoker, past smoker, non-smoker, categorically) and education (primary school, secondary school, university school, categorically). The use of increments has been selected for improving readability of results.

Analyses of the selected variables were performed for each sex separately. Tables 37 and 38 present the multiple regression results after under-reporters of energy intake have been excluded from the analysis. Tables 37 and 38 refer to 1,167 and 1,007 regular reporters respectively.

Table 37. Multiple linear regression coefficients for BMI as dependent variable (1167 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Energy intake	0.599	0.175	0.001	0.937	0.213	<0.001
Per 500 kcal						
Energy expenditure	-0.272	0.150	0.075	-0.353	0.205	0.084
Per 5 MET-hours/day						
Smoking status *(baseline current smoker)*						
Past smoker	0.559	0.424	0.188	0.029	0.628	0.963
Non- smoker	0.159	0.414	0.702	0.218	0.430	0.613
Education *(baseline university)*						
Secondary school	0.027	0.404	0.947	1.003	0.439	0.023
Primary school	0.844	0.569	0.139	3.037	0.485	<0.001
Age *(baseline 25-39)*						
40-54	0.520	0.451	0.249	1.879	0.419	<0.001
55-64	1.562	0.639	0.015	3.438	0.588	<0.001
65+	0.755	0.749	0.314	3.824	0.651	<0.001

* *exclusion of under-reporters based on study population specific EI/BMR cut-offs*

Table 38. Multiple linear regression coefficients for BMI as dependent variable (1007 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Energy intake	0.661	0.190	0.001	1.095	0.225	<0.001
Per 500 kcal						
Energy expenditure	-0.290	0.160	0.068	-0.380	0.210	0.075
Per 5 MET-hours/day						
Smoking status *(baseline current smoker)*						
Past smoker	0.699	0.453	0.124	-0.017	0.643	0.979
Non- smoker	0.119	0.445	0.790	0.250	0.449	0.578
Education *(baseline university)*						
Secondary school	0.001	0.422	0.998	0.950	0.449	0.035
Primary school	0.375	0.638	0.557	2.902	0.503	<0.001
Age *(baseline 25-39)*						
40-54	0.277	0.457	0.544	1.680	0.431	<0.001
55-64	1.840	0.682	0.007	4.268	0.618	<0.001
65+	0.865	0.804	0.283	3.505	0.735	<0.001

* *exclusion of under-reporters based on EI/BMR ≤1.1.4*

It is not surprising that the results summarised in Tables 39 and 40 are practically identical because, in fact, data overlap by approximately 90%. According to the results, BMI increases with age but the increase is much steeper among women than among men. There is a positive association between energy intake and BMI, with an 500 kcals increment corresponding to an increase of 0.599 kg/m^2 in men and 0.937 kg/m^2 in women. Concerning energy expenditure there is some indication of inverse association among both men and women, which however remained non-statistically significant. It seems that higher energy intake increases BMI and physical activity reduces BMI. However physical activity is considerably less efficient than reducing energy intake.

Smoking status does not appear to predict BMI neither in men or women when adjusted for the effect of age, energy expenditure and education. Finally, as far as education is concerned, the latter consists of a significant inverse predictor of BMI in women but not in men.

9. BMI IN RELATION TO NUTRIENT INTAKE

As already discussed in chapter 7.3.2, the analysis investigating the association between BMI and nutrient intake will be performed by both including and excluding subjects defined as under-reporters of energy intake.

9.1 Fat intake and passive/active overconsumption

As already discussed in the literature review chapter, two of the most plausible mechanisms that have been proposed for the involvement of fat in the aetiology of obesity are those of passive overconsumption, due to the higher energy density of fatty foods, and active overconsumption which may be due either to the enhanced taste and texture of fat-rich foods or to the weaker satiety and satiation effect of fat. Exploring this hypothesis in an epidemiological set-up is quite challenging. When a nutrient (fat intake) is associated with the outcome under study (obesity) through its effects to total energy intake (increase of total energy intake) the issue of adjusting for energy intake becomes even more complicated. Some authors have suggested that in such cases, one should not adjust for energy intake, since this would result in over-adjusting. In this chapter there will be an effort to explore further the hypothesis suggesting that people who cannot resist fatty foods, tend to consume more energy and thus become overweight. In order to evaluate this hypothesis energy intake should not be adjusted for in the analysis.

For this particular analysis body mass index was modelled as a function of age (25-29, 40-54, 55-64, 65+ categorically), proportion of total grams from fat (continuous), total grams intake - alcohol included - (continuous), energy expenditure (continuous), tobacco smoking (current smoker, past smoker, non-smoker, categorically) and education (primary school, secondary school, university school, categorically). Regression models were estimated using data from the total sample

(1,557 subjects) as well as regular reporters only. According to the study population specific EI/BMR cut-offs these are 1,167 participants, that is 451 men and 716 women. According to the Goldberg single cut-off point these are 1,007 participants, that is that is 387 men and 620 women.

This approach literally follows the statement that overweight persons tend to consume proportionately more fat for a given total amount of grams of energy generating nutrients, by introducing terms for both total grams intake and proportion of total grams from fat. Results from this model applied on the total sample of 1,557 subjects are shown in Table 39.

Table 39. Multiple linear regression coefficients for BMI as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
% Fat, grams	0.021	0.041	0.613	0.139	0.043	0.001
Total grams	0.002	0.001	0.210	0.003	0.002	0.137
Energy expenditure	-0.059	0.028	0.035	-0.096	0.037	0.010
Smoking status *(baseline current smoker)*						
Past smoker	0.335	0.379	0.377	0.057	0.585	0.923
Non- smoker	-0.263	0.368	0.475	0.150	0.402	0.710
Education *(baseline university)*						
Secondary school	0.101	0.357	0.777	1.106	0.416	0.008
Primary school	0.826	0.505	0.102	3.296	0.452	<0.001
Age *(baseline 25-39)*						
40-54	0.728	0.408	0.075	1.882	0.399	<0.001
55-64	0.932	0.574	0.105	3.588	0.536	<0.001
65+	0.006	0.660	0.992	3.257	0.596	<0.001

Tables 40 and 41 present the results after applying the model described above, to those subjects considered to regularly report their dietary intake (that is after excluding under-reporters). Table 40 refers to regular reporters defined by using to study population specific EI/BMR cut-offs and Table 41 refers to regular reporters defined by assuming the Goldberg single cut-off point of 1.14.

Table 40. Multiple linear regression coefficients for BMI as dependent variable (1167 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
% Fat grams	0.053	0.046	0.254	0.116	0.051	0.022
Total grams	0.007	0.002	0.001	0.010	0.002	<0.001
Energy expenditure	-0.056	0.031	0.069	-0.072	0.041	0.077
Smoking status *(baseline current smoker)*						
Past smoker	0.544	0.424	0.200	-0.007	0.627	0.990
Non- smoker	0.105	0.415	0.801	0.197	0.429	0.647
Education *(baseline university)*						
Secondary school	0.043	0.404	0.914	1.009	0.438	0.022
Primary school	0.912	0.574	0.112	3.135	0.488	<0.001
Age *(baseline 25-39)*						
40-54	0.569	0.456	0.212	1.889	0.419	<0.001
55-64	1.638	0.645	0.011	3.525	0.589	<0.001
65+	0.815	0.751	0.278	3.964	0.654	<0.001

* exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 41.Multiple linear regression coefficients for BMI as dependent variable (1007 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
% Fat grams	0.035	0.050	0.479	0.144	0.054	0.008
Total grams	0.007	0.002	<0.001	0.011	0.002	<0.001
Energy expenditure	-0.060	0.032	0.061	-0.076	0.042	0.072
Smoking status *(baseline current smoker)*						
Past smoker	0.690	0.453	0.128	-0.038	0.641	0.953
Non- smoker	0.074	0.446	0.868	0.223	0.447	0.618
Education *(baseline university)*						
Secondary school	0.009	0.422	0.984	0.966	0.447	0.031
Primary school	0.412	0.644	0.522	3.039	0.507	<0.001
Age *(baseline 25-39)*						
40-54	0.296	0.463	0.523	1.701	0.430	<0.001
55-64	1.869	0.687	0.007	3.372	0.618	<0.001
65+	0.902	0.807	0.264	3.654	0.736	<0.001

* exclusion of under-reporters based on EI/BMR ≤1.1.4

Overall, results seem to support the statement that overweight women (but not men) tend to consume proportionately more fat for a given total amount of grams of energy generating nutrients.

9.2 Fat (and other macronutrient) intake in iso-energetic diets

In this chapter there will be an effort to explore further whether energy equivalent amounts of energy-generating nutrients have variable effects on BMI. In order to evaluate this, energy intake should be adjusted for in the analysis. Five of the models that have been described in the Methods and Materials chapter have been performed to account for total energy intake when examining the effect of different macro-nutrient intake on obesity indices (the mathematical expressions of the models have been described in the Materials and Methods chapter).

Adjustment for total energy intake in the multivariate analysis would be sufficient and would compensate for the effect of under-reporting, if there was no problem of systematic bias and all macronutrients were equally under-reported. Since, however, there has been some evidence –although weak- that this is not so, analysis will be performed by both including and excluding under-reporters of energy intake and subsequently comparing the results.

For this particular analysis body mass index was regressed age (25-39, 40-54, 55-64, 65+ categorically), energy expenditure (continuous), tobacco smoking (current smoker, past smoker, non-smoker categorically) and education (primary school, secondary school, university school, categorically). The introduction of nutrient intake and energy intake in the models varies according to the model used for energy adjustment purposes.

9.2.1 Standard multivariate model

The results for different macronutrients, after applying the standard multivariate model among men and women, before and after excluding under-reporters, are summarised in Tables 42-44. In this approach, regression coefficients for nutrients represent the risk associated with 1g increase in particular nutrient when total energy intake is held constant. Total energy intake is essentially a surrogate for 'non-specific nutrient' energy and for this reason it is not interpretable in its original biological meaning.

Table 42. Multiple linear regression coefficients* for BMI as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	0.0005	0.011	0.964	0.045	0.017	0.009
MUF, g	0.012	0.018	0.516	0.072	0.025	0.004
PUF, g	-0.013	0.038	0.727	-0.005	0.046	0.907
SAT, g	-0.018	0.024	0.457	0.027	0.030	0.368
Carbohydrate, g	-0.0003	0.005	0.957	-0.016	0.006	0.010
Protein, g	0.018	0.017	0.305	0.090	0.021	<0.001
Alcohol, g	-0.003	0.009	0.727	-0.076	0.030	0.011

* adjusted for total energy intake, energy expenditure, age, education, smoking status

Table 43.Multiple linear regression coefficients* for BMI as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, g	0.004	0.012	0.748	0.026	0.018	0.145
MUF, g	0.014	0.019	0.467	0.074	0.026	0.004
PUF, g	-0.012	0.038	0.755	-0.078	0.048	0.100
SAT, g	-0.005	0.025	0.848	0.007	0.031	0.830
Carbohydrate, g	0.0005	0.005	0.925	-0.009	0.007	0.167
Protein, g	0.030	0.018	0.109	0.069	0.022	0.002
Alcohol, g	-0.008	0.009	0.361	-0.055	0.028	0.054

*adjusted for total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 44.Multiple linear regression coefficients* for BMI as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, g	0.0002	0.012	0.988	0.027	0.018	0.134
MUF, g	0.014	0.019	0.487	0.071	0.027	0.008
PUF, g	-0.027	0.041	0.506	-0.105	0.051	0.038
SAT, g	-0.015	0.026	0.575	0.023	0.032	0.469
Carbohydrate, g	0.002	0.005	0.670	-0.011	0.007	0.114
Protein, g	0.030	0.019	0.125	0.073	0.023	0.001
Alcohol, g	-0.008	0.009	0.362	-0.049	0.030	0.095

* adjusted for total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

With respect to nutrient intakes, exclusion of individuals who underreport their energy intake, however those have been operationally defined, resulted in some changes in the respective regression coefficients. However, findings remained essentially unchanged. For men none of the macronutrients per-se appear to affect BMI. However, for women it appears that the nutrient most affecting BMI is protein and to a lesser extent monounsaturated fat. It is interesting to note that the significant associations between total fat intake (+) and carbohydrate intake (-) turned weaker and non significant when under-reporters were excluded from the analyses.

9.2.2 Nutrient density model

The results after applying the nutrient density model for men and women, before and after excluding under-reporters are summarised in Tables 45-47. In the nutrient density model, macronutrient intake is expressed as percent of total energy intake; thus regression coefficient represents the risk associated with a difference in 1% of energy from the nutrient with the total energy intake kept constant.

Table 45. Multiple linear regression derived coefficients * for BMI as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, %	0.012	0.030	0.694	0.116	0.033	0.001
MUF %	0.032	0.046	0.477	0.141	0.048	0.004
PUF, %	-0.047	0.096	0.628	0.061	0.086	0.475
SAT, %	-0.008	0.059	0.890	0.102	0.060	0.087
Carbohydrate, %	-0.015	0.027	0.583	-0.096	0.028	0.001
Protein, %	0.065	0.094	0.487	0.386	0.090	<0.001
Alcohol, %	-0.003	0.029	0.916	-0.232	0.085	0.006

* adjusted for total energy intake, energy expenditure, age, education, smoking status

Table 46.Multiple linear regression coefficients* for BMI as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	0.029	0.034	0.392	0.092	0.040	0.021
MUF, %	0.038	0.054	0.476	0.146	0.058	0.012
PUF, %	-0.024	0.105	0.819	-0.124	0.104	0.231
SAT, %	0.058	0.069	0.400	0.118	0.066	0.076
Carbohydrate, %	-0.022	0.031	0.481	-0.067	0.033	0.045
Protein, %	0.082	0.114	0.471	0.273	0.109	0.013
Alcohol, %	-0.013	0.033	0.690	-0.152	0.084	0.071

* adjusted for total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 47.Multiple linear regression coefficients* for BMI as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	0.019	0.037	0.593	0.108	0.043	0.012
MUF, %	0.048	0.059	0.415	0.145	0.063	0.022
PUF, %	-0.089	0.119	0.452	-0.204	0.122	0.095
SAT, %	0.029	0.075	0.696	0.190	0.072	0.009
Carbohydrate, %	-0.008	0.034	0.822	-0.086	0.036	0.017
Protein, %	0.076	0.129	0.552	0.306	0.118	0.010
Alcohol, %	-0.019	0.036	0.597	-0.138	0.092	0.136

* adjusted for total energy intake, energy expenditure, age, education, smoking status
**exclusion of under-reporters based on EI/BMR ≤1.1.4

Exclusion of individuals who underreport their energy intake, however those have been operationally defined, resulted in some changes in the respective regression coefficients. However, findings remained virtually the same suggesting that the nutrient most affecting BMI is protein and to a lesser extent monounsaturated fat and total fat intake. Carbohydrate intake remained consistently inversely associated with BMI. These effects are profound in women but not in men.

9.2.3 Multivariate nutrient density model

In the multivariate nutrient density model, macronutrient intake is expressed as percent of total energy. Inclusion of total energy intake, as a separate term in the model addresses and gives a solution to some of the problems inherent with the use of the somehow complex density term (chapter 5.5.3). The results as those derived after applying the multivariate nutrient density model for men and women, before and after excluding under-reporters are summarised in Tables 48-51.

Table 48. Multiple linear regression derived coefficients* for BMI as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	0.011	0.030	0.714	0.112	0.034	0.001
MUF, %	0.038	0.046	0.415	0.148	0.048	0.002
PUF, %	-0.247	0.113	0.030	0.065	0.086	0.452
SAT, %	0.097	0.083	0.240	0.080	0.062	0.201
Carbohydrate, %	-0.007	0.038	0.855	-0.093	0.028	0.001
Protein, %	0.082	0.095	0.387	0.404	0.091	<0.001
Alcohol, %	-0.085	0.113	0.454	-0.242	0.085	0.005

* adjusted for total energy intake, energy expenditure, age, education, smoking status

Table 49.Multiple linear regression coefficients* for BMI as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	0.026	0.034	0.445	0.074	0.040	0.062
MUF, %	0.048	0.053	0.364	0.169	0.057	0.003
PUF, %	-0.029	0.103	0.778	-0.123	0.102	0.230
SAT, %	0.026	0.069	0.700	0.037	0.069	0.595
Carbohydrate, %	-0.010	0.031	0.747	-0.055	0.033	0.096
Protein, %	0.160	0.114	0.163	0.306	0.108	0.005
Alcohol, %	-0.030	0.033	0.374	-0.159	0.083	0.057

* adjusted for total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 50.Multiple linear regression coefficients* for BMI as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	0.012	0.036	0.735	0.088	0.042	0.039
MUF, %	0.052	0.058	0.373	0.180	0.062	0.004
PUF, %	-0.097	0.117	0.410	-0.026	0.119	0.059
SAT, %	-0.009	0.075	0.910	0.099	0.074	0.185
Carbohydrate, %	0.007	0.033	0.840	-0.072	0.035	0.041
Protein, %	0.177	0.130	0.174	0.350	0.116	0.003
Alcohol, %	-0.035	0.036	0.327	-0.149	0.091	0.099

* adjusted for total energy intake, energy expenditure, age, education, smoking status
**exclusion of under-reporters based on EI/BMR ≤1.1.4

Once more, exclusion of individuals who underreport their energy intake, however those have been operationally defined, resulted in some changes in the respective regression coefficients. It appears that the nutrient most affecting BMI is protein and to a lesser extent monounsaturated fat and total fat intake. This effect is profound in women but not in men.

9.2.4 Nutrient residuals model

The results after applying the nutrient residuals model for men and women, before and after excluding under-reporters are summarised in tables 51-53.

Table 51. Multiple linear regression derived coefficients* for BMI as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	0.0005	0.011	0.964	0.045	0.017	0.009
MUF, g	0.012	0.018	0.516	0.072	0.025	0.004
PUF, g	-0.013	0.038	0.727	-0.005	0.046	0.907
SAT, g	-0.018	0.024	0.457	0.027	0.030	0.368
Carbohydrate, g	-0.0003	0.005	0.957	-0.016	0.006	0.010
Protein, g	0.018	0.017	0.305	0.090	0.021	<0.001
Alcohol, g	-0.003	0.009	0.727	-0.076	0.030	0.011

* adjusted for total energy intake, energy expenditure, age, education, smoking status

Table 52.Multiple linear regression coefficients* for BMI as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	0.004	0.012	0.748	0.026	0.018	0.145
MUF, g	0.014	0.019	0.467	0.074	0.026	0.004
PUF, g	-0.012	0.038	0.755	-0.078	0.048	0.100
SAT, g	-0.005	0.025	0.848	0.007	0.031	0.830
Carbohydrate, g	0.001	0.005	0.925	-0.009	0.007	0.167
Protein, g	0.030	0.018	0.109	0.069	0.022	0.002
Alcohol, g	-0.008	0.009	0.361	-0.055	0.028	0.054

* adjusted for total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 53.Multiple linear regression coefficients* for BMI as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	0.0002	0.012	0.988	0.027	0.018	0.134
MUF, g	0.014	0.019	0.487	0.071	0.027	0.008
PUF, g	-0.027	0.041	0.506	-0.105	0.051	0.038
SAT, g	-0.015	0.026	0.575	0.023	0.032	0.469
Carbohydrate, g	0.002	0.005	0.670	-0.011	0.007	0.114
Protein, g	0.030	0.109	0.125	0.073	0.023	0.001
Alcohol, g	-0.008	0.009	0.362	-0.049	0.030	0.095

* adjusted for total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

An interesting observation is that the results are identical to those of the standard multivariate model. However, in terms of interpretability, since residuals are uncorrelated with energy intake the nutrient residuals model allows direct estimation of the effect of varying a given macronutrient on disease risk at constant total energy intake.

9.2.5 Energy decomposition model

The results from the energy decomposition model among men and women before and after excluding under-reporters of energy intake are summarised in Tables 54-56. These tables show the regression coefficient of BMI on each of the energy generating nutrients in turn, plus the regression coefficient of the sum of the remaining energy generating nutrients (including alcohol). To improve readability, each regression coefficient and the corresponding standard error, were multiplied by 500. In this approach, regression coefficients for nutrients represent the risk associated with 500 kcal increase in fat with non-fat energy held constant (i.e. total energy will vary). Regression coefficient for non-fat energy represents the risk associated with 500 kcal increase in non-fat energy intake with fat-energy held constant i.e. the risk associated with non-fat energy. However, in this particular model it is not adequate simply not to note the nutrient coefficients (standard errors and p values). Focus should be on whether the magnitude of the coefficient for the specific nutrient is actually different from the coefficient for other sources of energy (i.e. $b_1 Cal_F - b_2\ Cal_{X-F}$).

With respect to nutrient intakes, exclusion of individuals who definitely underreport their energy intake resulted in minor changes of the respective regression coefficients. However, findings remained essentially unchanged. Thus, from Tables 54-56, it appears that the nutrient most affecting BMI is protein and to a lesser extent monounsaturated fat and total fat intake. This effect is profound in women but not in men.

Table 54. Multiple linear regression coefficients * for BMI as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, kcal	0.191	0.780	0.607	1.571	0.527	0.003
All the rest	*0.142*	*0.252*	*0.642*	*-0.875*	*0.470*	*0.064*
MUF, kcal	0.709	0.779	0.364	3.418	1.082	0.002
All the rest	*0.018*	*0.253*	*0.943*	*-0.610*	*0.346*	*0.079*
PUF, kcal	-0.450	2.000	0.822	0.050	2.500	0.976
All the rest	*0.200*	*0.200*	*0.269*	*0.300*	*0.200*	*0.199*
SAT, kcal	-0.500	1.000	0.603	1.500	0.003	0.251
All the rest	*0.030*	*0.250*	*0.213*	*0.010*	*0.500*	*0.976*
Carbohydrate, kcal	0.150	0.500	0.716	-1.000	0.500	0.050
All the rest	*0.150*	*0.500*	*0.495*	*1.000*	*0.500*	*0.002*
Protein, kcal	2.126	1.862	0.254	9.940	2.268	<0.001
All the rest	*-0.119*	*0.299*	*0.692*	*-1.235*	*0.389*	*0.002*
Alcohol, kcal	-0.025	0.500	0.963	-5.000	2.000	0.016
All the rest	*0.200*	*0150*	*0.211*	*0.350*	*0.150*	*0.037*

* adjusted for total energy intake, energy expenditure, age, education, smoking status

Table 55.Multiple linear regression coefficients* for BMI as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	0.715	0.416	0.076	1.658	0.500	0.003
All the rest	*0.480*	*0.337*	*0.154*	*0.239*	*0.546*	*0.637*
MUF, kcal	1.244	0.828	0.134	4.158	1.137	<0.001
All the rest	*0.427*	*0.284*	*0.135*	*0.065*	*0.376*	*0.863*
PUF, kcal	0.068	2.000	0.973	-3.025	2.500	0.216
All the rest	*0637*	*0200*	*0.004*	*1.207*	*0.500*	*<0.001*
SAT, kcal	0.463	1.000	0.699	1.294	0.500	0.123
All the rest	*0.627*	*0.500*	*0.020*	*0.876*	*0.500*	*0.019*
Carbohydrate, kcal	0.621	0.500	0.161	0.162	0.500	0.775
All the rest	*0.564*	*0.500*	*0.044*	*1.369*	*0.500*	*<0.001*
Protein, kcal	3.895	2.037	0.057	8.340	2.370	<0.001
All the rest	*0.139*	*0.322*	*0.667*	*-0.237*	*0.421*	*0.575*
Alcohol, kcal	0.060	0.500	0.921	-2.929	2.000	0.146
All the rest	*0.635*	*0150*	*<0.001*	*0.969*	*0.200*	*<0.001*

* adjusted for total energy intake, energy expenditure, age, education, smoking status

** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 56.Multiple linear regression coefficients* for BMI as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	0.664	0.410	0.109	1.858	0.562	0.001
All the rest	*0.629*	*0.357*	*0.079*	*0.342*	*0.525*	*0.514*
MUF, kcal	1.297	0.853	0.130	4.229	1.178	<0.001
All the rest	*0.489*	*0.305*	*0.109*	*0.256*	*0.389*	*0.511*
PUF, kcal	-0.500	2.000	0.760	-4.500	2.500	0.106
All the rest	*1.000*	*0.250*	*0.002*	*1.500*	*0.500*	*<0.001*
SAT, kcal	0.050	1.000	0.956	2.000	1.500	0.126
All the rest	*1.000*	*0.500*	*0.002*	*1.000*	*0.500*	*0.028*
Carbohydrate, kcal	1.000	0.500	0.076	0.200	0.500	0.743
All the rest	*0.500*	*0.500*	*0.058*	*1.500*	*0.500*	*<0.001*
Protein, kcal	3.996	2.155	0.064	8.960	2.440	<0.001
All the rest	*0.203*	*0.339*	*0.552*	*-0.150*	*0.435*	*0.729*
Alcohol, kcal	0.100	0.500	0.861	-2.500	2.000	0.254
All the rest	*0.500*	*0.200*	*<0.001*	*1.000*	*0.200*	*<0.001*

* adjusted for total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

9.2.6 Conclusions

In conclusion, similar inferences have been drawn after data were modelled through different energy adjustment models. Results support the fact that, in women, the nutrient most affecting BMI is protein and to a lesser extent monounsaturated fat, the latter most probably being responsible for an apparent higher efficiency of energy from total fat. Whether this reflects a genuine biological phenomenon or variable patterns of misclassification among nutrients (what is referred to as 'correlation dilution') is not immediately obvious.

10. WIIR, WC IN RELATION TO ENERGY INTAKE AND EXPENDITURE

In this chapter there will be an effort to evaluate the effect on WHR and WC (in cm) of energy intake and physical activity controlling not only for age, gender, education and smoking status, but also for BMI.

Controlling for BMI was considered important in order to allow us to determine whether associations between WHR and WC were independent of overall body size; preliminary analysis indicated that BMI contributed to the majority of the variability in WHR and WC. In one aspect, introduction of BMI among the predictor variables inherently controls for confounding by underreporting, since the consequences of under-reporting reflect the joint association of this characteristic with high BMI and low energy intake. Thus, models based on regular reporters and all participants should be essentially similar.

Analysis was separately done for WHR, because of its popularity, and on WC alone, because it generates more understandable results.

10.1 WHR in relation to energy intake and expenditure

Results on the association between the basic elements of the energy balance equation, that is energy intake and energy expenditure, with WHR, for men and women after under-reporters have been excluded from the analysis are shown in Tables 57 and 58. Table 57 refers to regular reporters defined by using the study population specific EI/BMR cut-off points and Table 58 refers to regular reporters defined by assuming the Goldberg single cut-off point of 1.14.

Table 57. Multiple linear regression coefficients for WHR as dependent variable (1167 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Energy intake	-0.002	0.025	0.520	-0.001	0.002	0.870
Per 500 kcal						
Energy expenditure	-0.004	0.002	0.102	-0.005	0.001	0.049
Per 5 MET-hours/day						
BMI	0.009	0.001	<0.001	0.005	0.001	<0.001
Smoking status *(baseline current smoker)*						
Past smoker	-0.009	0.007	0.183	0.009	0.010	0.348
Non- smoker	-0.009	0.006	0.163	0.006	0.007	0.353
Education *(baseline university)*						
Secondary school	0.008	0.007	0.231	0.003	0.006	0.596
Primary school	0.023	0.009	0.009	0.025	0.007	0.001
Age *(baseline 25-39)*						
40-54	0.050	0.007	<0.001	0.030	0.006	<0.001
55-64	0.059	0.011	<0.001	0.062	0.009	<0.001
65+	0.038	0.013	0.003	0.065	0.010	<0.001

* exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 58 .Multiple linear regression coefficients for WHR as dependent variable (1007 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Energy intake	-0.0001	0.001	0.970	- 0.001	0.004	0.771
Per 500 kcal						
Energy expenditure	-0.005	0.005	0.052	-0.005	0.005	0.079
Per 5 MET-hours/day						
BMI	0.009	0.001	<0.001	0.005	0.001	<0.001
Smoking status *(baseline current smoker)*						
Past smoker	-0.007	0.007	0.335	0.006	0.010	0.522
Non- smoker	-0.006	0.007	0.404	0.005	0.007	0.442
Education *(baseline university)*						
Secondary school	0.008	0.007	0.242	0.004	0.007	0.536
Primary school	0.033	0.010	0.001	0.025	0.008	0.001
Age *(baseline 25-39)*						
40-54	0.050	0.007	<0.001	0.029	0.006	<0.001
55-64	0.059	0.011	<0.001	0.059	0.009	<0.001
65+	0.038	0.013	0.003	0.064	0.011	<0.001

* exclusion of under-reporters based on EI/BMR ≤1.1.4

According to the results WHR increases with age, even after adjustment for the increase in BMI, but the increase is much steeper among women than among men. Additionally, in men there is a peak in the age group of 55-64 followed by a decline in measures. This is not the case for women who are characterised by a continuous increase in WHR measures. There seems to be no association between energy intake and WHR in men or in women. There is an inverse association between energy expenditure and WHR with a 5 METs hour increment corresponding to a decrease of about 0.005 in both men and women after controlling for age, education, smoking status and BMI. It seems that higher physical activity level is associated with lower WHR.

10. 2 WC in relation to energy intake and expenditure

Results on the association between the basic elements of the energy balance equation, that is energy intake and energy expenditure, with WC, for men and women, after under-reporters have been excluded from the analyses are summarised in Tables 59 and 60. Table 59 summarises results on regular reporters defined by using the study population specific EI/BMR cut-offs and Table 60 summarises results on regular reporters defined by assuming the Goldberg single cut-off point of 1.14.

It is interesting to note that although WC increases with age in women, the opposite is the case in men. There seems to be a positive association between energy intake and WC in men but not in women. Accordingly, there seems to be an inverse between energy expenditure and WC in men (with a 5 METs hour increment corresponding to a decrease of about 0.6 cm) but not in women when controlling for age, education, smoking status and BMI.

Table 59.Multiple linear regression coefficients for WC as dependent variable (1167 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Energy intake	0.514	0.500	0.073	0.249	0.500	0.388
Per 500 kcal						
Energy expenditure	-0.505	0.245	0.041	-0.434	0.055	0.112
Per 5 MET-hours/day						
BMI	2.158	0.076	<0.001	1.788	0.050	<0.001
Smoking status *(baseline current smoker)*						
Past smoker	-1.009	0.682	0.139	-0.112	0.838	0.894
Non- smoker	-0.958	0.665	0.150	0.065	0.574	0.910
Education *(baseline university)*						
Secondary school	0.617	0.691	0.372	-0.007	0.588	0.907
Primary school	1.689	0.916	0.066	1.238	0.665	0.063
Age *(baseline 25-39)*						
40-54	4.170	0.725	<0.001	2.296	0.568	<0.001
55-64	5.328	1.033	<0.001	5.427	0.805	<0.001
65+	4.845	1.204	<0.001	5.357	0.891	<0.001

* exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 60.Multiple linear regression coefficients for WC as dependent variable (1007 regular reporters)*

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Energy intake	0.743	0.500	0.020	0.324	0.500	0.298
Per 500 kcal						
Energy expenditure	-0.560	0.260	0.032	-0.435	0.285	0.131
Per 5 MET-hours/day						
BMI	2.165	0.084	<0.001	1.785	0.055	<0.001
Smoking status *(baseline current smoker)*						
Past smoker	-0.959	0.744	0.198	-0.260	0.869	0.765
Non- smoker	-0.877	0.729	0.230	0.009	0.607	0.988
Education *(baseline university)*						
Secondary school	0.617	0.691	0.372	0.005	0.609	0.994
Primary school	2.689	1.044	0.010	1.172	0.698	0.094
Age *(baseline 25-39)*						
40-54	4.100	0.748	<0.001	2.467	0.590	<0.001
55-64	5.174	1.128	<0.001	5.503	0.854	<0.001
65+	3.396	1.318	0.010	5.416	1.012	<0.001

* exclusion of under-reporters based on EI/BMR ≤1.1.4

10.3 Conclusions

In conclusion, it seems that there is evidence that physical activity tends to disproportionately reduce waist circumference rather than hip circumference and therefore, WHR. The effect is more profound in men than in women. This may actually be a hidden reason for the advantageous effect of physical activity on several ill health conditions.

11. WHR AND WC IN RELATION TO NUTRIENT INTAKE

In this chapter there will be an effort to examine the differential effects, if any, of energy generating nutrients on WHR and WC, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI.

Five of the models that have been described in the Methods and Materials chapter have been performed for accounting for total energy intake when examining the effect of different macro-nutrients' intake in WHR and WC. In order to compensate for the problem of any systematic bias in under-reporting, regression models were estimated using data from the total sample (1,557 subjects) as well as from regular reporters only. According to the study population specific cut-offs these are 1,167 participants, that is 451 men and 716 women. According to the Goldberg single cut-off point these are 1,007 participants, that is that is 387 men and 620 women.

11.1 WHR in relation to nutrient intake

11.1.1 Standard Multivariate model

Tables 61-63 summarise the regression coefficients of WHR on each of the energy generating nutrients in turn, after applying the standard multivariate model. Results refer to men and women separately, before and after under-reporters of energy intake have been excluded from the analysis.

With respect to nutrient intakes, exclusion of individuals who definitely underreport their energy intake resulted in some minor changes in the respective regression coefficients. Overall, WHR does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI.

There is some evidence, although weak and not consistent, that protein increases WHR among women and mono-unsaturated fat decreases WHR among men.

Table 61. Multiple linear regression coefficients * for WHR as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, g	-0.0002	0.0002	0.305	0.00003	0.0002	0.885
MUF, g	-0.0003	0.0003	0.248	-0.000003	0.0004	0.992
PUF, g	-0.0004	0.001	0.407	-0.0002	0.001	0.767
SAT, g	-0.0002	0.0004	0.598	0.0002	0.0004	0.556
Carbohydrate, g	-0.00007	0.00007	0.348	-0.00003	0.00009	0.728
Protein, g	0.0004	0.0003	0.100	0.0007	0.0003	0.021
Alcohol, g	0.0002	0.0001	0.144	-0.0005	0.0004	0.244

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 62.Multiple linear regression coefficients* for WHR as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, g	-0.0003	0.0002	0.133	0.00009	0.0003	0.713
MUF, g	-0.0005	0.0003	0.097	-0.00009	0.0004	0.816
PUF, g	-0.0004	0.001	0.498	0.0002	0.001	0.820
SAT, g	-0.0003	0.0004	0.415	0.0004	0.0004	0.362
Carbohydrate, g	-0.00001	0.00008	0.861	-0.00005	0.0001	0.635
Protein, g	0.0004	0.0003	0.137	0.0005	0.0003	0.091
Alcohol, g	0.0002	0.0001	0.224	-0.0003	0.0004	0.438

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 63.Multiple linear regression coefficients* for WHR as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, g	-0.0003	0.0002	0.080	0.00008	0.0003	0.759
MUF, g	-0.0006	0.0003	0.044	-0.00007	0.0004	0.859
PUF, g	-0.0006	0.001	0.357	0.0003	0.001	0.665
SAT, g	-0.0003	0.0004	0.536	0.0003	0.0005	0.508
Carbohydrate, g	-0.00002	0.00008	0.790	-0.00002	0.0001	0.854
Protein, g	0.0005	0.0003	0.104	0.0004	0.0003	0.304
Alcohol, g	0.0002	0.0001	0.133	-00004	0.0004	0.374

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
**exclusion of under-reporters based on EI/BMR ≤1.1.4

11.1.2 Nutrient density model

Tables 64-66 show the regression coefficients of WHR on each of the energy generating nutrients in turn, after applying the nutrient density model. Results refer to men and women before and after under-reporters of energy intake have been excluded from the analysis.

Table 64. Multiple linear regression derived coefficients* for WHR as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	-0.0002	0.001	0.732	0.00007	0.0005	0.879
MUF %	-0.0005	0.001	0.473	-0.0002	0.001	0.818
PUF, %	-0.0007	0.002	0.679	-0.0007	0.001	0.540
SAT, %	-0.0006	0.001	0.573	0.0009	0.001	0.271
Carbohydrate, %	-0.0004	0.0004	0.270	-0.0002	0.0004	0.626
Protein, %	0.002	0.001	0.201	0.003	0.001	0.009
Alcohol, %	0.0006	0.0004	0.176	-0.002	0.001	0.202

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 65.Multiple linear regression coefficients* for WHR as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	-0.001	0.001	0.363	0.001	0.001	0.190
MUF %	-0.0002	0.001	0.125	-0.0002	0.001	0.817
PUF, %	-0.001	0.002	0.778	-0.0001	0.002	0.942
SAT, %	-0.001	0.001	0.190	0.001	0.001	0.227
Carbohydrate, %	0.00003	0.001	0.955	-0.0004	0.001	0.428
Protein, %	0.002	0.002	0.255	0.003	0.002	0.032
Alcohol, %	0.0006	0.001	0.265	-0.001	0.001	0.360

* adjusted for BMI, total energy intake, energy expenditure, education, smoking status

** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 66.Multiple linear regression coefficients* for WHR as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	-0.001	0.001	0.142	0.0009	0.001	0.175
MUF %	-0.002	0.001	0.019	-0.0001	0.001	0.886
PUF, %	-0.002	0.002	0.444	0.00004	0.002	0.985
SAT, %	-0.001	0.001	0.339	0.001	0.001	0.360
Carbohydrate, %	-0.0001	0.001	0.814	-0.0002	0.001	0.670
Protein, %	0.003	0.002	0.205	0.002	0.002	0.182
Alcohol, %	0.001	0.001	0.067	-0.002	0.001	0.262

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

With respect to nutrient intakes, exclusion of individuals who definitely underreport their energy intake resulted in some minor changes in the respective regression coefficients but the results remained virtually unchanged. Overall, WHR does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI. There has been some evidence that needs to be replicated, that protein increases WHR among women.

11.1.3 Multivariate nutrient density model

Tables 67-69 summarise regression coefficients of WHR on each of the energy generating nutrients in turn, after applying the multivariate nutrient density model. Results refer to men and women before and after under-reporters of energy intake have been excluded from the analysis.

Table 67. Multiple linear regression derived coefficients* for WHR as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	-0.0002	0.001	0.718	0.001	0.001	0.313
MUF, %	-0.0005	0.001	0.484	-0.0001	0.001	0.898
PUF, %	-0.001	0.002	0.684	-0.0004	0.001	0.738
SAT, %	-0.001	0.001	0.538	0.001	0.001	0.443
Carbohydrate, %	-0.0004	0.0004	0.280	-0.0002	0.0004	0.694
Protein, %	0.002	0.001	0.144	0.004	0.001	0.006
Alcohol, %	0.001	0.0004	0.183	-0.002	0.001	0.173

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 68.Multiple linear regression coefficients* for WHR as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	-0.001	0.001	0.345	0.001	0.001	0.236
MUF, %	-0.001	0.001	0.115	-0.0002	0.001	0.803
PUF, %	-0.001	0.002	0.765	-0.0001	0.002	0.945
SAT, %	-0.001	0.001	0.217	0.001	0.001	0.190
Carbohydrate, %	-0.00001	0.001	0.988	-0.0004	0.0004	0.420
Protein, %	0.002	0.002	0.302	0.003	0.002	0.033
Alcohol, %	0.001	0.001	0.222	-0.001	0.001	0.362

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 69.Multiple linear regression coefficients* for WHR as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat %	-0.001	0.001	0.115	0.001	0.001	0.219
MUF, %	-0.002	0.001	0.019	0.0002	0.001	0.885
PUF, %	-0.002	0.002	0.425	-0.0001	0.002	0.976
SAT, %	-0.001	0.001	0.337	0.001	0.001	0.301
Carbohydrate, %	-0.0001	0.001	0.809	-0.0002	0.001	0.655
Protein, %	0.003	0.002	0.198	0.002	0.002	0.190
Alcohol, %	0.001	0.001	0.064	-0.001	0.001	0.267

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

Once again, WHR does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI. There is some evidence, that protein increases WHR among women.

11.1.4 Nutrient residuals model

Tables 70-72 summarise the regression coefficients of WHR on each of the energy generating nutrients in turn, after applying the energy adjusted model. Results refer to men, and women, before and after under-reporters of energy intake have been excluded from the analyses.

Table 70. Multiple linear regression derived coefficients* for WHR as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	-0.0002	0.0002	0.305	0.00003	0.0002	0.885
MUF, g	-0.0003	0.0003	0.248	-0.000003	0.0004	0.992
PUF, g	-0.0004	0.001	0.407	-0.0002	0.001	0.767
SAT, g	-0.0002	0.0004	0.598	0.0002	0.0004	0.556
Carbohydrate, g	-0.00007	0.00007	0.348	-0.00003	0.00009	0.728
Protein, g	0.0004	0.0003	0.100	0.001	0.0003	0.021
Alcohol, g	0.0002	0.0001	0.144	-0.001	0.0004	0.244

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 71.Multiple linear regression coefficients* for WHR as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	SE	P	β	SE	P
Fat g	-0.0003	0.0002	0.133	0.0001	0.0003	0.713
MUF, g	-0.001	0.0003	0.097	-0.0001	0.0004	0.816
PUF, g	-0.0004	0.001	0.498	0.0002	0.001	0.820
SAT, g	-0.0003	0.0004	0.415	0.0004	0.0004	0.362
Carbohydrate, g	-0.00001	0.00008	0.861	-0.00005	0.0001	0.635
Protein, g	0.0004	0.0003	0.137	0.001	0.0003	0.091
Alcohol, g	0.0002	0.0001	0.224	-0.0003	0.0004	0.438

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 72.Multiple linear regression coefficients* for WHR as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	-0.0003	0.0002	0.080	0.0001	0.0003	0.759
MUF, g	-0.001	0.0003	0.044	-0.00011	0.00041	0.859
PUF, g	-0.001	0.001	0.357	0.0003	0.001	0.665
SAT, g	-0.0003	0.0004	0.536	0.0003	0.001	0.508
Carbohydrate, g	-0.00002	0.00008	0.790	-0.00002	0.0001	0.854
Protein, g	0.0005	0.0003	0.104	0.0004	0.0003	0.304
Alcohol, g	0.0002	0.0001	0.133	-00004	0.0004	0.374

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

The results are identical to those of the standard multivariate model.

11.1.5 Energy decomposition model

The results from the energy decomposition model among men and women, before and after under-reporters have been excluded from the analysis are summarised in Tables 73-75. These tables show the regression coefficient of WHR on each of the energy generating nutrients in turn, plus the regression coefficient of the sum of the remaining energy generating nutrients (including alcohol). To improve readability, each regression coefficient and the corresponding standard error, per kcal, was multiplied by 500.

With respect to nutrient intakes, WHR does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI.

Table 73. Multiple linear regression coefficients * for WHR as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	-0.005	0.005	0.393	0.004	0.007	0.608
All the rest	*0.005*	*0.005*	*0.318*	*0.002*	*0.005*	*0.755*
MUF, kcal	-0.010	0.010	0.306	0.003	0.015	0.871
All the rest	*0.004*	*0.004*	*0.318*	*0.003*	*0.005*	*0.514*
PUF, kcal	-0.020	0.030	0.532	-0.005	0.035	0.828
All the rest	*0.002*	*0.003*	*0.577*	*0.004*	*0.004*	*0.246*
SAT, kcal	-0.005	0.015	0.664	0.015	0.020	0.478
All the rest	*0.002*	*0.004*	*0.629*	*0.001*	*0.005*	*0.854*
Carbohydrate, kcal	0.005	0.006	0.418	0.0003	0.005	0.975
All the rest	*0.004*	*0.004*	*0.363*	*0.005*	*0.045*	*0.364*
Protein, kcal	0.045	0.027	0.096	0.100	0.030	0.018
All the rest	*-0.005*	*0.005*	*0.169*	*-0.009*	*0.005*	*0.120*
Alcohol, kcal	0.015	0.010	0.140	-0.030	0.030	0.288
All the rest	*-0.001*	*0.002*	*0.784*	*-0.025*	*0.003*	*0.149*

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 74.Multiple linear regression coefficients* for WHR as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	-0.009	0.009	0.106	0.002	0.005	0.794
All the rest	*0.005*	*0.005*	*0.349*	*-0.003*	*0.005*	*0.703*
MUF, kcal	-0.022	0.015	0.089	-0.005	0.015	0.772
All the rest	*0.004*	*0.005*	*0.424*	*0.001*	*0.005*	*0.890*
PUF, kcal	-0.022	0.030	0.479	0.007	0.035	0.846
All the rest	*0.001*	*0.004*	*0.848*	*-0.001*	*0.004*	*0.816*
SAT, kcal	-0.015	0.019	0.389	0.020	0.020	0.404
All the rest	*0.001*	*0.004*	*0.909*	*-0.004*	*0.005*	*0.434*
Carbohydrate, kcal	-0.003	0.005	0.684	-0.004	0.010	0.609
All the rest	*0.001*	*0.005*	*0.785*	*0.002*	*0.005*	*0.749*
Protein, kcal	0.045	0.030	0.152	0.058	0.035	0.099
All the rest	*-0.008*	*0.005*	*0.108*	*-0.009*	*0.006*	*0.127*
Alcohol, kcal	0.010	0.010	0.328	-0.023	0.030	0.430
All the rest	*-0.003*	*0.003*	*0.320*	*-0.0002*	*0.008*	*0.962*

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 75.Multiple linear regression coefficients* for WHR as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	-0.010	0.007	0.121	0.002	0.005	0.880
All the rest	*0.008*	*0.006*	*0.152*	*-0.003*	*0.005*	*0.702*
MUF, kcal	-0.025	0.015	0.056	-0.005	0.015	0.789
All the rest	*0.005*	*0.005*	*0.150*	*0.0001*	*0.040*	*0.983*
PUF, kcal	-0.030	0.035	0.369	0.015	0.040	0.702
All the rest	*0.002*	*0.004*	*0.650*	*-0.002*	*0.005*	*0.650*
SAT, kcal	-0.010	0.020	0.560	0.010	0.020	0.574
All the rest	*0.002*	*0.005*	*0.704*	*-0.004*	*0.005*	*0.518*
Carbohydrate, kcal	-0.002	0.005	0.800	-0.003	0.005	0.773
All the rest	*0.001*	*0.005*	*0.870*	*-0.00003*	*0.005*	*0.996*
Protein, kcal	0.050	0.035	0.104	0.035	0.035	0.327
All the rest	*-0.005*	*0.005*	*0.167*	*-0.005*	*0.005*	*0.311*
Alcohol, kcal	0.015	0.009	0.157	-0.030	0.030	0.360
All the rest	*-0.002*	*0.003*	*0.627*	*-0.001*	*0.004*	*0.876*

* adjusted for BMI total energy intake, energy expenditure, age, education, smoking status
**exclusion of under-reporters based on EI/BMR ≤1.1.4

11.2 WC in relation to nutrient intake

11.2.1 Standard multivariate model

Tables 76-78 summarise the regression coefficients of WC on each of the energy generating nutrients in turn, after applying the standard multivariate model. Results refer to men and women, before and after data on under-reporters have been excluded from the analysis.

With respect to nutrient intakes, WC does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI. There has been some evidence that mono-unsaturated fat intake reduces WC in men.

Table 76. Multiple linear regression derived coefficients * for WC dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	-0.019	0.017	0.269	-0.003	0.023	0.888
MUF, g	-0.050	0.027	0.064	-0.00004	0.033	0.999
PUF, g	-0.023	0.057	0.683	-0.098	0.061	0.106
SAT, g	-0.002	0.036	0.948	0.036	0.040	0.371
Carbohydrate, g	0.003	0.007	0.692	-0.001	0.009	0.939
Protein, g	0.042	0.026	0.104	0.033	0.028	0.247
Alcohol, g	0.003	0.013	0.832	-0.015	0.039	0.714

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 77.Multiple linear regression coefficients* for WHR as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	-0.028	0.019	0.146	-0.013	0.024	0.572
MUF, g	-0.064	0.030	0.034	-0.025	0.035	0.467
PUF, g	-0.017	0.062	0.786	-0.054	0.064	0.395
SAT, g	-0.017	0.040	0.675	0.022	0.041	0.583
Carbohydrate, g	0.006	0.008	0.439	0.003	0.009	0.761
Protein, g	0.047	0.030	0.286	0.021	0.030	0.462
Alcohol, g	0.003	0.014	0.845	-0.002	0.038	0.968

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 78.Multiple linear regression coefficients* for WHR as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	-0.032	0.020	0.105	-0.018	0.025	0.453
MUF, g	-0.078	0.032	0.014	-0.036	0.037	0.323
PUF, g	-0.027	0.067	0.690	-0.033	0.069	0.628
SAT, g	-0.007	0.043	0.870	0.013	0.043	0.753
Carbohydrate, g	0.005	0.008	0.584	0.005	0.009	0.553
Protein, g	0.059	0.032	0.066	0.003	0.031	0.929
Alcohol, g	0.0002	0.000	0.133	0.008	0.015	0.609

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

11.2.2 Nutrient density model

Tables 79-81 summarise the regression coefficients of WC on each of the energy generating nutrients in turn, after applying the nutrient density model. Results refer to men and women before and after under-reporters of energy intake have been excluded from the analysis.

Table 79. Multiple linear regression derived coefficients * for WC as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, %	-0.035	0.046	0.443	0.004	0.045	0.930
MUF, %	-0.107	0.070	0.129	0.009	0.064	0.886
PUF, %	-0.028	0.147	0.851	-0.250	0.113	0.027
SAT, %	0.013	0.091	0.885	0.123	0.079	0.121
Carbohydrate, %	-0.003	0.042	0.944	-0.011	0.038	0.769
Protein, %	0.153	0.144	0.290	0.175	0.122	0.150
Alcohol, %	0.022	0.045	0.629	-0.073	0.113	0.517

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 80.Multiple linear regression coefficients* for WC as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, %	-0.075	0.054	0.168	-0.002	0.053	0.964
MUF, %	-0.167	0.085	0.051	-0.052	0.077	0.498
PUF, %	0.009	0.166	0.957	-0.107	0.137	0.434
SAT, %	-0.063	0.109	0.561	0.115	0.088	0.192
Carbohydrate, %	0.029	0.050	0.569	-0.008	0.044	0.863
Protein, %	0.181	0.181	0.318	0.163	0.145	0.262
Alcohol, %	0.024	0.053	0.652	-0.032	0.112	0.778

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 81.Multiple linear regression coefficients* for WC as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, %	-0.097	0.060	0.105	-0.015	0.057	0.800
MUF, %	-0.257	0.095	0.007	-0.097	0.085	0.252
PUF, %	0.008	0.193	0.968	-0.033	0.162	0.840
SAT, %	-0.009	0.123	0.942	0.111	0.097	0.252
Carbohydrate, %	0.001	0.054	0.982	0.010	0.048	0.842
Protein, %	0.248	0.210	0.238	0.036	0.158	0.820
Alcohol, %	0.068	0.058	0.233	-0.015	0.123	0.906

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

Once again, with respect to nutrient intakes, WC does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI. There has been some evidence that monounsaturated fat intake decrease BMI in men and poly-unsaturated fat intake reduces WC in women.

11.2.3 Multivariate nutrient density model

Tables 82-84 summarise regression coefficients of WC on each of the energy generating nutrients in turn, after applying the multivariate nutrient density model. Results refer to men and women before and after under-reporters of energy intake have been excluded from the analysis.

Once again, with respect to nutrient intakes, WC does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI. There has been some evidence that mono-unsaturated fat intake reduces WC in men and poly-unsaturated fat intake reduces WC in women.

Table 82. Multiple linear regression derived coefficients * for WC as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, %	-0.037	0.045	0.410	-0.002	0.045	0.961
MUF, %	-0.092	0.070	0.190	0.017	0.064	0.792
PUF, %	-0.025	0.146	0.863	-0.247	0.113	0.030
SAT, %	-0.030	0.092	0.746	0.097	0.083	0.240
Carbohydrate, %	0.011	0.042	0.785	-0.007	0.038	0.855
Protein, %	0.206	0.145	0.155	0.196	0.122	0.110
Alcohol, %	0.004	0.045	0.928	-0.085	0.113	0.454

* adjusted for BMI, total energy intake, energy expenditure, education, smoking status

Table 83.Multiple linear regression coefficients* for WC as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, %	-0.077	0.054	0.154	-0.007	0.053	0.897
MUF, %	-0.158	0.085	0.064	-0.046	0.077	0.553
PUF, %	-0.004	0.166	0.981	-0.108	0.137	0.432
SAT, %	-0.091	0.110	0.406	0.101	0.092	0.270
Carbohydrate, %	0.039	0.050	0.438	-0.005	0.044	0.913
Protein, %	0.254	0.184	0.168	0.175	0.146	0.231
Alcohol, %	0.010	0.054	0.845	-0.034	0.112	0.758

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 84.Multiple linear regression coefficients* for WC as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, %	-0.105	0.059	0.078	-0.020	0.057	0.729
MUF, %	-0.252	0.095	0.008	-0.087	0.085	0.308
PUF, %	-0.003	0.192	0.986	-0.042	0.162	0.797
SAT, %	-0.052	0.123	0.676	0.090	0.101	0.372
Carbohydrate, %	0.017	0.055	0.753	0.013	0.048	0.788
Protein, %	0.373	0.213	0.081	0.053	0.159	0.741
Alcohol, %	0.053	0.059	0.370	-0.020	0.123	0.873

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

11.2.4 Nutrient residuals model

The results from different macronutrients from the energy adjusted model among men and women before and after under-reporters of energy intake have been excluded from the analysis are summarised in tables 85-87. The results are practically identical to those of the standard multivariate model.

Table 85. Multiple linear regression derived coefficients * for WC as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, g	-0.019	0.017	0.269	-0.003	0.023	0.888
MUF, g	0.050	0.027	0.064	-0.00004	0.033	0.999
PUF, g	-0.023	0.057	0.683	-0.098	0.061	0.106
SAT, g	-0.002	0.036	0.948	0.036	0.040	0.371
Carbohydrate, g	0.003	0.007	0.692	-0.001	0.009	0.939
Protein, g	0.042	0.026	0.104	0.033	0.028	0.247
Alcohol, g	0.003	0.013	0.832	-0.015	0.039	0.714

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 86.Multiple linear regression coefficients* for WC as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat, g	-0.032	0.020	0.105	-0.018	0.025	0.453
MUF, g	-0.064	0.030	0.034	-0.025	0.035	0.467
PUF, g	-0.027	0.067	0.690	-0.033	0.069	0.628
SAT, g	-0.007	0.043	0.870	0.013	0.043	0.753
Carbohydrate, g	0.005	0.008	0.584	0.005	0.009	0.553
Protein, g	0.059	0.032	0.066	0.021	0.030	0.462
Alcohol, g	0.0002	0.000	0.133	0.008	0.015	0.609

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 87.Multiple linear regression coefficients* for WC as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat g	-0.028	0.019	0.146	-0.013	0.024	0.572
MUF, g	-0.078	0.032	0.014	-0.036	0.037	0.323
PUF, g	-0.017	0.062	0.786	-0.054	0.064	0.395
SAT, g	-0.017	0.040	0.675	0.022	0.041	0.583
Carbohydrate, g	0.006	0.008	0.439	0.003	0.009	0.761
Protein, g	0.047	0.030	0.286	0.003	0.031	0.920
Alcohol, g	0.003	0.014	0.845	-0.002	0.038	0.968

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

Once again, with respect to nutrient intakes, WC does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI. There has been some evidence that mono-unsaturated fat intake reduces WC in men.

11.2.5 Energy decomposition model

Tables 88-90 summarise the regression coefficient of WC on each of the energy generating nutrients in turn, plus the regression coefficient of the sum of the remaining energy generating nutrients (including alcohol). To improve readability, each regression coefficient and the corresponding standard error, per kcal, was multiplied by 500.

Table 88. Multiple linear regression derived coefficients* for WC as dependent variable (1557 subjects)

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	-0.045	0.500	0.936	0.200	0.500	0.750
All the rest	*1.000*	*0.500*	*0.034*	*0.400*	*0.500*	*0.524*
MUF, kcal	-1.495	1.189	0.209	0.300	1.500	0.827
All the rest	*1.098*	*0.385*	*0.005*	*0.350*	*0.500*	*0.467*
PUF, kcal	-0.500	3.000	0.861	-5.000	3.000	0.131
All the rest	*0.500*	*0.500*	*0.032*	*0.640*	*0.500*	*0.031*
SAT, kcal	0.500	1.500	0.735	2.000	2.000	0.293
All the rest	*0.500*	*0.500*	*0.140*	*0.005*	*0.500*	*0.989*
Carbohydrate, kcal	0.100	0.500	0.226	0.250	0.500	0.719
All the rest	*0.400*	*0.500*	*0.288*	*0.350*	*0.500*	*0.451*
Protein, kcal	5.140	2.841	0.071	3.816	3.056	0.212
All the rest	*-0.130*	*0.457*	*0.774*	*-0.227*	*0.521*	*0.664*
Alcohol, kcal	0.500	1.000	0.414	-0.500	3.000	0.802
All the rest	*0.500*	*0.200*	*0.019*	*0.350*	*0.200*	*0.135*

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

Table 89.Multiple linear regression coefficients* for WC as dependent variable (1167 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	-0.337	0.500	0.602	-0.140	0.500	0.849
All the rest	*1.172*	*0.500*	*0.031*	*0.596*	*0.500*	*0.380*
MUF, kcal	-2.114	1.326	0.112	-0.870	1.541	0.572
All the rest	*1.226*	*0.457*	*0.008*	*0.567*	*0.505*	*0.262*
PUF, kcal	-0.235	3.000	0.942	-2.613	3.500	0.434
All the rest	*0.557*	*0.500*	*0.116*	*0.443*	*0.500*	*0.221*
SAT, kcal	0.157	2.000	0.935	1.216	2.000	0.516
All the rest	*0.624*	*0.500*	*0.602*	*0.050*	*0.500*	*0.920*
Carbohydrate, kcal	1.003	0.500	0.159	0.449	1.000	0.553
All the rest	*0.235*	*0.500*	*0.602*	*0.123*	*0.500*	*0.810*
Protein, kcal	5.685	3.284	0.084	2.554	3.213	0.427
All the rest	*-0.186*	*0.517*	*0.720*	*0.109*	*0.566*	*0.848*
Alcohol, kcal	0.690	1.000	0.480	0.140	2.500	0.959
All the rest	*0.485*	*0.500*	*0.100*	*0.247*	*0.500*	*0.386*

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status
** exclusion of under-reporters based on study population specific EI/BMR cut-offs

Table 90.Multiple linear regression coefficients* for WC as dependent variable (1007 regular reporters)**

Predictor variables	Men			Women		
	β	*SE*	*P*	*β*	*SE*	*P*
Fat kcal	-0.250	0.500	0.729	-0.200	1.000	0.789
All the rest	*1.500*	*0.500*	*0.010*	*0.800*	*0.700*	*0.262*
MUF, kcal	-2.500	1.392	0.078	-1.500	1.500	0.437
All the rest	*1.500*	*0.497*	*0.001*	*0.750*	*0.550*	*0.149*
PUF, kcal	-0.500	3.500	0.886	-1.500	3.500	0.686
All the rest	*1.000*	*0.500*	*0.037*	*0.450*	*0.500*	*0.249*
SAT, kcal	0.500	2.000	0.789	1.000	2.000	0.652
All the rest	*1.000*	*0.500*	*0.105*	*0.200*	*0.500*	*0.681*
Carbohydrate, kcal	1.000	1.000	0.140	0.500	1.000	0.346
All the rest	*0.500*	*0.500*	*0.264*	*0.050*	*0.500*	*0.906*
Protein kcal	7.240	3.538	0.041	0.596	3.364	0.859
All the rest	*-0.100*	*0.556*	*0.824*	*0.278*	*0.593*	*0.640*
Alcohol, kcal	1.000	1.000	0.229	0.500	3.000	0.839
All the rest	*0.650*	*0.300*	*0.039*	*0.300*	*0.500*	*0.306*

* adjusted for BMI, total energy intake, energy expenditure, age, education, smoking status

**exclusion of under-reporters based on EI/BMR ≤1.1.4

With respect to nutrient intakes, WC does not seem to be differentially affected by energy equivalent amounts of energy generating nutrients, after controlling for energy intake, energy expenditure, age, gender, education, smoking status as well as BMI.

SECTION IV. DISCUSSION AND CONCLUSIONS

12. General discussion

The current investigation sought to evaluate the relationships between dietary intake and obesity, after controlling for potential confounding factors such as age, sex, physical activity, education, smoking status, in a cross-sectional design. It is a fairly large study involving 1,557 apparently healthy adults. As in virtually all clinical and epidemiological studies of this nature, ethical considerations limit the study sample participants to volunteers but, since there has been a conscious effort to include subjects who reflect a wide range of social-demographic parameters, none of the plausible selection factors can be thought of as interacting with any of the associations under investigation. Demonstration of well-known associations of obesity indices with gender, age, and some environmental factors like educational status (see below), argues in favor of the validity of other study results. However, the all-but-unavoidable utilization of a cross-sectional design for the study of these relations in a large dataset imposes great caution in the interpretation of the results.

12.1 Assessment of energy balance components

As far as the associations between the key variables of this study are concerned, it is important to note that this study can not completely overcome problems which are inherent in the epidemiologic investigation of the association between dietary intake on the one hand and obesity indices on the other (Lissner & Heitmann, 1995, Margetts & Nelson 1997, Willett, 1998, Freudenheim, 1999). The most basic methodological constraint involves the difficulty of measuring habitual energy intake. It is indeed very difficult to find a dietary assessment tool that can be efficiently used in a large population and which is still highly reliable and valid. The estimates of individual dietary intake in the present study have been

derived from a semi-quantitative FFQ and it is assumed to represent habitual energy and nutrient intake. The features as well as the advantages, limitations and potential sources of measurement error in FFQ have been discussed previously by several investigators (Briefel et al, 1992, Bingham et al, 1994, Willett, 1998d, Nelson & Bingham, 1997, Sempos et al, 1999). The FFQ used in the current study has been validated against twelve 24-hour recalls and biochemical markers, and, as has already been extensively discussed in the methods section, it provides a reasonably reliable measure of intake for the nutrients which are relevant to the current investigation (Chapter 4.1.1.1). Moreover, the questionnaire was administered by specially trained and experienced interviewers, which is believed to improve further data quality. The later notion has been supported by recent scientific evidence suggesting that nutritionist review of a self-administered food frequency questionnaire improves data quality (Caan et al, 1999). Moreover, the food composition tables used for the calculation of energy and nutrient intake may have introduced misclassification errors in the analysis, but these are unlikely to threaten the validity of the results, except by introducing underestimation of the observed associations.

The energy expenditure questionnaire has not been formally validated, but assessment of physical activity has relied on a detailed set of questions covering type, frequency, intensity and duration of a broad range of activities. One potential source of misclassification concerns accuracy in describing the frequency, intensity or duration of different physical activity components. Since, however, calculation of METs is the grand sum of several weighted activities, it is unlikely that there would be biases towards the same direction throughout them, particularly because the total time of the average day provides a built-in constraint. The effect of a random misclassification, would be to reduce the ability to control for its confounding effect as a covariate and would tend to cause an underestimation of the observed associations.

12.2 Assessment of obesity

Another important issue is that of obesity classification. In the present analysis general obesity was assessed through BMI and central adiposity was assessed through both WHR and WC. These were treated both as continuous as well as binary or categorical variables. Both analytical approaches are considered important, since they provided different insights into the problem. The use of continuous variables takes advantage of the richer information offered by continuous data, whereas graded classification of obesity is valuable for a number of reasons such as: a) it allows meaningful comparisons within and between populations; b) it facilitates identification of individuals and groups at increased risk of morbidity and mortality; c) it helps identifying priorities at individual and community intervention; and finally d) provides a firm basis for evaluating interventions (WHO Report on Obesity, 1997).

It has been obvious from the introduction as well as the methods sections, that besides BMI, about which there has been a relative consensus on its validity for classifying general adiposity (WHO, Report on Physical Status, 1995), there is a considerable lack of consistency concerning anthropometric indicators of abdominal obesity. The latter issue is critical, considering that the importance of abdominal obesity as a risk factor for cardiovascular diseases, hypertension, stroke and non-insulin dependent diabetes, has been broadly recognised since the 1980s and onwards (Molarius & Seidell, 1998). During the last few years particular emphasis has been given to the hazardous effect of visceral fat accumulation (James & Ralph, 1999). The latter fact combined with the evidence that WC has been proven to be strongly correlated with visceral fat as measured through computed tomography (Despres et al, 1991, Pouliot et al, 1994) has led to the suggestion that WC makes a suitable choice for the 'optimal' indicator of risk for abdominal adiposity. The latter suggestion was raised questions and a central issue concerning the central role of visceral fat in disease aetiology. In a critical review undertaken by Seidell and Bouchard (Seidell & Bouchard, 1997), the authors concluded that the evidence is rather circumstantial and far from

conclusive. They argued that visceral fat may be more important for some diseases such as NIDDM (Carey et al, 1997) and stroke (Walker et al, 1996) whereas general adiposity may be an important risk factor for some others, such as cardiovascular diseases (Rimm et al, 1995) and general mortality (Folshom et al, 1993). The issue is complicated and no clear answers seem to be available. A systematic evaluation of the proposed indicators which would take into account possible differences between genders, age groups, ethnic groups as well as differences in morbidity and mortality is needed. Universal recommendation for an optimal indicator for abdominal obesity would be very helpful in terms of public health recommendations and interpretation of epidemiological data.

Similar considerations to the ones mentioned above relate to the use of appropriate cut-off points to determine abdominal obesity. It must be noted that the selection of cut-off points is particularly problematic, because the risk of disease often increases gradually, which implies that selection of specific cut-off points may be rather 'arbitrary'. This is actually the case for the suggested cut-off points for the WHR (Molarius & Seidell, 1997). These have been based on rather arbitrary criteria, whilst they have derived from a small number of subjects in the context of cross-sectional investigations (Bjorntrop, 1985, Bray, 1987). The cut-off points suggested for WC have the advantage that the authors have attempted to justify the selected cut-off points (Lean et al, 1995, Lemieux et al, 1996). However, there are methodological shortcomings since these cut-off points have been based on other arbitrary classifications, such as the WHR classification (Lean et al, 1995) or visceral fat 'critical amount' (Lemieux et al, 1996).

In conclusion, it is obvious that there is an urgent need to put the diversity of anthropometric indicators as well as the cut-off points, currently suggested and used, into perspective. A more comprehensive assessment of possible indicators is needed to derive an optimal indicator for abdominal obesity. Such an effort requires: a) better understanding of the different roles of abdominal and general adiposity ; b) prospective studies, large enough to test possible differences

between genders, age categories and ethnic groups; as well as c) intervention studies to reveal whether proposed indicators and cut-off points are responsive to change by lifestyle or other interventions, since a reduction in the indicators should be able to predict a reduction at risk (Molarius & Seidell, 1998, Bouchard et al, 1990).

12.3 Assessment of confounding variables

Concerning control for factors which may confound the associations between dietary intake and obesity, we applied eligibility criteria when defining our sample so as to exclude pregnant women or individuals on diets of any type, so as to be able to assume weight stability. We also controlled for the potential confounding effects of age, physical activity, cigarette smoking, and education in the analysis. It should be noted however that this study is limited by a lack of consideration for the strong familial and genetic factors that are known to be implicated in the aetiology of obesity.

Current results supported what has been previously shown (Seidell, 1995, James, 1996, Seidell & Flegal, 1997) that is, obesity is more common among women and the percentage of women relative to men, increases with severity of obesity. Thus, in the age groups 40-65 the prevalence of obesity (BMI≥30) has been higher among women than among men, although overweight (BMI=25-29.9) tends to be more common among men than among women of the same age (Table 15, Chapter 6.2). Also, mean BMI was found to increase with age in both men and women. Trends in WHR and WC were similar to trends in BMI. A slight decrease in mean BMI and mean values of central obesity indicators was observed in men but not in women over 65 years of age (Tables 11-12, Chapter 6.1). These results are consistent with those reported from other studies (Prescott-Clarke & Primatesta, 1998). The age-related increase in obesity is associated with a number of changes in body composition, including reduction in lean body mass

and increase in body fat which all have been well documented (Evans & Cyr-Campbell, 1997). Increased obesity in older ages is also likely to be promoted by the fact that most people reduce the frequency, duration and intensity of physical activity very considerably as they age (Prentice, 1992). The latter notion has actually been confirmed by our data (Table 16, Chapter 6.2). The most profound increase in obesity prevalence noticed in women over the age of 55 may well imply menopausal status effects. It has been well documented that menopause is a high-risk time for weight gain in women. Although the average woman gains about 1 to 3 kg during menopausal transition, some women are at risk for greater weight gains (Lovejoy, 1998).

Moreover, crude data suggested that the prevalence of obesity is usually inversely related to educational level. The association was more consistent among women than among men (Table 20, Chapter 6.2). In the regression models, however, lower educational level was a predictor of obesity for women but not for men. This is consistent with findings suggesting that in other European countries, obesity is usually inversely associated with education and socio-economic status, particularly among women (Seidell, 1995, Sorensen, 1995, Seidell & Flegal, 1997). One of the plausible reasons for the observed inverse association is the 'unhealthy' dietary patterns that are most commonly adopted by people at lower socio-economic and education groups. These frequently provide cheap energy dense foods such as meat products, full cream milk, fats, sugar, preserves, potatoes and cereals which most probably lead to increased adiposity compared to dietary patterns characterized with increased consumption of vegetables, fruit and wholemeal bread, which are commonly adopted by higher socio-economic and education groups (James et al, 1997). Further to the 'non healthy' dietary patterns adopted by less educated individuals, there has been evidence suggesting that physical inactivity in modern societies is more common among lower socio-economic and education groups (Prentice & Jebb, 1995, James, 1997). The latter may actually play a dominant role in the development of obesity by greatly reducing energy needs (Prentice & Jebb, 1995).

Finally, crude data from this study suggested that female current smokers are less likely to be obese compared to non smokers or past smokers. The association was not significant for men (Table 21, Chapter 6.2). After adjustment for the effects of energy intake, physical activity, age and educational level, smoking status was not found to be a predictor of increased adiposity either for men or women. However, the inverse association between tobacco smoking and relative body weight has been revealed in several studies (Fehily et al, 1984, Bolton-Smith & Woodward, 1994, Colhoun & Prescott-Clarke,1996). Several mechanisms have been proposed for the observed association. According to these smoking induces an acute rise in metabolic rate and tends to reduce food intake (Dalosso & James, 1984). Moreover, tobacco smoking may cause a longer term increase in RMR, although evidence for the latter has been conflicting (Hofstetter et al, 1986, Warwick et al, 1995). Recently Nicklas (Nicklas et al, 1999) suggested that cigarette smoking directly elevates circulating plasma leptin concentrations, and this increase may be the reason of the lower body weight of smokers compared to non-smokers. The observed inconsistency between our results and previous findings may be attributed to the known difficulty of separating the effects of social class and smoking habit, since these two are so closely correlated (Fehily et al, 1984).

12.4 Statistical methods used for data analysis

Concerning data analysis, this study took advantage of the earlier gained experience and the substantial literature that has been developed. The study considered some fundamental methodological issues and applied techniques to adjust associations for errors in measurement. There are two methodological issues, adjustment for energy intake and under-reporting, which have been brought into prominence by this analysis. In regard to adjustment for energy intake, we operated five different techniques which were all considered appropriate and fitted the data well: the standard multivariate model; the nutrient density model; the multivariate nutrient density model; the nutrient residual

model; and the energy decomposition model. Summary results for the nutrients which turned out to be consistent predictors of BMI, WHR and WC are shown in Tables 94-99 and 100-109. All five models yielded similar results. To facilitate subsequent discussion the example of fat will be used to draw some important points; the same points apply to all macronutrients. Actually the standard multivariate, the nutrient residual method and the energy decomposition method are mathematically identical and that is the reason they actually give identical results. However, the interpretation of the coefficients is not the same across the models (Mackeras, 1996, Willett & Stampfer, 1998). In both, the standard multivariate model and the nutrient residual model, the regression coefficient for fat can be interpreted as the effect of 1 gr increase of fat, while holding constant total energy intake. The latter however, can only occur if there is a simultaneous decrease of the energy intake equivalent to 1g of fat in non-fat energy sources. Therefore, regression coefficients refer to the net effect of this change; it is not the risk for changing fat intake alone. Actually, although the correlation between fat and the fat residual is not high (Table 91) the scaling among those with the same total energy intake is preserved and this is the reason both models give identical results. In the energy decomposition model, the regression coefficient of fat represents the effect of 500 kcal increase of fat while keeping constant the remaining energy sources; it therefore represents the effect of 'adding fat' since total energy intake will vary. In the case of the energy decomposition model, however, an extra calculation is needed in order to derive the net effect while holding energy constant. For example the effect associated with an iso-energetic increase of 1g of fat (9 kcal) must include both the change associated with increasing fat kcal by 9 and decreasing non-fat kcal by 9. Thus allowing for the expression of fat in grams in standard multivariate model and kcals in energy decomposition model we calculate 9 x (regression coefficient for fat – regression coefficient for non-fat energy) from the energy decomposition model. The result of this calculation actually equals the regression coefficient for fat in the standard multivariate model. (Note that since in our case, energy decomposition models have been multiplied by 500, the first step should always be to divide the coefficient given in tables by 500) (Mackeras, 1996, Willett & Stampfer, 1998).

In both versions of nutrient density models (nutrient density model and multivariate nutrient density model), the nutrient coefficients can be interpreted as the effect of a 1 percent increase of fat. The nutrient density method is intended to ensure (as in the residual model) that persons with low fat intake relative to their energy intake will be grouped at the same end of the distribution (Mackeras, 1996). As the fat residual and the fat % are highly correlated (Table 91), they place the individuals in a very similar order. However, regression coefficients in the nutrient density models are different, since these are expressed in different units (% instead of g). It has been suggested that if the correlation between total energy intake and fat density is not zero (Table 91) total energy intake should be included in the model to control for remaining confounding. When the correlation is low the bias in the effect of fat if energy intake is omitted from the model is not very serious. Actually, in our data inclusion of total energy intake did not cause major differences in values derived from the two models (Mackeras, 1996, Willett & Stampfer, 1998).

Table 91.Correlation between energy intake & different models of expressing nutrient intake

Energy or nutrient (units)	Nutrient (units)	Pearson's correlation coefficient*	
		Men	*Women*
Energy (kcal)	x fat (kcal or g)	0.92	0.95
	x nonfat kcal	0.94	0.95
	x fat (% energy)	0.79	0.23
	x fat (residual)	-0.001	0.20
Nonfat (kcal)	x fat (kcal or g)	0.73	0.81
Fat (% energy)	x fat (g)	0.45	0.50
	x fat (residual)	0.95	0.95
Fat (residual)	x fat (g)	0.40	0.50

* Correlation coefficients refer to the whole sample

In the past decade there has been considerable debate over the most appropriate method for accounting for total energy intake in the analysis (Mackeras, 1996, Willett & Stampfer, 1998) . Our empirical comparisons of methods for energy adjustment suggest that although similar conclusions are drawn from various

analyses, the interpretation of the findings is not the same across different methods. Analysing the data using alternative approaches for energy adjustment, while paying careful attention to the interpretation of the coefficients, helps to gain insight into the diet-disease relationships.

As far as 'under-reporting' is concerned, it has been shown that the prevalence of under-reporters depends on the method used for determining energy and the cut-off points adopted for the evaluation of the EI/BMR ratio. In the current data, it ranges from about 35%, when the single Goldberg EI/BMR cut-off is applied, to about 25% when the population sex and age specific EI/BMR cut-off points are applied instead. The use of lower cut-off points for determining under-reporters was theoretically justified by the fact that participants in the current study had (or reported) lower physical activity levels. The latter observation however, raises an issue about the validity of physical activity data. The possibility that a fraction of study participants may have under-estimated their physical activity could not be excluded (Black et al, 1991, Black et al 1996). However, an assumption like this lies rather in contrast to what it would 'normally' have been expected, and also scientifically suggested (Lichtman et al, 1993), that is that people tend to overestimate their physical activity. Since relevant data have not yet been validated in the Greek socio-cultural context, the results from the more detailed analysis, should be interpreted with caution. Nevertheless, the fact that analysis was conducted using both methods for determining under-reporters, and results were practically similar, is supportive to the notion that our findings are qualitatively sound and the implications correct, even if the underreporting is not fully captured through the indicated procedures.

12.5 Energy intake and energy expenditure in relation to BMI

The results suggested that higher energy intake increases BMI and physical activity reduces BMI, but physical activity is considerably less efficient in reducing BMI compared to a decrease in energy intake.

Tables 92 and 93 summarise crude as well as adjusted results on the relationships between energy intake, energy expenditure and BMI. Control for potential confounding factors has strengthened the positive association between energy intake and BMI whereas it weakened the association between energy expenditure and BMI. Actually, inverse associations between energy expenditure and BMI were only marginally significant. The latter observation may be attributed to the fact that energy expenditure is highly correlated with energy intake (see Tables 13-14, Chapter 6.1).

Table 92.Summary of results on the association between energy balance components & BMI

Energy balance components	Men					
	Population specific EI/BMR cut-offs (450 men)			*Goldberg EI/BMR cut-off (396 men)*		
	β	SE	P value	β	SE	P value
Energy intake	0.152	0.145	0.296	0.246	0.172	0.152
Adjusted energy intake *	0.599	0.175	0.001	0.661	0.190	0.001
Energy expenditure	-0.055	0.025	0.030	-0.060	0.027	0.028
Adjusted energy expenditure **	-0.272	0.150	0.075	-0.290	0.160	0.068

* adjusted for energy expenditure, age, education, smoking status

** adjusted for energy intake, age, education, smoking status

Table 93.Summary of results on the association between energy balance components & BMI

Energy balance components	Women					
	Population specific EI/BMR cut-offs (715 women)			*Goldberg EI/BMR cut-off (620 women)*		
	β	SE	P value	β	SE	P value
Energy intake	-0.189	0.214	0.377	0.239	0.238	0.316
Adjusted energy intake *	0.937	0.213	<0.001	1.095	0.226	0.001
Energy expenditure	-0.141	0.042	0.001	-0.117	0.045	0.009
Adjusted energy expenditure **	-0.353	0.205	0.084	-0.380	0.210	0.075

* adjusted for energy expenditure, age, education, smoking status

** adjusted for energy intake, age, education, smoking status

Our results concerning the positive association between energy intake and BMI are consistent with recent observational epidemiologic evidence reported by

Richards et al (2000) and by Popkin et al (1995). Moreover, experimental work by Prentice (Prentice et al, 1996) suggests that the energy needs of obese persons are elevated since their energy expenditure is substantially and progressively raised; raised levels of total energy expenditure (TEE) must be supported by similar levels of energy intake. This finding contradicts the notion that has been stated in many studies, that obesity develops and is maintained in spite of low levels of energy intake (Romieu et al, 1988, Dreon et al 1988, Miller et al, 1990, Slattery et al, 1992, Miller et al, 1994, Macdiarmid et al, 1996, Andersson et al, 1996). This conflicting evidence is probably due to weaknesses in study design, the effects of non-dietary confounders, methodological flaws, measurement error and most importantly failure to control for the effect of under-reporting (Prentice et al, 1986, Black et al, 1991, Prentice et al, 1996).

Concerning the role of physical activity in reducing BMI, there is some evidence, although weak, that obese people are less active than non-obese, after controlling for the potential effect of energy intake, age, tobacco smoking and education level. Several cross-sectional studies (Williamson et al, 1993, Rising et al, 1994, Schultz et al, 1994, DiPietro, 1995, Ching et al, 1996, Westerterp et al, 1997) have reported lower body weights and indices of body fatness (skinfolds measures and BMI) among individuals with self-reported high levels of physical activity or fitness. Several prospective studies (Williamson et al, 1993, French et al, 1994, Popkin et al, 1995) have reported inverse relationships between physical activity and subsequent weight gain. However, one study found that physical activity was inversely associated with weight gain in women but not in men (Klesges, 1992) and another one showed that a low level of physical activity was a risk factor for weight gain only in those women consuming a high fat diet (Lissner et al, 1997). Also, there have been several comprehensive reviews (Rippe & Hess, 1998, Jebb & Moore, 1999) which have explored the relationship between physical activity and obesity. These reviews have reached the conclusion that there is a good reason to believe that there may be a causal association between low levels of physical activity and obesity. Definite statements, however, are limited by the methodological flaws of such studies, particularly

with respect to the quantification of physical activity. Moreover, Prentice (Prentice et al, 1996) have indicated that this is a rather difficult issue to resolve, since it implicates difficulties, such as appropriately adjusting for differences in body weight and composition. The authors concluded that except in massive obesity, patterns of physical activity are quite similar at different levels of BMI. The latter suggests that perhaps a similar behaviour pattern is shared both by obese and normal weight individuals.

In conclusion, we interpret our results as suggesting that in cross-sectional data, BMI is positively associated with energy intake. Concerning the association between physical activity and BMI, there is some evidence, although weak, that BMI is inversely associated with physical activity. Anyway, increasing physical activity appears to be less effective than decreasing energy intake in reducing BMI in men and considerably less in women. The latter of course does not imply that physical inactivity is not a risk factor for the development of obesity.

12.6 Nutrient intake in relation to BMI

The results suggested that energy equivalent amounts of energy-generating nutrients may have variable effects on BMI. Thus, in our study population total fat and mono-unsaturated fat intake were predictors of BMI in women, but not in men. Tables 94 and 95 summarise crude and adjusted results on the relationships between total fat intake and BMI.

Table 94. Summary of results on the association between fat intake and BMI

	Men								
	All sample (596 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value
Fat intake, g	-0.002	0.004	0.614	0.004	0.005	0.482	0.005	0.006	0.347
Fat intake, %	-0.008	0.029	0.775	-0.002	0.033	0.946	-0.009	0.035	0.809
Adjusted Fat intake[a]	0.0005	0.011	0.964	0.004	0.012	0.748	0.0002	0.012	0.988
Adjusted Fat intake[b]	0.012	0.030	0.694	0.029	0.034	0.392	0.020	0.037	0.593
Adjusted Fat intake[c]	0.011	0.030	0.714	0.026	0.034	0.445	0.012	0.036	0.735
Adjusted Fat intake[d]	0.0005	0.011	0.964	0.004	0.012	0.748	0.0002	0.012	0.988
Adjusted Fat intake[e]	0.191	0.780	0.607	0.715	0.416	0.076	0.664	0.410	0.109
All the rest	*0.142*	*0.252*	*0.642*	*0.480*	*0.337*	*0.154*	*0.629*	*0.357*	*0.079*

Adjusted for physical activity, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e: energy decomposition method (values multiplied by 500)

Table 95. Summary of results on the association between fat intake and BMI

	Women								
	All sample (961 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value
Fat intake, g	-0.023	0.006	0.001	-0.009	0.007	0.182	0.004	0.008	0.622
Fat intake, %	-0.045	0.035	0.196	-0.067	0.042	0.115	-0.029	0.045	0.517
Adjusted Fat intake[a]	0.045	0.017	0.009	0.026	0.018	0.145	0.027	0.018	0.134
Adjusted Fat intake[b]	0.141	0.048	0.004	0.092	0.040	0.021	0.108	0.043	0.012
Adjusted Fat intake[c]	0.112	0.034	0.001	0.074	0.040	0.062	0.088	0.042	0.039
Adjusted Fat intake[d]	0.045	0.017	0.009	0.026	0.018	0.145	0.027	0.018	0.134
Adjusted Fat intake[e]	1.571	0.527	0.003	1.658	0.500	0.003	1.858	0.562	0.001
All the rest	*- 0.875*	*0.470*	*0.064*	*0.239*	*0.546*	*0.637*	*0.342*	*0.525*	*0.514*

Adjusted for physical activity, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e: energy decomposition method (values multiplied by 500).

Tables 96 and 97 summarise the results on the relationships between mono-unsaturated fat intake and BMI. Once again mono-unsaturated fat intake seems to be positively related to BMI in women but not in men.

Table 96.Summary of results on the association between monounsaturated fat intake & BMI

	Men								
	All sample (596 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR Cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value
MUF intake, g	-0.001	0.009	0.894	0.009	0.010	0.356	0.014	0.011	0.199
MUF intake, %	0.020	0.045	0.666	0.010	0.052	0.854	0.022	0.058	0.708
Adjusted MUF intake[a]	0.012	0.018	0.516	0.014	0.019	0.467	0.014	0.019	0.487
AdjustedMUF intake[b]	0.033	0.046	0.477	0.038	0.054	0.476	0.048	0.059	0.415
Adjusted MUF intake[c]	0.039	0.046	0.415	0.048	0.053	0.364	0.052	0.058	0.373
AdjustedMUF intake[d]	0.012	0.018	0.516	0.014	0.019	0.467	0.014	0.019	0.487
AdjustedMUF intake[e]	0.709	0.779	0.364	1.244	0.828	0.134	1.297	0.853	0.130
All the rest	*0.018*	*0.253*	*0.943*	*0.427*	*0.284*	*0.135*	*0.489*	*0.305*	*0.109*

Adjusted for physical activity, age, education, smoking status. a : standard multivariate method, b: nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e: Energy decomposition method (values multiplied by 500)

Table 97.Summary of results on the association between monounsaturated fat intake & BMI

	Women								
	All sample (961 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR Cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value
MUF intake, g	-0.041	0.012	0.001	-0.007	0.015	0.639	0.022	0.016	0.170
MUF intake, %	0.013	0.052	0.798	0.023	0.064	0.716	0.065	0.069	0.346
Adjusted MUF intake[a]	0.072	0.025	0.004	0.074	0.026	0.004	0.071	0.027	0.008
AdjustedMUF intake[b]	0.141	0.048	0.004	0.146	0.058	0.012	0.145	0.063	0.022
Adjusted MUF intake[c]	0.148	0.048	0.002	0.169	0.057	0.003	0.180	0.062	0.004
AdjustedMUF intake[d]	0.072	0.025	0.004	0.074	0.026	0.004	0.071	0.027	0.008
AdjustedMUF intake[e]	3.418	1.082	0.002	4.158	1.137	0.001	4.229	1.178	0.001
All the rest	*-0.610*	*0.346*	*0.079*	*0.065*	*0.376*	*0.863*	*0.256*	*0.389*	*0.511*

Adjusted for physical activity, age, education, smoking status. a : standard multivariate method, b: nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e: energy decomposition method (values multiplied by 500).

Our results indicating a positive association between fat and mono-unsaturated fat intake with increased BMI are consistent with previously reported data from other epidemiological investigations (Kromhout et al, 1983, Miller et al, 1994). Various potential mechanisms have been proposed to explain why fat intake

should lead to increased relative weight (Prentice, 1995, Lissner, 1997). These include mechanisms implicating 'passive overconsumption' because of the greater density of high-fat diet or/and the weaker effect of fat on satiation and satiety, "active overconsumption" due to greater flavour and palatability of high-fat foods. Finally there has been evidence for more efficient metabolism of this nutrient relative to other macronutrients; fat, is more likely to be stored rather than oxidised whilst storage costs are lower for fat than for carbohydrate. Evidence relating to the association between dietary fat and obesity has been extensively reviewed in Chapter 2. As far as passive overconsumption is concerned, current study provided evidence to support the notion that overweight women (but not men) tend to consume proportionately more fat for a given total amount of grams of energy-generating nutrients.

Concerning sex differences in BMI predictors, these may indicate greater error among the estimates of nutrient intake in men. For example men self-reports of dietary intake may be less reliable compared to those of women or alternatively that there are some other unmeasured predictors (e.g metabolic rate) that are more efficient in ingesting dietary intake from fat in men but not in women.

Taking in mind however, that there have not been results indicating any associations between other fat fractions, especially saturated fat intake, and BMI, an association which has been reported before (Romieu et al, 1988, Doucet et al, 1998), and given the fact that the associations between MUF intake and BMI are stronger and more consistent than those observed for total fat intake, there is a possibility that it is the mono-unsaturated fat intake which is mainly responsible for the apparent association between total fat intake and obesity. The latter reasonable assumption combined with the fact that olive oil (the main source of mono-unsaturated fat intake in the Greek diet) is a valued commodity in Greece, makes it possible that the extent of non-differential misclassification is lower for mono-unsaturated fats than for other nutrients, thus explaining the higher regression coefficient for the former. Some support for this explanation may be

found in the fact that the high regression coefficient for olive oil is mostly evident among women, who traditionally in Greece, take care of the preparation of meals. From Tables 98-99, it appears that the nutrient most affecting BMI is protein. The association is once again gender specific with protein being a strong and consistent predictor of BMI in women but not in men.

Table 98. Summary of results on the association between protein intake and BMI

	Men								
	All sample (596 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value
Protein intake, g	-0.001	0.007	0.947	0.009	0.008	0.245	0.014	0.009	0.134
Protein intake, %	0.044	0.094	0.636	0.044	0.113	0.700	0.030	0.128	0.817
Adjusted Protein intake[a]	0.018	0.017	0.305	0.030	0.018	0.109	0.030	0.019	0.125
Adjusted Protein intake[b]	0.065	0.094	0.487	0.082	0.114	0.471	0.076	0.129	0.552
Adjusted Protein intake[c]	0.082	0.095	0.387	0.160	0.114	0.163	0.177	0.130	0.174
Adjusted Protein intake[d]	0.018	0.017	0.305	0.030	0.018	0.109	0.030	0.019	0.125
Adjusted Protein intake[e]	2.126	1.862	0.254	3.895	2.037	0.057	3.996	2.155	0.064
All the rest	*-0.119*	*0.299*	*0.692*	*0.139*	*0.322*	*0.667*	*0.203*	*0.339*	*0.552*

Adjusted for physical activity, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 99. Summary of results on the association between protein intake and BMI

	Women								
	All sample (961 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value
Protein intake, g	-0.024	0.009	0.007	0.0004	0.011	0.971	0.024	0.012	0.048
Protein intake, %	0.313	0.100	0.002	0.193	0.121	0.110	0.265	0.128	0.039
Adjusted Protein intake[a]	0.090	0.021	<0.001	0.069	0.022	0.002	0.073	0.023	0.001
Adjusted Protein intake[b]	0.386	0.090	<0.001	0.273	0.109	0.013	0.306	0.118	0.010
Adjusted Protein intake[c]	0.404	0.091	<0.001	0.306	0.108	0.005	0.350	0.116	0.003
Adjusted Protein intake[d]	0.090	0.021	<0.001	0.069	0.022	0.002	0.073	0.023	0.001
Adjusted Protein intake[e]	9.940	2.268	<0.001	8.340	2.370	<0.001	8.960	2.440	<0.001
All the rest	*-1.235*	*0.389*	*0.002*	*-0.237*	*0.421*	*0.575*	*-0.150*	*0.435*	*0.729*

Adjusted for physical activity, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

We were first surprised by the results. So far little attention has been paid to the risks associated with increased protein consumption excess; even though food contains three nutrients, in most papers related to overweight and obesity, only two (fat and carbohydrate) are considered. However, although the association between protein intake and increased BMI has not been frequently reported, there have been some studies that have reported positive associations (Slattery et al, 1992, Richards, 2000). As these studies were cross-sectional, high protein intake was not clearly interpreted as a possible causal factor promoting fatness development. However, there has been systematic work referring to childhood obesity suggesting that high protein intake in early life can increase the risk of obesity and various other pathologies (Rolland-Cachera et al, 1995). Among the possible mechanisms proposed for the association is that the high protein intake in early life may alter hormonal status. It may promote accelerated growth and induce an enhanced cell proliferation in various tissues, including muscle and adipose tissue. In addition the low fat intake and accelerated growth recorded in infancy may reduce energy balance and create a metabolic adaptation to this relative deficit. Subsequent changes from reduced to adequate or positive energy balance may promote risk factors for various metabolic disorders (Rolland Cachera et al, 1995). Moreover, there has been a study by Appleby et al (1998) suggesting that non-meat eaters are thinner than meat-eaters. Although the main interest of the investigators in this study has been animal fat, one might not exclude the possibility that it might be the combination of animal fat and protein found in meat which is implicated in the etiology of obesity. Since people do not eat nutrients in isolation but foods, a value for protein or fat intake may be the same for individuals but may be obtained from entirely different sources. Different food sources may contribute to differences in intake of other nutrients which may modify the effects of the primary nutrient on the endpoint. There is always the possibility of a strong degree of interdependence among nutrients (animal fat and protein). A better understanding of dietary intake and its associations with anthropometric, physiological and disease endpoints can only be made by a description of dietary patterns expressed as combinations of foods consumed. Unfortunately, in the context of the present study we were not able to

explore this potential mechanism further, since data on food level was not available.

Anyway, since the association between protein intake and increased BMI has never been investigated for its own sake, but most of the time emerges as one of the findings in studies designed to explore associations between dietary intake and disease, we should be cautious of accepting or rejecting this particular finding. It may well reflect a genuine biological phenomenon, but it may also be the result of variable patterns of misclassification among nutrients (what is referred as 'correlation dilution'). Thus, if protein was, for some reason, recorded systematically in a more accurate way, compared to other nutrients, this might have lead to strengthening the protein intake-obesity associations. It has been actually suggested that individuals tend to remember better the main component of a meal, which is very frequently of protein origin (meat or milk), than snack type foods that accompany the dish and are preferentially forgotten (Heitmann & Lissner 1995, Poppitt et al, 1995). Finally, another fact that should not be ignored is that in the current analysis there has been some evidence that nutrient imbalances, although minor, in under-reporting may exist.

To summarise, our results have revealed that the nutrient most affecting BMI is protein and to a lesser extent mono-unsaturated and total fat intake. The associations were profound in women but not in men. Sex differences as a possible moderator variable in the energy balance equation have also been discussed before by Klesges et al (1992), Slattery et al (1992). Possible implications of current findings are: a) either men and women are differentially affected by components of the energy balance equation; or b) there is a greater error among the estimates of nutrient intake in men (for example men self-reports of dietary intake may be less reliable compared to those of women). The proposition of a causal relationship between protein, mono-unsaturated and total fat intake and BMI must be accepted with caution, due to complex methodological issues involved in the analysis of the cross-sectional studies.

However, there has been sufficient epidemiological and experimental evidence suggesting that regular exercise may diminish the risk of independent metabolic abnormalities that are associated with abdominal obesity, in particular abdominal subcutaneous and visceral fat, and are most likely to precede cardiovascular disease and type II diabetes. The basis for this effect of exercise may be its ability to diminish or prevent insulin resistance, hyperinsulinaemia, and increases in intra-abdominal adipose mass (Rudermann et al, 1992, Buemann & Tremblay, 1996). Thus, Mourier (Mourier et al, 1997) conducted a randomised controlled trial to explore the effects of exercise alone on visceral and subcutaneous fat per se. The authors observed large reductions in both visceral (about 48%) and abdominal subcutaneous adipose tissue (about 18%) in response to moderate exercise performed three times per week for 8 week in men and women with type II diabetes. In that study no parallel weight loss was observed. Additionally in a review by Buemann and Tremblay physical training was suggested as a non-pharmacological tool for the treatment of abdominal obesity and associated metabolic disorders (Buemann & Tremblay, 1996). Concerning the more profound effect of physical activity on men, Despres (Despres et al, 1988) observed and suggested that when aerobic exercise training is used to induce weight loss, men generally lose more fat than women. Moreover, in a recent review on the effects of diet and exercise induced weight loss on visceral adipose tissue in men and women, Ross (Ross, 1997) reported that it appears to be some kind of resistance to visceral adipose tissue reduction in obese women, whereas exercise induced weight loss is associated with significant reductions in visceral adipose tissue in men.

The current results lend credence to the notion that physical activity is related to reduced WHR and WC. This may actually be a hidden reason for the advantageous effect of physical activity in health. Moreover, the stronger and more consistent effect observed in men, provides a plausible answer to the question why physical activity seems to be more important for health among men than among women. In fact, although there has been conclusive evidence that physical activity increases longevity and reduces the incidence of cardiovascular

diseases among both men and women, it has been suggested that the efficiency of physical activity in reducing cardiovascular risk may be higher among men than among women; the current study provides a plausible explanation for this differential effect.

12.8 Nutrient intake in relation to WHR and WC

The results from the present study suggest that WHR and WC are not differentially affected by energy equivalent amounts of energy generating nutrients controlling for BMI. Concerning an association between dietary fat intake and abdominal obesity, this was not supported from this analysis. Our results are consistent with those from other studies reporting either weak or no associations (Larson et al, 1996, Samaras et al, 1998, Singh et al, 1998). However, there have been other epidemiological studies which have provided evidence for a positive association between dietary fat and abdominal obesity (George et al, 1990, Tucker & Kano, 1992, Miller et al, 1994, Nelson et al, 1996).

This study revealed some evidence that protein increases WHR and WC in women and mono-unsaturated fat intake reduces WHR and WC in men. Tables 102-109 summarise the crude and adjusted results on the relationships between mono-unsaturated fat intake, WHR and WC.

Lack of consistency between results across sexes and different methods used, raise the possibility that statistical associations may be from either chance or from methodological biases. Anyway, it should be noted that there have been studies exploring regional adiposity features, relating protein excess with fat located at the abdominal level rather than other sites (Rolland-Cachera et al, 1996a). Once again evidence has risen from investigators working on childhood obesity. Nicklas (Nicklas et al, 1993), in the Bogalusa Heart Study, reported an increasing prevalence of obesity in children of 10 years of age. This increase was parallel with decreases both in energy intake and in percentage of energy from fat.

Rolland-Cachera, (1996b) observed similar changes. Between 1978 and 1995, the prevalence of obesity (actually, a more centralized pattern of adipose tissue), in 10 years old French children, has doubled and energy from fat has decreased from 38.7% to 37%. The percentage of protein though had increased from 13.5% to 15.3%.

There has also been parallel evidence derived from studies investigating associations between nutrient intake, central fat distribution and several metabolic diseases. Particularly, it has been suggested that there is an association between protein excess and metabolic complications of obesity such as insulin resistance and cardiovascular disease (Bjorntrop, 1996, Gannon & Nuttall, 1995). Associations between nutrient intake and hormonal status, which could explain the relations between excess protein and metabolic disorders were discussed previously (Rolland-Cachera et al, 1996).

Table 100. Summary of results on the association between energy balance components and WHR - WC

Energy balance Components	Men											
	WHR						WC					
	Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value	β	SE	P value
Energy intake	-0.011	0.035	<0.001	-0.008	0.005	0.027	-0.210	0.500	0.608	0.375	0.001	0.446
Adjusted energy intake *	-0.002	0.025	0.520	-0.0001	0.001	0.970	0.514	0.500	0.073	0.743	0.500	0.020
Energy expenditure	-0.014	0.002	<0.001	-0.014	0.005	<0.001	-1.570	0.350	<0.001	-1.510	0.385	<0.001
Adjusted energy expenditure **	-0.004	0.002	0.102	-0.005	0.005	0.052	-0.505	0.245	0.041	-0.560	0.260	0.032

* adjusted for BMI energy expenditure, age, education, smoking status
** adjusted for BMI energy intake, age, education, smoking status

Table 101. Summary of results on the association between energy balance components and WHR - WC

Energy balance Components	Women											
	WHR						WC					
	Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value	β	SE	P value
Energy intake	-0.017	0.005	<0.001	-0.012	0.0004	0.002	-1.285	0.500	0.011	-0.180	0.500	0.749
Adjusted energy intake *	-0.0005	0.002	0.870	-0.001	0.004	0.771	0.249	0.500	0.388	0.324	0.500	0.298
Energy Expenditure	-0.018	0.005	<0.001	-0.015	0.005	<0.001	-2.345	0.495	<0.001	-1.950	0.525	<0.001
Adjusted energy Expenditure **	-0.005	0.001	0.049	-0.005	0.005	0.079	-0.434	0.055	0.112	-0.435	0.285	0.131

* adjusted for BMI energy expenditure, age, education, smoking status
** adjusted for BMI energy intake, age, education, smoking status

Table 102. Summary of results on the association between monounsaturated fat intake & WHR

	Men								
	All sample (596 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value
MUF intake, g	-0.0007	0.0002	<0.001	-0.0009	0.0002	<0.001	-0.0007	0.0002	0.001
MUF intake, %	-0.001	0.001	0.197	-0.002	0.001	0.018	-0.003	0.001	0.004
Adjusted MUF intake[a]	-0.0003	0.0003	0.248	-0.0005	0.0003	0.097	-0.0006	0.0003	0.044
Adjusted MUF intake[b]	-0.0005	0.001	0.473	-0.0002	0.001	0.125	-0.002	0.001	0.019
Adjusted MUF intake[c]	-0.0005	0.001	0.484	-0.001	0.001	0.115	-0.002	0.001	0.019
Adjusted MUF intake[d]	-0.0003	0.0003	0.248	-0.0005	0.0003	0.097	-0.0006	0.0003	0.044
Adjusted MUF intake[e]	-0.010	0.001	0.306	-0.022	0.015	0.089	-0.025	0.015	0.056
All the rest	*0.004*	*0.004*	*0.318*	*0.004*	*0.005*	*0.424*	*0.005*	*0.005*	*0.150*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 103. Summary of results on the association between monounsaturated fat intake & WHR

	Women								
	All sample (961 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value
MUF intake, g	-0.002	0.0002	<0.001	-0.001	0.0002	<0.001	-0.0008	0.0003	0.002
MUF intake, %	-0.002	0.001	0.008	-0.002	0.001	0.044	-0.001	0.001	0.340
Adjusted MUF intake[a]	-0.000003	0.0004	0.992	-0.00009	0.0004	0.816	-0.00007	0.0004	0.859
Adjusted MUF intake[b]	-0.0002	0.001	0.818	-0.0002	0.001	0.817	-0.0001	0.001	0.886
Adjusted MUF intake[c]	-0.0001	0.001	0.898	-0.0002	0.001	0.803	-0.0002	0.001	0.885
Adjusted MUF intake[d]	-0.000003	0.0004	0.992	-0.00009	0.0004	0.816	-0.00007	0.0004	0.859
Adjusted MUF intake[e*]	0.003	0.015	0.871	-0.005	0.015	0.772	-0.005	0.015	0.789
All the rest	*0.003*	*0.005*	*0.514*	*0.001*	*0.005*	*0.890*	*0.0001*	*0.040*	*0.983*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 104. Summary of results on the association between protein intake and WHR

	Men								
	All sample (596 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value
Protein intake, g	-0.0005	0.0001	<0.001	-0.0006	0.0002	<0.001	-0.004	0.0002	0.042
Protein intake, %	0.001	0.002	0.578	-0.0001	0.002	0.949	-0.0005	0.003	0.856
Adjusted Protein intake [a]	0.0004	0.0003	0.100	0.0004	0.0003	0.137	0.0005	0.0003	0.104
Adjusted Protein intake [b]	0.002	0.001	0.201	0.002	0.002	0.255	0.003	0.002	0.205
Adjusted Protein intake [c]	0.002	0.001	0.144	0.002	0.002	0.302	0.003	0.002	0.198
Adjusted Protein intake [d]	0.0004	0.0003	0.100	0.0004	0.0003	0.137	0.0005	0.0003	0.104
Adjusted Protein intake [e*]	0.045	0.027	0.096	0.045	0.030	0.152	0.050	0.035	0.104
All the rest	*-0.005*	*0.005*	*0.169*	*-0.008*	*0.005*	*0.108*	*-0.005*	*0.005*	*0.167*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 105. Summary of results on the association between protein intake & WHR

	Women								
	All sample (961 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value
Protein intake, g	-0.0007	0.0001	<0.001	-0.0007	0.0002	<0.001	-0.0004	0.0002	0.030
Protein intake, %	0.003	0.002	0.046	0.003	0.002	0.141	0.003	0.002	0.152
Adjusted Protein intake [a]	0.0007	0.0003	0.021	0.0005	0.0003	0.091	0.0004	0.0003	0.304
Adjusted Protein intake [b]	0.003	0.001	0.009	0.003	0.002	0.032	0.002	0.002	0.182
Adjusted Protein intake [c]	0.004	0.001	0.006	0.003	0.002	0.033	0.002	0.002	0.190
Adjusted Protein intake [d]	0.001	0.0003	0.021	0.001	0.0003	0.091	0.0004	0.0003	0.304
Adjusted Protein intake [e*]	0.100	0.030	0.018	0.058	0.035	0.099	0.035	0.035	0.327
Al the rest	*-0.009*	*0.005*	*0.120*	*-0.009*	*0.006*	*0.127*	*-0.005*	*0.005*	*0.311*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 106. Summary of results on the association between mono-unsaturated fat intake and WC

	Men								
	All sample (596 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value
MUF intake, g	-0.042	0.024	0.083	-0.040	0.028	0.153	-0.017	0.032	0.611
MUF intake, %	-0.121	0.126	0.339	-0.245	0.148	0.099	-0.318	0.164	0.053
Adjusted MUF intake [a]	-0.050	0.027	0.064	-0.064	0.030	0.034	-0.078	0.032	0.014
Adjusted MUF intake [b]	-0.107	0.070	0.129	-0.167	0.085	0.051	-0.257	0.095	0.007
Adjusted MUF intake [c]	-0.092	0.070	0.190	-0.158	0.085	0.064	-0.252	0.095	0.008
Adjusted MUF intake [d]	-0.050	0.027	0.064	-0.064	0.030	0.034	-0.078	0.032	0.014
Adjusted MUF intake [e*]	-1.495	1.189	0.209	-2.114	1.326	0.112	-2.500	1.392	0.078
All the rest	*1.098*	*0.385*	*0.005*	*1.226*	*0.457*	*0.008*	*1.500*	*0.497*	*0.001*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 107. Summary of results on the association between mono-unsaturated fat intake and WC

	Women								
	All sample (961 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value
MUF intake, g	-0.143	0.028	<0.001	-0.090	0.035	0.010	-0.019	0.039	0.630
MUF intake, %	-0.121	0.125	0.333	-0.152	0.151	0.314	-0.069	0.164	0.672
Adjusted MUF intake [a]	-0.00004	0.033	0.999	-0.025	0.035	0.467	-0.036	0.037	0.323
Adjusted MUF intake [b]	0.009	0.064	0.886	-0.052	0.077	0.498	-0.097	0.085	0.252
Adjusted MUF intake [c]	0.017	0.064	0.792	-0.046	0.077	0.553	-0.087	0.085	0.308
Adjusted MUF intake [d]	-0.00004	0.033	0.999	-0.025	0.035	0.467	-0.036	0.037	0.323
Adjusted MUF intake [e*]	0.316	1.445	0.827	-0.870	1.541	0.572	-1.260	1.617	0.437
All the rest	*0.335*	*0.461*	*0.467*	*0.567*	*1.505*	*0.262*	*0.766*	*0.529*	*0.149*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 108. Summary of results on the association between protein intake and WC

	Men								
	All sample (596 men)			Population specific EI/BMR cut-offs (450 men)			Goldberg EI/BMR cut-off (396 men)		
	β	SE	P value	β	SE	P value	β	SE	P value
Protein intake, g	-0.017	0.019	0.376	-0.009	0.023	0.700	0.022	0.027	0.407
Protein intake, %	0.144	0.262	0.582	0.073	0.319	0.820	0.058	0.365	0.879
Adjusted Protein intake [a]	0.042	0.026	0.104	0.047	0.030	0.286	0.059	0.032	0.066
Adjusted Protein intake [b]	0.153	0.144	0.290	0.181	0.181	0.318	0.248	0.210	0.238
Adjusted Protein intake [c]	0.206	0.145	0.155	0.254	0.184	0.168	0.373	0.213	0.081
Adjusted Protein intake [d]	0.042	0.026	0.104	0.047	0.030	0.286	0.059	0.032	0.066
Adjusted Protein intake [e *]	5.140	2.841	0.071	5.685	3.284	0.084	7.240	3.538	0.041
All the rest	*-0.130*	*0.457*	*0.774*	*-0.186*	*0.517*	*0.720*	*-0.100*	*0.556*	*0.824*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

Table 109. Summary of results on the association between protein intake and WC

	Women								
	All sample (961 women)			Population specific EI/BMR cut-offs (715 women)			Goldberg EI/BMR cut-off (620 women)		
	β	SE	P value	β	SE	P value	β	SE	P value
Protein intake, g	-0.086	0.021	<0.001	-0.044	0.026	0.094	0.012	0.029	0.669
Protein intake, %	0.615	0.238	0.010	0.407	0.287	0.157	0.465	0.304	0.127
Adjusted Protein intake [a]	0.033	0.028	0.247	0.021	0.030	0.462	0.003	0.031	0.929
Adjusted Protein intake [b]	0.175	0.122	0.150	0.163	0.145	0.262	0.036	0.158	0.820
Adjusted Protein intake [c]	0.196	0.122	0.110	0.175	0.146	0.231	0.053	0.159	0.741
Adjusted Protein intake [d]	0.033	0.028	0.247	0.021	0.030	0.462	0.003	0.031	0.929
Adjusted Protein intake [e *]	3.816	3.056	0.212	2.554	3.213	0.427	0.596	3.364	0.859
All the rest	*-0.227*	*0.521*	*0.664*	*0.109*	*0.566*	*0.848*	*0.278*	*0.593*	*0.640*

Adjusted for BMI energy expenditure, age, education, smoking status. a : standard multivariate method, b : nutrient density method, c: multivariate nutrient density method, d: nutrient residuals method, e*: energy decomposition method (values multiplied by 500).

13. CONCLUSIONS AND RECOMMENDATIONS

Clearly, the relationship between dietary intake, energy expenditure, environmental factors and body weight is complex and many aspects of this relationship remain to be clarified. Future research, through clinical as well as epidemiological settings, needs to further explore and subsequently link the dietary factors with other non-dietary factors, both biologic and non biologic, which interact to determine body weight and occurrence of obesity in populations.

In this context, nutritional epidemiology has a critical role to play. It may contribute to better understanding of different aspects of obesity aetiology, and thus provide a critical link in the causal chain of inference between nutrient intake and obesity. Concerning different study designs, until recently, and for practical reasons, the epidemiological approach to the problem has been limited either to cross-sectional or short-term metabolic or intervention studies. These studies have provided valuable preliminary evidence. Caution, however, is needed so as to avoid the temptation of over-interpreting these epidemiological findings. This need is dictated (implied) by our incomplete understanding of the mechanisms involved in obesity aetiology, as well as the knowledge that methodological problems may interfere and limit the interpretation of results derived from epidemiological studies, particularly observational ones. The most important limitation is that the key variables, dietary intake and energy expenditure are measured only by proxy and are usually subject to important bias. There are also issues regarding the extent to which a study is able to account for potential sources of confounding factors, especially when these are unmeasured, measured with error, or are so closely correlated with the dietary exposure of interest that the dietary factor and the correlated factor cannot be distinguished.

The next step in exploring dietary intake – obesity associations should be through specifically designed long-term intervention studies or prospective studies using unbiased and repeated estimates of energy balance components as well as relevant covariates. The advantage of prospective studies compared to

intervention ones is perhaps that these are conducted in realistic environmental settings. Repeated measures of energy balance components as well as relevant covariates are desirable and critical, since they may capture changes in dietary patterns over time and allow etiological associations of credibility to be established. Future studies should be targeted to evaluate associations between diet and development of obesity in specific population subgroups, defined by characteristics such as age, sex and race. For example, prospective studies among children and young adults are of particular importance in a sense that early stage of growth may consist of a critical period for obesity development. Moreover, a broader recognition and appreciation of the fact that there is considerable interaction among nutrients, both within dietary sources and in their effects on the human system, suggests that it may sometimes be more appropriate to examine the effects of foods, food groups or even dietary patterns rather than the effects of a single nutrient. Thus, dietary data need to be presented and analysed in several different ways (e.g., analyses by foods, food groups, or nutrients, with or without adjustments for other dietary factors).

Concerning methodological tools to be used in the context of these studies, there is a need to have access to better dietary data which would allow the magnitude of the between-person variation to emerge and thus would lead to more accurate results and valid conclusions. Further research and more validation studies are needed to: a) determine the most appropriate dietary assessment techniques for population subgroups; b) fully identify the features and effects of different sources of measurement errors and biases; and c) develop and evaluate techniques to efficiently control or adjust for measurement error. Furthermore, addition of biochemical measures of nutritional status and dietary intakes might consist of an effective means of obtaining more powerful and unbiased estimates of the relations between nutrition and health.

Concerning the assessment of energy expenditure, further research is needed to: a) distinguish the effect of different dimensions of energy expenditure on increased adiposity; b) determine the most efficient methods for measuring

different dimensions of physical activity; c) determine appropriate measurement techniques for population subgroups; d) identify factors that enhance the recall of physical activity in survey procedures for the entire population and for population subgroups; and e) identify the features and effects of different sources of measurement errors and biases.

Concerning the use of different indicators for obesity classification purposes, it would be valuable to have an accurate and validated indicator for total as well as central adiposity. It has been obvious that there is an urgent need to put the diversity of anthropometric indicators as well as the cut-off points currently suggested into perspective. A more comprehensive assessment of possible indicators is needed to derive an optimal indicator for abdominal obesity. Such an effort requires: a) better understanding of the different roles of abdominal and general adiposity; b) prospective studies, large enough to test possible differences between genders, age categories and ethnic groups; as well as c) intervention studies to reveal whether proposed indicators and cut-off points are amenable to change by lifestyle or other interventions since a reduction in the indicators should be able to predict a reduction at risk.

There is also need for research on gene-environmental interactions which is probably more relevant to obesity than to any other disease. It would be of value to determine whether genetic susceptibility to obesity is mediated through individual preferences in food or activity patterns and to determine whether these are influenced by physiological factors or whether they are entirely socio-behavioural phenomena.

It is a challenge for nutritional epidemiologists to find ways to cope with these methodological issues, through developing better ways to conduct nutritional epidemiological studies and better understanding of how to analyse and interpret data. Methodological advances are needed if our ability to accurately assess the foods we eat and evaluate the effects of measurement error on relations between dietary intake estimates and obesity are to improve.

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