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# METHODOLOGICAL ISSUES IN RISK ASSESSMENT OF HUMAN EXPOSURE TO ARTIFICIAL SWEETENERS IN FOOD

by

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**to**

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**Department of Clinical Medicine,**

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**Dublin.**

**November 2000**

## DECLARATION

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**Muireann Gearóidín Cullen**

**Summary.**

The primary aim of this thesis is in estimating food chemical intakes with a specific interest in the area of intense sweeteners. To date all studies of intense sweeteners have been extremely detailed both in the estimate of the presence or absence of the target sweetener in each food and the level of sweetener used. Brand level data are required for definitive identification of the presence or absence of a sweetener.

The Dietary and Nutritional Survey of British Adults food intake data are recorded at brand - level. This provides a unique opportunity to examine this issue of the incremental value, or otherwise, of recording brand - level food intake data. The first hypothesis of this thesis was that the retention of brand level data in food consumption databases is essential for accurate assessment of human exposure to food – borne chemicals. The precise number of subjects required reliably to establish food intake at the $97.5^{th}$ percentile of the distribution of intakes was taken to be 60. Of the 1,363 brands examined, only 85 had $\geq 60$ consumers. Another important finding was that the multiple of 1.3 times the food category $97.5^{th}$ percentile intake provides a robust worst - case analysis. In this study it can be seen that there is limited incremental value in retaining brand - level food intake data in food consumption databases to estimate intakes of brands among consumers and food consumption databases that do not retain brand - level data are not materially disadvantaged in assessing food additive intake.

The methods used in previous intense sweetener studies have been quite detailed. Article 4 in the 94/35/EC Directive on sweeteners states that 'within three years of its adoption, Member States shall establish a system of consumer surveys to monitor sweetener intake'. Whilst the previous studies gave detailed and accurate data, the methods employed are not suitable for routine monitoring due to participant burden and also the issue of 'commercial sensitivity' for concentration data. The second hypothesis of this thesis was that a simple food frequency questionnaire, with a food list identified from a national food ingredient database as containing target intense sweeteners, would provide a reliable means of routine surveillance of sweetener intake in high users. Food consumption data was collected using a Semi Quantitative Food Frequency Questionnaire developed with a food list identified from an Irish national food ingredient database as containing target intense sweeteners. The FFQ was self - administered and also completed with the interviewer present by a diabetic and control

group. A 3 - day diary was also kept by subjects. Sweetener concentrations were taken from the MPLs in the Directive. Comparison across three methods for each subject showed significant differences, ($p$<0.001 - $p$ = 0.05). Comparisons of intense sweetener intake of diabetics versus controls for each method, there were significant differences between the two groups with diabetics showing higher intakes of all intense sweeteners ($p$<0.001 - $p$ = 0.05). Bland and Altman plots indicate that currently FFQs are not an appropriate method for routine monitoring when compared to the diary, as there was no directional bias in over or under - estimating intakes.

There is debate and concern that the use of intense sweeteners may result in an increase in % energy from fat as a result of the substitution of sugar with intense sweeteners. The third and final hypothesis of this thesis was that the use of high levels of intense sweeteners in potentially vulnerable groups such as insulin dependent diabetics will lead to a higher percent energy from fat in their diet. Nutrient and sweetener intakes were investigated using a 3 - day diary and the sweeteners MPLs from the Directive. Each investigation showed that diabetic has much higher usage levels of intense sweeteners in comparison to the controls ($p$ = 0.0001). Each investigation also showed no differences in fat % energy even though the diabetics had double the intake of the three indices of sweetener intake. Notwithstanding these more favourable patterns in diabetics, the fat tertile analysis clearly shows that the frequently observed inverse relationship between fat and sugar as a % of energy exists in both diabetics and controls. Thus the present cross sectional study therefore confirms the fat - sugar seesaw is present even among insulin dependent diabetics and it shows no evidence that the use of intense sweeteners leads to higher fat diets. No evidence has been produced in the present study to show that in a free - living population on a self - selected diet, intense sweetener intake influences macronutrient balance.

*To my parents for their continued love, support and encouragement throughout the years in everything I did.*

*We are all individuals and nowhere more so than over the matter of food.*

*Elsie Widdowson, 1962.*

(RIP 2000)

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## TABLE OF CONTENTS

## TABLES AND FIGURES

**FIGURES** **Page**

**CHAPTER 3**

## ABBREVIATIONS.

| | |
|---|---|
| % | Percent |
| %ile | Percentile |
| $90^{th}$%ile | $90^{th}$ percentile |
| $95^{th}$ %ile | $95^{th}$ percentile |
| $97.5^{th}$ %ile | $97.5^{th}$ percentile |
| ± | Plus or minus |
| < | Less than |
| ≤ | Less than or equal to |
| ≥ | Greater than or equal to |
| %cons | Percent consumers |
| % en | Percent energy |
| % en cho | Percentage energy from carbohydrates |
| % en fat | Percentage energy from fat |
| % en pro | Percentage energy from protein |
| % en starch | Percentage energy from starch |
| % en starch:sugars | Percentage energy from starch to percentage energy from sugars |
| 24h | 24 hour |
| 3 - d | 3 day |
| aces | Acesulfame K |
| ADI | Acceptable Daily Intake |
| *a priori* | From causes to effects |
| asp | Aspartame |
| ARMS | Adverse Reaction Monitoring System |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BDA | British Diabetes Association |
| bw | Body weight |
| C | Control group |
| CDC | Centre for Disease Control |
| CHO | Carbohydrates |
| Cont'd | Continued |
| Cyc | Cyclamic acid |

| | |
|---|---|
| D | Diabetic group |
| DNA | Deoxyribonucleic acid |
| DNSBA | Diet and Nutritional Survey of British Adults |
| *e.g.* | *'exempli gratia'*, for example |
| EI | Energy Intake |
| EI:BMR | Energy Intake: Basal Metabolic Rate ratio |
| EURODIAB | European Diabetes Study Group |
| *et al* | *'et alii'*, for 4 or more co - authors or co - workers |
| *etc.* | *'et cetra'*, and the rest |
| EC | European Commission |
| EEC | European Economic Community |
| ECOs | Expanded Clinical Observations |
| EU | European Union |
| FAO | Food and Agricultural Organisation |
| FDA | Food and Drug Administration |
| FFQ | Food Frequency Questionnaire |
| g | grams(s) |
| g/d | grams per day |
| g/household/yr | grams per household per year |
| g/kg bw/d | grams per kilogram body per day |
| Gen. Pop. | General Population |
| GI | Gastrointestinal |
| HPB | Health Promotion Board |
| *i.e.* | *' id est'*, that is |
| IDDM | Insulin Dependent Diabetes Mellitus |
| IEFS | Institute of European Food Studies |
| IgE | Immunoglobulin E |
| INFID | Irish National Food Ingredient Database |
| *in vitro* | In the test tube |
| IPFFQ | Interviewer Present Food Frequency Questionnaire |
| IUNA | Irish Universities Nutrition Alliance |
| ISA | International Sweeteners Association |
| ISI | Intense Sweetness Intake Index |

| | |
|---|---|
| JECFA | Joint FAO/WHO expert committee on food additives |
| kcal | Kilocalories |
| kg | kilogram |
| $kg/m^2$ | Kilogram per metre squared (BMI) |
| KJ | Kilojoule |
| lbs | pounds |
| LOAEL | Lowest Observed Adverse Effect Level |
| LOEL | Lowest Observed Effect Level |
| m | Metre |
| MAFF | Ministry of Agriculture. Fisheries and Food |
| mg | milligram |
| mg/d | milligrams per day |
| mg/kg bw/d | Milligrams per kilogram body weight per day |
| MJ | Megajoule |
| MJ/d | Megajoules per day |
| ml | Millilitre(s) |
| ml/d | Millitres per day |
| MNS | Macronutrient Substitute |
| MPL | Maximum Permitted Level |
| MRL | Maximum Risk Level |
| n | number |
| NC | Not calculated/given |
| NHDC | Neohespheridine DC |
| NIDDM | Non Insulin Dependent Diabetes Mellitus |
| NIH | National Institute for Health |
| NIEHS | National Institute for Environmental Health Sciences |
| NOAEL | No Observed Adverse Effect Level |
| NOEL | No Observed Effect Level |
| NS | Not significant |
| NSA | No Specified ADI |
| NSIFCS | North/South Ireland Food Consumption Survey |
| OECD | Organisation for Economic Co-operation and Development |
| *p* | probability |

| | |
|---|---|
| PMS | Post Marketing Surveillance |
| $r^2$ | R squared (coefficient of determination) |
| RF | Reduced fat |
| RNA | Ribonucleic acid |
| RS | Reduced sugar |
| sac | Saccharin |
| SAFFQ | Self Administered Food Frequency Questionnaire |
| SCF | Scientific Committee for Foods |
| SCOOP | Scientific Co-operation |
| SD | Standard Deviation |
| SI | Statutory Instrument |
| SPSS | Statistics Package for Social Sciences |
| TDS | Total Diet Study |
| th | Thaumatin |
| µg | Microgram |
| UK | United Kingdom |
| USA | United States of America |
| v | versus |
| WHO | World Health Organisation |
| x | Multiplied by |

## Publications

***The following publication has been derived from the work described in this thesis:***

**M. Cullen, J. Lambe, J. Kearney, M. Gibney, An analysis of the incremental value of retaining brand - level information in food consumption databases in estimating food additive intake. Food Additives and Contaminants, 1999, Vol. 16. No. 3, 93 - 97.**

# *Chapter 1*

# *Introduction.*

### 1.1 Introduction.

The primary aim of this thesis is to estimate food chemical intakes with a specific interest in the area of intense sweeteners. This chapter will take the format of four sections. Section A will deal with the introduction to food additives, Acceptable Daily Intakes (ADIs) and macronutrient substitutes (MNS). Section B will deal with the monitoring of human exposure to food additives. Section C looks at the previous sweetener studies that have been conducted, their methods, populations studied and results. Section D is the discussion.

## 1.2 Section A.

### 1.2.1 What are food additives?

***'Food Additive'*** *means any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods (EC 89/107, Article 1).*

Food additives are not a new discovery. For many years, salt, sugar and vinegar have been added to foods in order to preserve them. As a result of developments in food technology, there has been a move from natural to artificially - made chemicals. However, many substances produced this way are actually purely analogues of naturally occurring chemicals (Tennent, 1997). This move has resulted in the number of food additives increasing greatly and their function has become widespread, no longer used solely as preservatives.

There have been key growth factors in the increasing use of food additives

1. an increasing trend towards further processed foods (consumers want foods with minimum preparation time and no compromise on taste and quality).
2. to achieve this, production has become more complex and leads to inclusion of higher levels of additives.

3. health issues are increasingly important to consumers – the public now seeks foods reduced in fat/sugar for health reasons that also taste good.

Macronutrient substitutes (MNS) are a new area of food additives. They are food ingredients designed to replace the organoleptic (affecting the organs of sense) and / or functional properties of macronutrients such as fats or sugars in processed foods. They are unique due to the amounts that may be consumed, the nutritional or caloric compensations that may occur, and potential unique environmental fates (Fix and Allen, 1998). Several food additive categories are supported almost entirely by 'healthier' food and drink *e.g.* Sweeteners – replace sugar, decrease calories and increase dental safety and fat replacers – replace fat in foods, thus decreasing the caloric intake (Boyle, 1999).

The European Union (EU) legislation on food additives is based – as is much national legislation – on the 'positive' list system. This 'positive' list allows use of all food additives generally available commercially. Council Directive 89/107/EC of December 21, 1988 – the so-called Framework Directive – contains a list of approved categories of food additives for which more specific provisions are drawn up. Examples of these categories are Colours, Preservatives, Flavour enhancers and Sweeteners.

Companies may find new uses for these existing additives or develop new additives to replace ones already in existence, subject to the approval of the Scientific Committee for Food (SCF). Due to the fact that many food manufacturers and food retailers know only too well that their industry is 'consumer sensitive' there has been a growing trend away from the use of synthetic additives in recent years towards the use of equivalent substances extracted from natural products (Tennant, 1997). Whilst food additives mainly have one principal function, they can also be multi-functional. For example, in the Irish National Food Ingredient Database (INFID), sorbitol and sorbitol syrup (E420), were used as a sweetener, a humectant and a stabiliser depending on the manufacturer's requirements.

### 1.2.2 Advantages of food additives.

Food additives were developed by the food industry to enable certain functions to be carried out. One such function is the creation of a greater variety of foods for consumers

especially those with certain dietary restrictions *e.g.* diabetics. Another is to provide alternatives to the foods already available on the market for those requiring special diets *e.g.* diabetics, coeliacs *etc.* Food additives are also used to give processed food a natural look and taste or to replace attributes lost in processing *e.g.* colour and to improve shelf life of foods

### 1.2.3 Perceived concerns regarding food additives.

There are three perceived main health concerns with food additives. Firstly, intolerance *e.g.* allergic reactions that mostly have to related to exposure dose rather than immunological reactions. Secondly, food additives, particularly some artificial sweeteners are of concern in relation to being carcinogenic *i.e.* saccharin and cyclamate causing bladder cancer in rats at high doses. However if it were the case that they were carcinogenic in humans, their use would not be permitted. Finally, there is the issue of potential nutrient dilution of the diet in relation to high sugar diets for example and whether imbalance in the diet occurs as a result. The question remains as to whether or not this will apply in the case of intense sweeteners.

### 1.2.4 How are food additives evaluated?

All food additives must undergo extensive testing before they can be approved for use in food. This applies as much to natural substances as to synthetic analogues. Approval processes may vary from country to country but most authorities expect to be satisfied as to the safety and quality of food additives, as well as agreeing that there is a genuine need before giving approval for their use. Some 300 food additives are approved for use in food in Europe. Approval governs the foods in which additives may be used and limits the levels of use in each type of food. Government and industry work together to ensure that the risks associated with food additives are minimal (Tennent, 1997).

Some chemicals have the potential to be harmless and improve health or to cause harm and it is only the dose, which determines whether benefits or adverse effects will result. Hence the dose-response relationship is such an important part of risk assessment. Toxicological testing can throw light on the possible health consequences of exposure to chemicals in food. In the seventeenth report of the Joint FAO/WHO Expert Committee on

Food Additives of 1974, the principles governing toxicological evaluation of food additives were explained. There are two stages in the toxicological evaluation of a substance proposed for use as a food additive. The first is the collection of relevant data which are usually derived from experimental testing in the laboratory animals and whenever possible, from observations in man. The second is the interpretation and assessment of the data in order to arrive at a decision about the acceptability or otherwise as a food additive. Observations in man are of prime importance because of the differences between one species and another in reactions to toxic substances and the subsequent uncertainty when extrapolating data from animal experiments to human beings. These human studies are usually via post-marketing surveillance where the additive has already been approved and is used in foods that are available in the market.

In 1993 the EU issued a directive (93/35/EEC) stating that beginning in 1998, no cosmetic can be marketed in the EU if it was tested on animals. The 1993 National Institute for Health (NIH) Revitalization Act in the USA directs the NIH to conduct or support research into methods that do not require the use of animals or reduce the numbers of animals used. It specifically directs the National Institute of Environmental Health Sciences (NIEHS) to establish criteria for the validation and regulatory acceptance of alternative testing, and to recommend a process through which scientifically validated alternative methods can be accepted for regulatory use. The Organisation for Economic Co-operation and Development (OECD) promulgates harmonisation methods for use by its member countries for testing, labelling and registering chemicals in commerce, including food additives, pharmaceuticals and biocides. Many of the methods and guidelines under consideration by the OECD are designed to reduce animal use or replace laboratory animals with an *in vitro* system or with animals lower on the phylogenetic scale. cDNA microassay is a new and developing technology. It is used to analyse changes in genome-wide patterns of gene expression. cDNA microassays are used directly to compare the gene expression profiles of two RNA samples that are simultaneously hybridised to the chip. In other words, there is a control population and a treated population and RNA is isolated in both groups. The chip is then added to the RNA, mixed and then scanned for fluorescence, which indicates that the gene has been 'switched on'. Thus it can be determined what factors are involved in 'switching on' a gene. A problem with this method is that it can be

difficult to interpret as a number of genes rather than a single gene can be 'switched on', thus the ability to determine which particular gene is involved in a particular process *e.g.* formation of cancer cells *etc.* cannot be determined immediately.

### 1.2.5 Regulation of food additives.

Since the formation of the European Economic Community (EEC) and especially since Ireland joined the community in 1973, the range of foods and food products available to the consumer in Europe has increased significantly. With that in mind, in the late 1980s the European Union (EU), began the task of harmonising rules on the use of food additives, which can be seen as one of the major achievements of EU food law. The EU laws covering additives, colours and sweeteners are based on scientific and technological criteria, while at the same time ensuring that the protection of the consumers and public health are of paramount importance in any decision taken to authorise a particular food additive. In 1988, the European Commission came forward with a proposed framework Directive (89/107/EC), to cover all the major additives used in the manufacture of various food products. It is important to recognise that market forces provide an additional form of control. If there is a demand for foods, which are free of additives or certain categories of additives, then these can be made available. Consumer attitudes and perceptions can change rapidly and are not necessarily logical (nor is there any reason why they should be) and the industry can respond quickly. By contrast the system of legislative control is by its very nature fairly slow to respond and as far as possible should be based on a sound logical foundation (Wheelock, 1986).

### 1.2.6 Allergenicity of food additives.

A number of food additives are known to cause intolerance manifestations *e.g.* allergy and behavioural disorders like hyperactivity in susceptible individuals. Food additive intolerance *i.e.* urticaria after ingesting a formula containing tartrazine was first described in 1959 and behavioural disturbances in children in 1973. Although the list of additives used by the food industry is very extensive most of them have not been implicated in adverse food reactions (EC, 1997).

Additive intolerance is supposedly responsible for occasional putative cases of urticaria angioedema, anaphylaxis, asthma, atopic dermatitis, gastrointestinal disturbances (nausea, vomiting, diarrhoea, abdominal pain) and many other complaints such as migraine and hyperkinetic syndrome, for which no evident cause has been identified. In 1981, the Commission of the European Communities suggested a prevalence of food additive intolerance of 0.03-0.15%. Many estimates of the prevalence of additive reactions have been based on selected populations. The mechanisms of actions of additives in triggering adverse reactions are uncertain. Most evidence suggests that the action is most often pharmacological rather than immunological even if in a few cases a clear IgE – mediated reaction has been demonstrated *e.g.* reactions to carmine red colorant. Various mechanisms may be simultaneously responsible for adverse reactions in some foods.

In the last decades some studies have suggested a role of food additives in the genesis of some 'neurological' disturbances. Headache, epilepsy and childhood behavioural disorders, such as hyperkinetic syndrome, childhood hyperactivity and attention deficit syndrome, are the main entities reported as being likely related to a food additive intolerance (EC, 1997). No approval would be given for the use as a food additive, a substance causing serious or widespread hypersensitivity reactions. For food additives with an allergic potential, the inclusion on food labels of lists of the individual additives used has been implemented as a means of enabling sensitive individuals and their physicians to identify the possible sources of allergic reaction.

Self-diagnosis of food additive intolerance has become very common. In order to make a definite diagnosis there are three sequential steps involved. The first step is an elimination diet (a diet where all the additives have been removed). The cessation of reported symptoms must occur in order to be able to proceed with the second step. After deciding to proceed there is an open challenge with the suspected additive(s). If this challenge is positive, a double – blind placebo controlled challenge with the suspected food additive is performed. The gold standard for the diagnosis is a positive double-blind placebo-controlled challenge with the suspected additive (EC, 1997).

The fact is that a variety of common foodstuffs, as well as artificial colours and preservatives have been shown to produce allergies and / or other forms of reaction. The problem with food allergies will not be solved by banning those additives that cause a

reaction since this only represents a small proportion of those who are affected. Further more, those who are sensitive to additives can often react to other substances that are eaten or inhaled (Wheelock, 1986).

Aspartame and sucrose have been suspected of causing problems with behaviour and cognitive performance in children although many studies have been conducted that have not found consistent adverse effects (Wolraich, *et al.*, 1994). In contrast, Walton *et al.*, (1993), concluded from a double blind challenge study in 13 patients with a history of depression and controls with no such history, that there was a significant difference between aspartame and the placebo group in number and severity of symptoms. No such symptoms were recorded in individuals with any history of depression. Hence the inference is that individuals with mood disorders are particularly sensitive to this artificial sweetener and its use in this population should be discouraged.

### 1.2.7 Interpretation of findings.

The objective in assessing the toxicological data on food additives is to ensure their safety for the consumers on the basis of all evidence available to the JECFA Committee at the time. Future results with present methods or with techniques yet to be developed will necessitate re-assessments that may lead to changes in earlier decisions.

The general procedure adopted has been to establish an acceptable daily intake (ADI) for man for each food additive or groups of food additives. When the toxicological data are derived from animal experiments, their extrapolation to man involves the application of a safety factor to the highest 'no effect level' obtained in animal studies.

#### *1.2.7.1 No effect level/No Observed Adverse Effect Level (NOAEL)*

When applied to data from animal experiments, the term 'no effect level' refers to the level of a substance that can be included in the diet of a group of animals without toxic effects (FAO/WHO, 1974). At its meetings, the Committee has considered a variety of effects that in the present state of knowledge are not deemed to be of toxicological significance, provided they are fully attributable to normal physiological adjustment and are reversible. They include for example changes in intestinal flora, laxative effects due to bulk or osmotic load, caecal enlargement, diminished growth rate caused by high levels of

non-digestible substances, liver hypertrophy and induction of microbial enzymes due to gross overloading with certain metabolisable substances (FAO/WHO, 1974).

Routine safety studies have always contained end points (*e.g.* clinical observations, histopathology) relevant for neurotoxicity assessments. The Expanded Clinical Observations (ECOs) in toxicity studies were developed and outline detailed guidelines for the evaluation of neurotoxicity in the test animals. Studies have demonstrated that sophisticated functional tests could reveal significant effects of drugs or brain lesions in animals that had no gross motor or sensory impairment. Similar phenomena were demonstrated in animals treated with neurotoxicants. As agencies considered the new testing guidelines for neurotoxicity, functional changes emerged as important end points for detecting and characterising neurotoxic effects. It was concluded that a systematic assessment of function should be included in toxicity tests.

Many of the ECOs observations fall within the standard clinical observations (*e.g.* salivation, lacrimation) and additional end points included address nervous system functions (*e.g.* auditory startle, observation of gait). In the case of ECOs, the subject is walking in a standard arena (*i.e.* table - top or selected area for walking) and all observations are recorded on a scale and reported. Whereas with traditional observations, the subject is observed through a cage wall or hand held and only abnormalities are recorded (Ross, Mattson and Fix, 1998).

The ECOs allow the detection of more subtle changes in the test subjects regarding their nervous system functions, thus detecting neurotoxic effects at an earlier stage in comparison to the traditional method. This leads to greater confidence in the results found and the safety factor that is applied.

In the U.S., the Agency for Toxic Substances and Disease Registry (ATSDR) uses the highest no-observed adverse effect level (NOAEL) or less serious lowest observed adverse effect level (LOAEL) in their database to derive Minimum Risk Levels (MRLs). An MRL is 'an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure'. The hazardous substance can be one of environmental or food source. It contrasts with the ADI in that the ADI value is based *over a lifetime* and also is from a food source.

### 1.2.8 Extrapolation of data to man.

In the extrapolation of animal data to man, the application of a safety factor is required. This factor is to allow for any differences in sensitivity between animal species and man, to allow for wide variations in sensitivity among the human population and to allow for the fact that the number of animals tested is small compared with the size of the human population which may be exposed.

It was recognised by the Committee (FAO/WHO, 1974), that the expression of the ADI in terms of body weight (bw) does not reflect the relative exposure of animals of different sizes. However, in practice the method of expressing the dose in terms of mg/kg body weight has proved satisfactory.

A safety factor of 100, the figure recommended by the Committee in its second report, has been widely accepted (FAO/WHO, 1974). Safety factors are intended to provide an adequate margin of safety for the consumer. The safety factor of 100 is based on the assumption that the human is 10 times more sensitive to the substance than experimental animals and that there is a 10 – fold range in sensitivity within the human population (10 x 10) (Hermann and Younes, 1999). It would be unreasonable to apply this figure too rigidly, for example, in the case of substances that are normal constituents of the human diet or are normal intermediary metabolites. When there are adequate data to show that in the human body a substance is converted by digestion or metabolised to a normal constituent of the diet or that a substance is not absorbed from the gastrointestinal tract, these data are used in the evaluation. When toxicological data derived from experiments in man are available they may be used to provide a lower safety factor since they obviate the need for interspecies extrapolation.

On the other hand, there may be reasons to increase the safety factor, for example, when the amount and/or the quality of toxicological information is limited. Furthermore, the nature of the toxic effect produced by an additive at very high levels might demand an increase in the factor in order to ensure safety in use. The use of default uncertainty factors is a central part of risk assessment but the scientific basis of their original derivation is unclear. A major problem with the use of the standard default uncertainty factors has been the absence of a mechanism for allowing compound – specific data in a particular area of

uncertainty to contribute to the derivation of the relevant, compound specific uncertainty factor.

The issue of whether or not extrapolating animal data to humans is effective in protecting the human population has always been one of controversy. Knudsen in 1999 concluded in his study that 'the cellular makeup in terms of cellular energy supply, synthesis, cell proliferation and apotosis as well as DNA repair mechanisms appears to be very similar in all mammalian species, including humans. The life cycle for all species is focused on the strive for producing and safeguarding the next generation. Therefore, the biology of total lifetime matches the qualitative and quantitative challenges of life from one species to another in an equal manner, making the animal lifetime study both the most relevant and the most important instrument for toxicological safety assessment'. Clayson (1998) states that the low dose risk assessment is used to calculate the likely effects of carcinogens, identified by the rodent bioassay using very high exposures to the test agent, in terms of their ability to induce tumours at doses more equivalent to exposures suffered by humans. Four prudent default assumptions underlie the processes used so far:

1. all carcinogens identified in animals will express a carcinogenic effect in humans.
2. there is no safe level or threshold for any carcinogen.
3. linear low dose extrapolation is the appropriate method by which to calculate the probable response of humans based on the high exposures that constitute the animals bioassay data.
4. relatively few rodents are used in the bioassay and it is preferable to use upper confidence levels rather than the real data for extrapolation. While most rodent models have some merit for understanding mechanisms, no model accurately predicts the spectrum of human responses, assuming that humans respond at all.

   Therefore, well-planned experiments involving human cells and tissues should be undertaken whenever possible to determine the relevance of animal models. Only in this manner can we be confident that risk assessments based on animal bioassay data are meaningful (Casanova, 1996).

### 1.2.9 Acceptable daily intake (ADI) for man.

The concept of the ADI was originally developed in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) who defined it as ' an estimate of the amount of food additive, expressed on a body weight basis, that can be ingested daily *over a lifetime* without appreciable health risk'. The concept is based on the premise that toxicity is a threshold, that the organism has a limited capacity to adapt to chemical challenge and only when this capacity is exceeded are toxic effects manifested (Walker, 1998).

An ADI is allocated only to substances for which the available data include either the results of adequate short-term and long-term toxicological investigations or satisfactory information on the biochemistry and metabolic fate of the compound, or both. An ADI may be allocated temporarily pending the provision of additional data within a stated period of time. This measure implies that the toxicological data are adequate to ensure safety in use of the additive during the time for which the temporary ADI applies. If the additional data requested do not become available within the stated period, the temporary ADI may be withdrawn at a future meeting of the JECFA.

An ADI without an explicit indication of the upper limit intake ('not limited'), may be assigned to substances of very low toxicity especially those that are food constituents or that may be considered as foods or normal metabolites in man. An additive having an 'ADI not limited' must meet the criteria of good manufacturing practice – for example, it should have proven technological efficacy and be used at the minimum level of efficacy, it should not conceal inferior food quality or adulteration and it should not create a nutritional imbalance. In the opinion of the JECFA, an ADI provides a sufficiently large safety margin to ensure that there need be no undue concern about occasionally exceeding it provided the average intake over longer periods of time does not exceed it.

#### *1.2.9.1 The allocation of ADIs to additives that are related chemically and toxicologically.*

As a number of food additives are closely related chemically and toxicologically a system of grouping additives for the purposes of evaluation was devised (FAO/WHO, 1974). In such cases, the ADI level is expected to cover all specified members of the group that may be included in the diet. A given food additive may be related to two groups, in which case

the level in the diet must not exceed the maximum acceptable level for either group. The problem is not so complicated as it may appear at first sight, since many of the substances in a group of additives have technologically related functions and are therefore likely to be used as alternatives to each other.

*1.2.9.2 Exclusion from the ADI of amounts occurring naturally in foods.*

The ADI includes only the amounts of the substance used as an additive, excluding 'amount naturally present in food'. There is a single exception namely, the ADI for phosphates which includes all phosphate, whatever the source.

*1.2.9.3 Other Conclusions.*

Other conclusions on food additives may be summed up in the following terms:

(a) 'decision postponed' (pending clarification of matters related to technological use).

(b) 'no ADI allocated' (in the absence of sufficient information to establish safety or of adequate specifications).

(c) 'not to be used' (where there is sufficient information on which to base such a decision).

It has been pointed out that the ADI value, which depends on a series of factors, is not a constant, but a guide serving to calculate the admissible limits of diverse chemical agents incorporated in foodstuffs (Truhaut, 1992).

*1.2.9.4 Who is at risk of exceeding the ADI of food additives?*

A group 'at risk' is by definition a part of the population more exposed to hazard than the rest of the considered population. In the field of food safety, this notion includes, on the one hand, factors linked to the physiological or pathological characteristics of the sub-group and, on the other hand, factors linked to the composition of ingested foods (Verger, *et al*, 1999).

Food additives are used in the formulation and production of many food products on the market. When a food additive has been approved, it may be used in many products in conjunction with other food additives from the same category or other categories. The

function of the food additive needs to be examined. A question is whether or not the food additive is of specific importance to the general population or to sub-groups of the population that may be found to consume a greater level of the food additive due to special dietary needs or desires. Many groups within a population are at potential risk of being over exposed to a food additive or a group of additives, excluding babies and infants. In order to be able to determine the groups at risk, investigations of actual intakes by these groups needs to be carried out.

According to Renwick and Walker (1993), the questions that need to be considered are (a) what proportion of the population should be allowed to exceed the ADI, (b) to what extent can the ADI be exceeded without any real concern and (c) how long does the person need to exceed the ADI before there is a cause for real concern?

Use levels are generally set for 'high consumers' of the product who may consume two or three times the mean intake. If use levels are based on intakes in such high consumers, it is likely that 'extreme consumers' will exceed the ADI to a limited extent on some occasions. The real problems for individuals with the type of diet that would be likely to exceed the ADI are likely to be nutritional adequacy rather than an 'overdose' of food additives. To set use levels of foods additives to cover such individuals would not be logical and would not benefit the vast majority of the population who have a sensible mixed diet.

The significance of any pattern of intake in excess of the ADI must be assessed on a compound – by - compound basis and by reference to the NOEL, which is the basis for the ADI calculation. The magnitude of a non-significant intake in excess of the ADI should be related to (a) the difference between threshold (NOAEL) and NOEL, (b) the incidence and variability in the lesion in animals given the NOEL and (c) the slope of the dose-response. The duration of nonsignificant intakes in excess of the ADI should be related to (a) the duration of intake in animals necessary to produce toxicity and / or (b) the time required for accumulation of the compound in humans to give steady state body loads.

## 1.3 Macronutrient substitutes (MNS).

### 1.3.1 What are MNS?

Macronutrient substitutes (MNS) are a new and developing sector in the area of food additives. They are products used to replace the organoleptic and / or functional properties of sugars or fats in foods and which contain substantially less available energy. Macronutrient substitutes include a broad spectrum of products, and the safety evaluation of these substances must be flexible enough to accommodate a variety of properties and uses. The potentially broad use of this emerging class of food additives has created issues that need to be addressed during safety evaluation (Borzelleca, 1992). It is important to realise that 'most consumers refuse to sacrifice taste for perceived health benefits'. It is naïve to believe that significant fat reduction can be achieved in our diet without modifying foods to satisfy both taste and convenience' (Davidson, 1996).

Macronutrient replacement or reduction in foods promises a simple, 'painless' and readily maintained means for reducing sugar and fat intakes, but there has been little evaluation of the situation in which modified foods are consumed more casually as part of a 'normal' diet, as opposed to a diet aimed at specific weight loss or a diabetic or low fat diet (Gatenby *et al.*, 1997). Since dietary guidelines call for a reduction in fat and saturated fats and an increase in the intake of carbohydrates – the question arises as to whether or not macronutrient substitutes affect adherence to these guidelines. The major assumption is that people will compensate for the reduction in energy in their diets, so they will eat to appetite, but they will not specifically seek to replace fat with fat or sugar with sugar (Cheney, 1996).

A good deal of the stimulus for the development of these new food components has been the recognition that various dietary constituents are risk factors for the development of conditions such as atherosclerotic heart disease, obesity and dental caries. Some better known macronutrient substitutes include fat replacers, bulking agents, and bulk sweeteners. Fat replacers are represented by several classes of products including synthetic fat - like products, chemically modified fats, microparticulated proteins and products that are carbohydrate based. These include Olestra, Caprenin, Simplesse and a number of other products such as modified starches and the processed natural products carrageenan and pectin. Products that are used as bulking agents in conjunction with non - nutritive

sweeteners include polydextrose, modified starches and various types of cellulose. A significant degree of overlap exists between fat replacers and bulking agents as several products serve both functions. The bulk sweeteners include polyols such as lactitol, isomalt and sorbitol (Munro *et al.*, 1996).

### 1.3.2 Safety of MNS.

Traditional approaches to assure a 'reasonable certainty that no harm would result from the substance under its intended conditions of use' for this unique class of food ingredients are neither appropriate nor adequate. Some of the issues that must be addressed in developing an appropriate safety evaluation program for MNS include :

- a realistic estimate of consumption and consumption patterns,
- potential nutritional imbalances (caloric and / or nutritional),
- potential interactions with essential nutrients with physiological and / or pharmacological side effects (such as laxation),
- stability of the MNS during processing and storage,
- the biodisposition of the MNS and its transformation products in the body,
- safety of workers involved in the production and / or processing of the MNS,
- the impact of the MNS on the environment.

These new ingredients contain substantially less available energy (lower calories), may come from traditional foods such as starches, gums or pectins and proteins or may be new ingredients and may be consumed in large quantities (grams) daily. Due to the potential high consumption of MNS and the impact on nutrition, the use of traditional safety factors is inappropriate due to the practical limitations of administering very high doses to animals. The proposed approach incorporates some of the features of established safety evaluation programs: focuses on comparative (including human) biodispositional and mechanistic studies, stresses comparative nutritive and physiological effects and introduces appropriate evaluations in humans early in the process. The process begins with an evaluation of the potential benefits and risks to the consumer to be derived from introducing the MNS into the food supply and proceeds in a scientifically sound and logical testing sequence from safety studies in animals to appropriate studies in humans and concludes with postmarking surveillance.

The acquisition of information relevant to the areas of differences in species and differences in sensitivity between humans can lead to substantial reduction in the need for a large safety factor for a food ingredient. This is an important consideration because one of the basic dilemmas in assessing the safety of macronutrient substitutes is that it is often not possible to feed enough of the macronutrient substance to an animal to achieve dosages that are 100 fold greater that the amount expected to be consumed by humans (Munro *et al.* 1996).

A three tiered approach to determining the safety of the MNS is used. In the first phase, 'information gathering and assessment', the data is critically evaluated by the toxicologists and other appropriate individuals. If the available data are adequate to support an ADI, then one is determined and appropriate regulatory consideration addressed. After the macronutrient has been introduced into the market place and the PMS data show that the actual consumption is less than the ADI, then additional uses of the new ingredient could be considered. If the available data are considered inadequate to establish an ADI, then appropriate studies are designed and executed consistent with scientific principles of experimental design including identifying appropriate parameters and possible outcomes.

In phase two, 'the toxicological characterisation of the new ingredient', toxicologists critically evaluate the data and a determination to proceed is made or not. The toxicologist could conclude that further studies are not needed based on the NOAEL and an appropriate uncertainty or safety factor and an ADI is proposed and appropriate regulatory considerations are addressed. Postmarketing surveillance (PMS) should be initiated when adequate penetration into the market has occurred. If the data is inadequate to establish an ADI, the following studies are conducted: a repeat dosing study in humans and suitable reproduction / developmental toxicity studies. These data are critically evaluated and an ADI is determined. If the data are inadequate to establish an ADI, then appropriate special studies are conducted (phase 3).

In phase three, the need for lifetime studies in animals will be minimal, as there will be extensive data in humans and adequate genotoxicity data. All available data are critically evaluated and an ADI proposed. In these safety studies particular attention is focused on the effects of the ingredient on physiological functions, especially of the gastrointestinal tract and on nutrition (*i.e.* potential interactions with nutrients). The effects

of the ingredient are evaluated in humans as soon as the animal data support human testing to provide information on tolerability. Ingredients are considered on an individual basis (case – by – case), due to their uniqueness and studies are initiated only after benefits to the consumer have been identified. PMS will provide continuing assurance of the safety of the ingredient to the consumer, the regulatory agencies and the manufacturer (Borzelleca, 1996).

Human studies are introduced early in this approach because humans are the ultimate target species, because some biologically significant parameters cannot be adequately evaluated using current non-human models and because subjective and often subtle effects can only be determined and evaluated in humans. Human studies provide a more rational basis for selecting safe exposure conditions for consumers and determining human acceptability.

### 1.3.3 Effects of MNS.

According to Glinsmann (1996) 'with macro - additives of any kind, there is going to be a need to deal with a very small, if not really negligible, exposure safety factor before some reasonably predictable effect occurs'.

While effects on gastrointestinal function are not traditional toxicological end points, they need to be carefully evaluated within a general safety review of a new MNS food additive. The question is do we have sufficient knowledge to decide if reasonably short – term studies are adequate to predict long-term health outcomes when the MNS is associated with many dietary changes involving other components in the food supply that also impact on health.

Another question is if you have a variety of different macronutrient additives that have similar potential for an adverse effect on nutritional status or bowel function, or perhaps their effects are interactive, how do you take into account these actions when you deal with the cumulative effect of an additive which is the safety end point that you need to consider. Does this mean that effects of all like compounds that produce similar interferences with nutrient absorption or effects on laxation need to be taken into account? Many MNS have been derived from traditional food sources such as gums, proteins and dietary fibres. Considerable information exists on how they affect digestion and

gastrointestinal (GI) physiology. Many newer substitutes have novel structures, which are minimally or almost totally nonabsorbed. Their safety assessments are predicted on lack of absorption.

Their effects on gastrointestinal function can be quite considerable and there are few standardised animal models with GI tracts that are similar to that of the human. Some macronutrient replacements that have been approved or are being tested for approval certainly do have very significant effects on nutrition and laxation. Poorly absorbed or non-absorbable fat substitutes have the ability to interfere with the absorption of fat-soluble vitamins in a fairly complex manner. A safety factor of 2.5 was proposed in order to reflect the population at large as only a small number of humans are typically evaluated.

Poorly absorbed MNS may interfere with the absorption of nutrients or may alter GI functions. Human testing should be conducted along with appropriate animal or non-animal surrogate models that evaluate nutritional interferences, changes in GI physiology and changes in microbial ecology of the bowel. For food additives, including MNS there can be no risk to the consumer. These considerations make the evaluation of MNS unique. The hazards associated with a novel food should be no greater than that of the original product nor should it be required to be safer than the original product. In ensuring the safety of MNS the following elements are important:

(1) The chemical and physical nature of the material.
(2) The source and derivation.
(3) The similarity to approved and normal food ingredients.
(4) Stability during processing.

and in the final product, comparative biodisposition including metabolism in animals and humans specific end points (such as GI effects) and mechanisms of toxicity (Fix and Allen, 1998).

### 1.3.4 Evaluation procedures of the safety of MNS.

Most procedures for evaluating the safety of food additives were designed for additives consumed in small (<1g) quantities per day and form an insignificant part of the diet. MNS may be consumed in gram quantities per day (g / d) because they may replace energy-dense traditional food constituents and may form a substantial part of the diet (for example,

total replacement of fat in the diet could result in the MNS becoming 15 - 20 % of the diet by weight). These potentially large exposures are part of the uniqueness of the MNS compared with traditional food additives. If MNS were to be evaluated similarly to traditional additives, that is to say by testing for safety at substantial multiples of the anticipated human exposure, adverse effects caused by nutritional imbalances could confound the toxicological interpretation of the data unless there is caloric compensation (*e.g.* animals cannot tolerate much above 15 - 20% non-nutritive diet without developing nutritional problems). Even if the test substance is not absorbed, it could interfere with absorption of nutrients or alter the normal physiological functions of the GI tract. Nutrition is critical in the design of appropriate safety-evaluation studies of MNS.

The initial step in evaluating the safety of a MNS is test – material characterisation, exposure assessment and initial safety assessment. Test material characterisation involves generating and evaluating information on the uniqueness of the test material, its source or synthetic process involved in its production (in detail sufficient to enable an assessment of the toxicity of the starting material, contaminants, intermediate and interaction products), its properties (chemical, physical and functional), manufacturing specifications (including major and minor components, impurities, by-products and stabilisers) and its stability during processing, storage and use. It may be necessary to identify specific degradants and test them individually because macronutrients may be consumed in large quantities and amounts of these degradants in the diet could be substantial.

Anticipated consumption should be based on the maximum anticipated replacement or on the assumption that the test material will totally replace an approved food constituent. These data should be developed for realistic maximal exposure (*e.g.* the $90^{th}$ percentile because it reflects heavy users) to ensure safety. Potentially sensitive subpopulation groups should be identified and appropriate exposures anticipated for each group. Exposure estimation should consider age, sex and dietary habits of consumers. Consumption patterns may change in response to the introduction of other MNS (*e.g.* sugar substitutes) and should be considered in assessing potential exposure. Targeted human studies should also involve a rational sequence from early single – dose studies in healthy human volunteers to dose-ranging longer term trials in both a controlled and free-living environment. The dose-ranging studies should be followed by special population studies

(*e.g.* elderly, children *etc.*), that may have a higher incidence of adverse effects. The risks associated with the proposed food additive should be weighed against the potential health benefits and health care giving cost savings (Davidson, 1996).

### 1.3.5 Potential problems with MNS.

Nevertheless, some potential problems with fat substitutes can be dealt with through product modification, other through a combination of labelling, consumer education and careful PMS. For example, most products can be fortified to replace any loss of essential fatty acids and fat - soluble vitamins. Regarding the oft-mentioned laxative effect of certain fat substitutes, most adults can recognise and deal with laxation by altering their diet. For those people, principally children, who cannot recognise the association between laxation and their diet, special labelling may be workable (Heckman, 1996).

Compliance with a low fat diet is enhanced by the substitution of foods with the same organoleptic properties of fat, namely flavour, texture and mouth feel. The safety of MNS must be established prior to introduction into the food supply. As their use increases so will concerns about their safety (Borzelleca, 1996).

### 1.3.6 Tissue uptake and storage of macromolecular food additives.

Information on the tissue storage of macro-molecular materials is frequently lacking. Unlike lipid-soluble substances whose storage within the body is non-specific, some macromolecular materials are localised within lysosomes of cells of the reticuloendothelial system (*e.g.* degraded carageenan). The consequences of uptake and storage of macromolecular substances in reticuloendothelial cells are not well understood, but there is some indication that alterations of phagocyte function may occur. In view of the availability of a range of techniques (analytical, histochemical, ultrastructural and autoradiographical) to supplement light microscope observations, further studies to obtain information on the issue storage of macromolecular food additives should be carried out (FAO/WHO 1974).

### 1.3.7 Potential impact on nutritional balance by MNS.

Intense sweeteners can be described as a category of MNS since they are designed to replace sugar (which is a carbohydrate) in a food without affecting the sweet taste of the food. The public health issue of intense sweetener usage is always related to the ADI, which is based on detailed toxicological evaluation. However, it is possible that the use of intense sweeteners, displacing sugar in the diet, may lead to distortion of dietary macronutrient intakes. Fat and sugar, as a % of energy, have been shown in several studies to be inversely related, the so-called 'fat – sugar seesaw' (Gibney, 1990, Gibney, 1993, Gibney, 1995, Lewis *et al*, 1992, Moloney 1993, Naismith *et al*, 1995, Flynn *et al*, 1996). This raises the question that a decrease of sugar intake in a diet through the use of intense sweeteners could result in an increase in the % energy from fat in the diet. This could be regarded as an adverse effect of sweetener usage depending on the type of fat consumption and the level of increase in fat consumption in the diet. Several intervention and cross sectional studies have investigated this area and their results have been conflicting.

One such study has investigated the longer-term effects of covert substitution of aspartame for sugar or vice versa on patterns of nutrient intake in free-living subjects (Naismith and Rhodes, 1995). The covert removal of 500 kcal of sugar and its replacement by an equal level of aspartame (to provide the same level of sweetness), over 10 days among free living subjects led to an 8 % fall in energy intake and an 11 % rise in total fat intake. The respective covert substitution of aspartame with sugar increased energy intake by 8 % and decreased total fat intake by 5 %. In contrast, a study by Gatenby *et al* (1995), investigating the extended use of foods modified in fat and sugar content and the nutritional implications in 49 free living non-obese females aged 18 – 50 years old for 10 weeks. The use of reduced sugar foods led to significant reductions in sucrose intake but not in intake of total sugars. However, in a sub – set analysis of the data it was found that for subjects starting from a low fat intake, use of reduced fat foods prompted a marked increase in percent energy from fat. When subjects were classified by initial sucrose intake, high sucrose consumers changing to the use of reduced sugar foods were found to increase their fat intake. Analyses based on subjects' initial diets showed no significant difference in changes in total energy intake or body weights (Mela, 1997). Chen and Parham corroborate these findings as in a study of 135 healthy young people

which included a few serious dieters, scant evidence was found that the use of high intensity sweeteners conferred any nutritional benefits. They do however note that these findings may not apply to persons with diabetes and others who are committed to a restricted intake of sugars (1991). Anderson and Leiter (1996), also state that 'there is no evidence that present intense sweetener availability and use has had an impact on the macronutrient contents of diets and therefore consumption of high intensity sweeteners is not associated with obesity.

Thus, there is still debate as to whether or not the use of intense sweeteners can increase % energy from fat intake by displacing sugar. If it is the case, this phenomenon could also be as important as the ADI in evaluating the public health significance of widespread use of intense sweeteners.

## 1.4. SECTION B – Monitoring Human Exposure to Food Additives.

### 1.4.1 Why is it important to monitor?

Monitoring allows determination of the type, weight and frequency of consumption of foods containing the food additive under investigation. The results will demonstrate whether or not there is a particular group consuming the food additive and whether or not the eating patterns of the population have changed, resulting in an increased or decreased exposure level to a food additive. There is also the legal requirement to monitor as the 89/107/EEC Framework Directive states that 'all food additives must be kept under continuous observation and must be re-evaluated wherever necessary in the light of changing conditions of use and new scientific information'.

### 1.4.2 What methods are available to monitor intakes?

Measurement of food additive intakes is complex and a number of different approaches are possible. A number of aspects need to be considered in the design of the study, in order to produce useful and reliable data. There is a wide variability in the intake of food additives, both between individuals and in the same individual from day to day. The number of subjects and the nature of the population studied are critical. Intake studies also need to

consider any special groups identified *a priori* to be of importance, *e.g.* children and pregnant women, or in the case of diabetics who may have an above average intake of intense sweeteners (Renwick, 1999). The food consumption survey enables one to monitor in a meaningful manner the consumption of intense sweeteners and to evaluate the legal concentrations (Hulshof and Bouman, 1995). Food consumption data may be collected at the national, household or individual level.

The importance of assessments of dietary intake in characterising any potential risk posed by food additives and contaminants is recognised in the 49$^{th}$ report of the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA). The methods for assessing dietary intake are briefly explored and it is worked on the basis that the estimation of potential dietary intakes is the same for all food chemicals (JECFA, 1999).

In beginning a quantitative assessment of food chemical intake, it is generally agreed that the starting point should be with the crudest and most simple method. If such methods indicate that there is no basis for public health concern then no further analysis is needed. Increasing the refinement of cruder approaches is costly and time consuming and thus should be undertaken only when absolutely necessary. Refining crude data can be achieved by means of more precise intake data or more precise food additive concentration data (Gibney, 1999). In most cases, assessments of the intake of food additives are conducted using data on consumption of processed foods. Five main approaches for assessing dietary intakes of food additives have been used by countries at national level. These approaches usually over - estimate chronic (long-term) daily intake and this could compensate for potential differences in intake between population sub-groups and for day-to-day fluctuations in individuals (FAO/WHO, 1999). Food intake methodologies can be broadly classified into direct and indirect methods. Indirect methods are the likes of food balance sheets or household budget data. The advantage of using such methods is that they are inexpensive and provide reliable time trends in food intake patterns. The disadvantages for food additive intake of these methods are that the data is at household / national level and not individual. In the context of food chemical intake this estimate of consumer only intake is of critical importance (Gibney, 1999).

### 1.4.3 Screening methods.

A screening method is a conservative method by which it can be determined whether or not an additive can be prioritised for more detailed assessments. The advantages and disadvantages of each screening method are summarised in Table 1.1.

*1.4.3.1 The Danish Budget method.*

The budget method was developed by the Danish National Food Agency over twenty years ago as a simple, practical and inexpensive way of setting maximum levels of use of additives, ensuring that the ADI would not be exceeded. The method can be used as a first screening mechanism to identify additives for which the proposed levels of use require closer scrutiny. This method is based on the fact that there is a physiological requirement for energy and fluid. It is assumed that the intake of processed food does not exceed 12.5g / kg bw / d. If the additive were found in all consumed processed foods *i.e.* 12.5g /kg bw/ d, it would be safe to allow the ADI expressed in mg/kg bw in this amount of food: ADI mg per 12.5g of food is equivalent to 80 times the ADI mg/kg bw of food.

Similarly for beverages, it is assumed that the consumption of soft drinks does not exceed 25 ml /kg bw / d. If the level of a given additive does not exceed the ADI mg/25 ml of soft drink (40 x ADI mg/l), then again the ADI could not be exceeded. (Nutriscan, 1994). If an additive is found in both solid foods and beverages, the ADI is divided between the two and the same principles apply.

According to Douglass *et al*, (1997), the Budget Method appears to be a conservative first screen for establishing priorities for monitoring consumption and use of food additives based on potential lifetime average exposures. A theoretical maximum daily intake (TMDI), additive is estimated by dividing the MPL in mg/kg by a factor chosen from the budget method assumptions. If the additive is allowed in a wide range of foods, factor 80 is chosen, if the foods in which it is found do not form a major part of the diet, a factor of 160 is chosen. This is repeated for solids and beverages and the totals are compared to the ADI:

| **Solids** | | **Beverages** | | |
|---|---|---|---|---|
| MPL | + | MPL | = | TMDI |
| 160 (80) | | 40 | | |

If TMDI ≤ ADI, there is no cause for concern. If TMDI ≥ ADI, progress to next level of refinement.

However, because of its simplicity it is generally accepted as an appropriate screening tool at the international or national level for the identification of food additives that require further assessment (FAO/WHO, 1999). Verger *et al* (1998) states, that the Danish Budget Method is not appropriate for all additives, for example when a given additive is permitted in foods or food ingredients that are minor parts of the diet. Other approaches are then needed.

*1.4.3.2 The model diet approach.*

The Model Diet method is used to predict the intake of a non-average individual to determine whether all of the population is protected by nationally or internationally agreed standards. This method can be used to ascertain whether more information is required to make better assessments. A model diet is constructed using a hypothetical diet. To protect all segments of the population, exaggerated consumption values may be used to make up the model diet. These values are then combined with residue concentrations to estimate dietary intakes. This is used to construct a model diet from information on food consumption for a selected population subgroup.

Model diet methods are useful when limited information is available. They provide a simple means of screening chemicals to identify situations where no further assessment is required or a more refined approach is called for. Independent sources of residue data are used with model diets (usually derived from monitoring programs), which may not be representative of the foods used in the model diet itself. Another limitation is that the quantity of foods used to construct the model diet may be so high that they will produce correspondingly high intakes of questionable value (Parmar, Miller and Burt, 1997).

### 1.4.4 Indirect Methods

*1.4.4.1 The food balance sheet/household survey approach.*

Sufficient information is available in most countries to make a *per capita* estimate. These estimates are calculated by dividing the total quantity of food chemical available in the country by the total number of individuals in the population. This would take into account

production with allowances for quantities used for non-food purposes, exported or imported. Another method for estimating a *per capita* intake would be to sum the product of the per capita consumption estimate (food balance sheets) and the residue levels (permitted, analysed) for each individual food (Parmar, Miller and Burt, 1997).

This is based on the food available for consumption either at a national or household level. Food balance sheets are adjusted for the proportion of each raw commodity that is processed and likely to contain the food additive being studied. Food supply data at the national level such as food balance sheets or food disappearance data provide gross estimates of the national availability of food commodities.

This method has the advantage in that it is inexpensive and less time consuming when compared to other methods. It is a good method to use for initial screening purposes and to indicate cases where dietary intakes are high or to prioritise a long list of food chemicals which require further investigation (FAO/WHO, 1998). It may not be suitable for estimating intakes of some environmental contaminants as it assumes that these are evenly distributed and so localised contamination is not considered (Parmar, Miller and Burt, 1997). A major limitation of national food supply methods is that they reflect food availability rather than food consumption. Losses or other uses of foods are not easily accounted for. These data do not provide information on the distribution of foods among individual members of the household (FAO/WHO, 1998), thus any variations due to dietary habits will not be determined.

### *1.4.5 Direct methods*

#### *1.4.5.1 Total Diet or Market Basket Methods.*

The total diet study (TDS) or market basket is based on dietary intake by a defined population group, which construct the national average diet. All food items that are part of this national average diet are purchased and prepared according to standard household procedures. The foods are then separated into groups of like foods, usually between 15 and 25 groups, and each group is then available for analysis for a range of contaminants or nutrients. The measured concentrations are multiplied by the consumption estimates of typical diets to estimate the overall intake. A TDS may be carried out several times a year

to take into account seasonal variation or repeated every 2 – 5 years to monitor trends in consumption and intakes.

The main advantage of the TDS is that it is relatively more cost effective than duplicate diet studies. Repeated studies can show up long term trends in eating habits. The source of the contamination and hence the contribution to intake can be established with a TDS but is limited to food groups only. Another advantage to using a TDS is that it can provide estimates of background intake levels. The TDS is limited to looking at the diet of a typical or average consumer and so its results cannot be applied to specific individuals or consumers who consume higher than average amounts of food. The food groups are widely defined in a TDS, which makes it difficult to obtain intakes from individual foods. A proportion of the TDS may be based on production statistics or trade figures. As it is effectively a household based study, a TDS cannot be used to express intakes of critical groups of the population (Parmar, Miller, Burt, 1997).

*1.4.5.2 The Duplicate Diet.*

This method assesses actual intake of a chemical by a particular at risk group. It is based on the collection of all foods consumed by an individual over the duration of the study and subsequent analysis of the homogenised total diet for the contaminant of interest. The intake is estimated by multiplying the amount of food consumed daily by the concentration of contaminant detected in the duplicate portions provided. The duplicate diet method can be used to identify individuals who have high levels of intakes or if the estimates of individuals with a particular dietary habit or food preparation habits are required.

The advantage of using a duplicate diet is that direct and accurate information can be obtained from individuals regarding their intake of food contaminants since data on the actual amounts of foods consumed and actual levels of contaminants detected in the food are used. The main limitation to this method is that it can be very expensive and labour intensive especially for the participant. The method is therefore restricted to small groups and conducted over a shorter period of time and so lacks information on the participant variation in food consumption. Intakes devised from this method cannot be applied to large populations or long - term intakes. The duplicate diet method cannot identify the sources of

contamination or the contribution of food groups to individual daily exposure (Parmar, Miller and Burt, 1997).

## 1.5 Individual Intakes based on food consumption survey data.

There are five major methods for assessing dietary intake for individuals. The advantages and disadvantages of each method are summarised in Table 1.2:

### *1.5.1 Individual dietary records*

There are two methods used in dietary records, weighed and estimated. Dietary records use data from representative national surveys of individual consumption.

### *1.5.2 Weighed food records.*

In this method, the subjects are taught to weigh and record the food and its weight immediately before eating and to weigh any leftovers. Not every item needs to be weighed. Where weighing would interfere with normal eating habits, it is usual to accept a descriptive record of the foods consumed *e.g.* in a restaurant.

### *1.5.3 Estimated food records/diaries.*

The subject is required to report all foods and beverages consumed for a period of time. If food additive assessment is the objective of the study, the amounts of foods and drinks consumed are to be recorded. The amounts are determined by weighing the food / drink or estimated by using portion sizes and / or a photographic atlas (FAO/WHO, 1999).

### *1.5.4 24 hour recall.*

These methods recall the actual intake on a specific day. The subject is required to list all foods and beverages consumed for the preceding 24 - 48 hours to the interviewer. A trained interviewer in this method solicits dietary information from the subject, as memory recall is the source of information. The interview either takes place face – to face or by telephone (FAO/WHO, 1999), using either an open form, a precoded questionnaire, a tape recorder or a computer program. The purpose of the study should be so well known by the

interviewer that it is easy for the interviewer to judge what information must be collected (Bingham *et al.*, 1988).

### *1.5.5 Food Frequency Questionnaire (FFQ).*

This consists of a structured listing of the foods and drinks of interest in the study. The subject is asked about the frequency of consumption based on the frequency categories provided (number of times per day up to yearly intake). The quantity of food may not be of relevance, have been determined before the interview (semi-quantitative), or the subject may be asked to estimate the quantity using household measures (fully quantitative). The questionnaire can be self – administered or an interviewer may be present. Some questionnaires include questions regarding food preparation, use of dietary supplements or brands of foods, depending on what the objective of the study is (FAO/WHO, 1999).

### *1.5.6 Diet history.*

This method is meal based to assess usual intake. Lists of commonly consumed food at each meal are given for a defined period, usually a week. A trained interviewer elicits the usual pattern of food intake on each day. The reference timeframe is often the past month or several months or reflects seasonal changes using the past year (FAO/WHO, 1999). Each meal is discussed in turn to find out which foods were used and how often, what alternatives might be used on other days of the week and any irregularities in the eating pattern, so that a menu can be established for 7 days or for a month. Usual portion sizes are estimated with the aid of food models or replicas and given information can be cross-checked using a list of individual foods as a memory aid. As with the 24-hour recall, the purpose of the study should be very well known by the interviewer.

### *1.5.7 Combined methods.*

There is no best method for all study purposes. As has been described, each method has specific advantages and disadvantages. A combination of two methods might give more information or might make a study easier to carry out (Bingham, *et al.*, 1988). It is becoming more common to use more than one method. The more detailed method along

with the FFQ, is expected to provide more accurate estimates of intake distribution (FAO/WHO, 1999).

## 1.6 Use of food consumption databases for estimating food chemical intake.

A fundamental requirement of the estimation of food additive intake is the availability of food consumption data. There is general agreement that in a cost-effective risk management approach, it is sufficient to begin with relatively crude data and proceed to more refined data only if required, as judged by the estimated intake relative to the acceptable daily intake (ADI). The closer the estimated intake to the ADI, the greater the need for more refined data either in terms of intakes of the foods in question or of the usage levels of additives in these foods. One area where intake data can be refined is at the level of food coding where the highest level of refinement involves the recording of food intake at brand - level.

Although brand-level information is routinely collected in food consumption surveys, its main use lies in assigning a suitable food code for nutrient analysis using food composition tables. The latter necessarily contains fewer codes than foods available to consumers. The rate at which new food brands enter the market are such as to preclude a detailed nutritional analysis of each to be entered onto a food composition database and hence be assigned a code. Such databases, therefore, must be confined to major foods of nutritional importance and / or to foods, which are broadly representative of others for which codes do not exist. Thus a database might contain one code and appropriate nutritional analysis for 'carbonated soft drinks containing sugar' to which all appropriate brands can be assigned. This is aggregation of foods into a general food category. Despite the fact that the composition of these beverages may differ, the information on the intakes of particular brands is lost. While brand level data are recorded in most food consumption studies and are used to assist in assigning an appropriate nutrition analysis code, the brand-level data are generally not retained in the ensuing food consumption - nutrient intake database.

Where the estimated intake of an additive is found to approach the ADI, it could be argued that while the intake from the aggregated food category is below the ADI, the intake for some brands might exceed the ADI. At present, there are no objective data

available to determine whether or not such a criticism is fair in the context of protecting consumer health. For example, if an additive used in biscuits is found to be consumed at 65 % of the ADI by biscuit consumers, it could be argued that, were sub-categories of biscuits to be considered (*e.g.* sweet, savoury, filled, chocolate coated, *etc.*), one of these sub-categories might lead to an intake which exceeds this figure and comes closer to the ADI. Even if further analysis were to reveal that of the sub-categories for which intake data can be determined, some fall below 65 % of the ADI and some reach 80 % of the ADI, the argument can still be made that, were individual brands to be considered, some brands could lead to intakes in excess of the ADI. Without objective data to address these possible criticisms, the strength of food additive intake data may, in certain circumstances, be called into question (Cullen *et al*, 1999). The question is that if brands were not recorded, is the database at a disadvantage in comparison to those that have and maintain their brand level data?

### 1.6.1 Estimating concentration and occurrence of food additives in the food supply.

Having decided on the method to be used to collect the food consumption data, the next issues to be dealt with are those of the occurrence and concentrations of the food additive of interest in the diet of the population being studied.

### 1.6.2 Occurrence

Occurrence means how often in foods does the additive of concern appear. The Irish National Food Ingredient Database (INFID) was developed in Trinity College between 1995 – 1999 (Lambe, 2000). It consists of 5,684 processed foods and the ingredient list for each food. In order to find out what foods contain the additive(s) of concern, a search using the name of the additive is conducted. The outcome of this search is a list of foods and their brands containing the additive in question. If the additive appears in no or few foods then it need not be assessed, whereas if it has widespread use, then an investigation of the concentration level in the foods needs to be determined.

### 1.6.3 Concentration

The concentration of additives can be determined by a number of methods, again one proceeds from the crude to the more refined methods. This is summarised in Table 1.3.

*1.6.3.1 Use of Maximum Permitted Levels.*

When using maximum permitted levels in conjunction with food consumption data, the assumption is made that the additive permitted is at its maximum level in foods containing it. In the majority of foods, this is not the case so an over-estimation of intake in the result. MPL use in estimating intakes help determine whether or not the levels in the regulations, provide sufficient protection for consumers when a greater level of detail of usage in unidentified.

*1.6.3.2 Use of technological levels.*

Assuming good manufacturing practice within the food industry, information regarding levels of additives required to achieve technological functions within foods helps to refine estimates. Again, the levels found are often less than the stated MPLs. Using this method allows refining of food chemical intake estimates as estimation of additive use in specific foods rather than the broad groups defined in the Directives can be performed.

*1.6.3.3 Use of manufacturer's data.*

Manufacturers often do not use food additives at the technological level for two reasons: one, additives are in combination with others or two, that they are not warranted at all. Food manufacturers can provide the information regarding actual use of additives in food products. There are problems with trying to get this information and also when it has been given:

1. Often manufacturers are not too willing to give out usage levels due to the 'commercial sensitivity' of the business.
2. Product formulations can change quite often, thus information can become out – of – date quite quickly.
3. Similar products may have different formulations depending on the manufacturer.
4. There may be large number of small manufacturers who might be difficult to locate.

*1.6.3.4 Use of analytical data.*

If the previous additive usage methods have not been successful in determining whether or not one is at risk of exceeding the ADI, then analytical data may be required. The analysis may be carried out on individual foods or food groups and on the consumer and non-consumer type diet of the additive of concern. The resulting intake can be compared to the ADI. These types of studies are expensive and best suited to food chemicals that are commonly used in unpredictable quantities *e.g.* environmental contaminants or food chemicals that are greatly reduced in concentration in processing or cooking *i.e.* pesticides. With additives and pesticides, analytical data of levels of occurrence in foods exists at a national level. These data are generated through monitoring programmes, which assess observance with control regulations and in the future could provide a useful data bank on concentration levels.

Having decided on what methods of monitoring to use, the foods to be investigated due to occurrence levels and the concentration levels to be used, another area of surveillance is that of post-marketing.

## 1.7 Postmarketing surveillance and food consumption patterns.

Postmarketing surveillance (PMS) is designed to identify previously unrecognised adverse effects of newly marketed drugs, medical devices and food additives. The premarketing determination of the ADI of a food additive and the projection of its potential intakes (*i.e.* estimated daily intake), provides assurance that the food additive is safe for its intended use. PMS may be used to obtain a more reliable measure of food additive intake and to identify and evaluate reports of idiosyncratic responses (Butchko, Tschanz and Kotsonis, 1994).

In a final report to the EU (Nutriscan, 1992), it is stated that 'when a new product is to be launched onto the market estimates of potential consumption can be made based on current consumption of a similar products. However, this assumption takes no account of the effect of the new product itself. The introduction of artificially sweetened drinks increased the overall consumption of soft drinks and it is anticipated that the same pattern may follow the introduction of foods containing fat substitutes. Dietary surveys would need to be carried out on a yearly basis if monitoring was to be undertaken, again an

impractical solution to such a problem. Market survey data, however, provides - up – to – date information on trends in consumption and they may be a useful adjunct in the intervening years between 'national dietary surveys'. Alternatively if food additives could be prioritised in terms of needs for exposure data, then certain foods could be targeted for particular attention and effort taken to monitor their consumption in yearly household budget surveys. At present there is no structure available that can adequately address the question of PMS, however, it may be possible to initiate research into the use of historical data on food consumption to derive simulation models that would help predict changes in consumption.

PMS of consumption patterns of MNS has been proposed as a condition of approval of MNS and / or extending permitted uses. PMS should be conducted on a representative sample of the population, should allow comparisons over time and should collect data on food consumption patterns, nutrient intakes and frequency of consumption of macronutrient consumption. This is a more detailed and costly form of PMS than measuring actual product consumption (Cheney, 1996). Several methods (*e.g.* food disappearance data, dietary recall, food diaries and duplicate diaries) are available for determining postmarketing estimate of consumption (Butchko, Tschanz and Kotsonis, 1994).

The PMS could either be active PMS that is initiated by the manufacturer or passive PMS that is initiated by the consumer and facilitated by the presence of a contact number to be called for information or for reporting associated adverse effects. The extent and quality of the information obtained from active versus passive PMS will differ significantly. Active PMS is very costly and time consuming and the data may be proprietary. How the data are used and the role of the regulatory agencies in the PMS is currently being addressed. The co-operative efforts of the manufacturers, the regulatory agencies and the consuming public are essential if PMS is to become an integral component of a continuing safety evaluation program (Borzelleca, 1996).

Overall, PMS of Olestra (a fat substitute) has not revealed adverse side effects thus far, and the symptoms that have been reported appear to be self-limited. While the numbers of individuals exposed to olestra – containing foods is unknown, based on the

amounts distributed in the test-market cities, tens of thousands of consumers are likely to have ingested these foods (Freston *et al.*, 1997).

### 1.7.1 Medical postmarketing surveillance.

The experience with PMS in the pharmaceutical industry in the past 30 – 40 years has indicated that even extensive pre - approval testing in animals and humans cannot always predict the occurrence of very rare or delayed types of adverse health effects, in part because of the relatively limited number of subjects in premarketing clinical trials. Thus passive surveillance systems were developed in the 1950s to monitor spontaneous reports of adverse health effects for marketed drugs. The systematic collection and evaluation of such reports of adverse reactions to marketed drugs provide a more complete picture of safety than is possible before a compound is marketed and such PMS is required from a regulatory standpoint for marketed drugs.

Although generally there are no regulatory requirements for PMS systems for foods and food additives, regulatory agencies such as the Food and Drug Authority (FDA) and Health Promotion Board (HPB) have acknowledged the usefulness of such systems in the continued assurance of the safety of newly approved foods and food additives. For example, medical complaints associated with aspartame were reviewed in 1984 by the Centers for Disease Control (CDC) at the request of the FDA and the FDA established a system, the Adverse Reaction Monitoring System (ARMS) to document and evaluated anecdotal reports of adverse health events associated with foods, food additives, colour additives and vitamin / mineral supplements. It has been argued that such surveillance systems are less necessary for food additives than for drugs given the nature of food additives and the large safety margins required for their approval. However, once a food additive gains widespread use and extensive data are collected the usefulness of continued medical PMS significantly diminishes (Butchko, Tschanz and Kotsonis, 1994).

## 1.8 SECTION C.

The EC Directive 94/35/EC states that each member state should monitor the intakes of sweeteners. Many countries have already carried out surveys on sweetener consumption.

The purpose of this section is to prepare a detailed review of such studies. Tables 1.4 and 1.5 summarise the methods, sweeteners, concentration data and populations studied.

### 1.8.1 Austria (Elmadfa, Zarfl and König, 1996).

A consumption survey investigating the intakes of acesulfame K, aspartame, saccharin, cyclamate, neohespheridine DC and thaumatin was completed by a nationally representative group of the Austrian population. They were aged from < 6 to 96 years. Those that were < 18 and > 60 years completed a 7 - day diary, those aged 18 - 60 completed a 24 - hour recall. Food manufacturers provided the data regarding usage levels of sweetener(s) in a product. Average weights were assigned to each age group: ≤ 6 years = 25 kg, 7 – 9 years = 30 kg, 10 – 12 years = 40 kg, 13 – 14 years = 50 kg, 15 – 19 years = 60 kg and from 20 to over 50 years = 70 kg. At the 97.5$^{th}$ percentile the highest intakes as a percent of the ADI were recorded at 33.3 % for acesulfame K, 12.9 % for aspartame, for saccharin 38 %, 44 % of the ADI for cyclamate and 19.3 % of the ADI for neohespheridine DC. It can be seen that none of the groups studied are at risk of exceeding the ADI for each of the sweeteners, no intake even approached the ADI.

### 1.8.2 Brazil (Toldeo and Ioshi, 1995).

In this study the potential intake of aspartame, cyclamate and saccharin were evaluated. The survey took place during July to September 1990 (winter) and December to March 1991 (summer) from the Campinas and Curitiba cities. 674 respondents completed a demographic questionnaire and any special dietetic practice of the participant related to the consumption of intense sweetener containing foods and beverages. In the food diary, the frequency of consumption of all types of food and drink likely to contain sweeteners as well as of table - top sweeteners was recorded on a daily, weekly or monthly basis, depending on the dietary habit of the respondent. All soft drinks and table - top sweeteners recorded in the questionnaire were chemically analysed to determine the actual concentration of the added sweeteners. For a number of products with relatively low levels of consumption – generally diet yoghurts, jellies and chocolate – mean sweetener concentrations were obtained from the declaration on the package label.

Individual total daily intakes of aspartame, cyclamate and saccharin were calculated from the sweetener content of foods, soft drinks and table top products that were known to contain these additives, and were expressed on a body weight basis (mg / kg bw / d). Based on individual intakes mean, median and extreme daily intakes of each sweetener were calculated for individual users. Although for all sweeteners the median intakes are well below the corresponding acceptable toxicological limit, individual intakes exceeding the ADI were recorded, particularly for diabetics through the consumption of table - top sweeteners containing saccharin and cyclamate. 16 (2.4 %) consumers had intakes above the ADI. Of these 6 exceeded the ADI of cyclamate, 2 exceeded the ADI of saccharin and 8 exceed both ADIs. No consumer exceeded the ADI for aspartame. The data of this survey indicated that, daily intakes of aspartame, cyclamate and saccharin are within their respective ADI.

1.8.3 Canada (Heybach and Ross, 1989).

Aspartame was the sweetener of concern for this study. A sample of approximately 5,000 people aged 2 + years in the two way study participated. A 7 - day diary was used. Quantities of aspartame per milligram of food or beverage as consumed were provided by the manufacturer of all 145 individual food and beverage products that contained aspartame.

In wave I, the mean, 90$^{th}$ and 95$^{th}$ percentiles of intake for the total aggregate of eaters only are all below 14.4 mg / kg bw / d. Wave II intakes tends to be slightly higher for the eater, mean intakes tend to be the same or slightly lower. Diabetics tended to have slightly higher intakes of aspartame at the 90$^{th}$ and 95$^{th}$ percentiles than the rest of the population.

The results of this study indicate that the intake of aspartame in the general population is below the acceptable daily intake, established by the Health Protection Branch and other regulatory organisations. At the 90$^{th}$ and 95$^{th}$ percentiles of intake indicate, even the extremes of APM intake in each of these population subgroups are below the currently recommended acceptable daily intake. Consumers only sample at 95$^{th}$ percentile, 6.8 and 7.7mg / kg bw / d and for diabetics, 6.2 and 14.4mg / kg bw / d for wave I and wave II.

1.8.4. Denmark (Renwick, 1999).

In 1991, the ISA conducted a survey of intense sweetener consumption between April and June. The objective of the survey was to measure the consumption of intense sweeteners in a representative sample of the population (n = 1,233) aged 1+ years, as part of their total diet. This survey reports the consumption of acesulfame – K, aspartame, saccharin and cyclamate from all foodstuffs. Sweetener concentration estimates were determined by three methods: 1) laboratory analysis, 2) information from the manufacturer and 3) MPLs from the Danish positive list of food additives.

Individuals were recruited to record their total consumption of food and drink in and out of the home, over a consecutive 7-day period in a purposely - designed diary. Data from this study were expressed on a user – population basis in order to estimate more precisely the intake among those actually consuming sweeteners.

The data show that the 7- day average for each sweetener did not exceed nor even come close to the respective ADI for that sweetener for any group. At the 97.5$^{th}$ percentile, the ADI was approached for cyclamate on a number of occasions (based on each group), and exceeded in the male (127 % of ADI), and diabetic groups (333 % of the ADI). The consumption study showed that the permitted levels of sweetener in Denmark ensure that the dietary intakes of sweeteners on average are far below the ADI. Those very few individuals, who in the particular week of the study had a high consumption of one sweetener, were of very different ages, and the sources of the intake were totally different (weight reduction diet / table - top sweetener / non - carbonated non-citrus beverage). Therefore, it is not likely that a high intake of an individual sweetener at a young age will continue at a constant high level over a lifetime.

1.8.5 Finland (Virtanen *et al.*, 1988).

The dietary intakes and sources of sucrose, fructose, sorbitol, xylitol, saccharin, cyclamate and aspartame of 152 diabetic and 74 non-diabetic adolescents were studied by means of two 24 - hour recall interviews for 2 school days and 2 weekend days. The use of all sweeteners and sweetened foods during the 4 days were thoroughly investigated. The amount of sweeteners in ready - made foods was either checked from food labels or enquiries were made to the manufactures. Information about the amount of sweeteners in

home - made foods was obtained from the adolescents or their parents. The school kitchen personnel gave data about the sweeteners used in foods served at the school. The total intakes and intakes per kg bw / d of fructose, sorbitol, xylitol, saccharin, cyclamate and aspartame from different food item were calculated. The intake of alternative sweeteners by the controls was very small. Only three non - diabetic adolescents had used sorbitol, three used xylitol, six used saccharin and three used aspartame during the 4 days of the study.

In the present study the mean intakes of all non-nutritive sweeteners was well below the acceptable daily intakes saccharin (11 % of the ADI), cyclamate (10 % of the ADI), aspartame (3 % of the ADI), and none of the adolescents exceeded them. In conclusion, the intake of alternative sweeteners by the Finnish diabetic adolescents was moderate, and they had used several different sweeteners in accordance with the recommendations for diabetics.

### 1.8.6 France (CRÉDOC, 1994).

The intake of intense sweeteners (acesulfame, aspartame, saccharin) by French consumers was evaluated using purchase data of foods containing such sweeteners (except 'light' jams and marmalades and chewing gum). Data were produced from households panel (6,914 households in 1992).

Individual intakes were evaluated (mean per member of households), and expressed in mg / kg bw /d (60 - kg person), in order to compare to acceptable daily intake. In 1992, the ADI was never reached for aspartame (maximum intake about 10 mg/kg bw / d, ADI = 40 mg/kg bw / d) and acesulfame (maximum intake 1.5 mg/kg bw / d). For saccharin, three people (over more than 19,000 in panel) could have intake slightly over ADI, they were members of households with extremely high intake of warm beverages sweetened by sucrose substitutes.

### 1.8.7 Germany (Bär and Bierman, 1992).

2,291 individuals from the general population completed a questionnaire on some individual characteristics including any special dietetic practices and a 24 - hour food diary was mailed to all participants. The intakes of aspartame, cyclamate and saccharin

were calculated from the reported intakes of foods, beverages and table - top products that were known to contain these compounds. Data on the sweetener content of the different products were obtained from the respective food manufacturers, from the declaration on the package label or in a few cases by chemical analysis. Of the 822 participants who reported an intake of sweeteners during the 1-day examination, those individuals who ingested more than 75 % of the ADI of cyclamate or saccharin were selected. Of the 41 people selected, 40 were available to be surveyed over a 7 - day period. For the group of 2,291 participants, the mean intakes of aspartame, cyclamate and saccharin on a body weight basis were 0.052, 0.0939 and 0.090mg / kg bw / d respectively. Because less than half of the participants consumed a sweetener on the day the median for each sweetener is zero. This means that even for the heavy consumer intake levels of all three sweeteners were well below the respective ADIs. Comparison of the intake data for each sweetener with the corresponding ADI values shows that 16 out of 2,291 persons exceeded the ADI of cyclamate and 1 exceeded the ADI of saccharin on the examination day. Of the 16 subjects who exceeded the ADI in the 1 - day record, only three of them had a 7 - day average intake that was also above the ADI. The data on saccharin intake is similar. All three subjects who exceeded 75 % of the ADI in the 1 - day study had a 7 - day average daily intake of saccharin lower in relation to its ADI than that of cyclamate. A tendency for higher intakes was seen for cyclamate but not for saccharin. Only five out of the 40 subjects reported an intake of aspartame during the 7 - day survey, these intakes were still well below the ADI. Overall conclusion is that there is no need to be concerned regarding individuals having an intake of intense sweeteners that occasionally exceeds the ADI due to the safety factor applied when formulating the ADI.

### 1.8.8 Italy (Leclerq *et al.*, 1999).

This study looks at the intake of saccharin, aspartame, acesulfame K and cyclamate in 212 teenagers aged 13 – 19 in 1996 using 14 - day diaries. As well as the diary, the students completed a demographic questionnaire including questions on whether students had any special habitual dietary regimen or whether they were on a diet during the survey. All the sweeteners content reported are those declared by manufacturers. No subject declared themselves to be a diabetic, 15 female subjects declared that they were on a slimming diet

during the survey. Daily intakes among users were on average less than 1% of the ADI for all sweeteners, varying from 0.07 % for aspartame to 0.2 % for saccharin. Daily intakes among consumers of each single sweeteners were on average slightly higher for cyclamate (2 % of ADI) and saccharin (4 %ADI). The maximum daily intake observed in the study sample was 1 %, 2.5 %, 6 % and 11 % of the ADI for aspartame, acesulfame K, cyclamate and saccharin respectively. At present the risk of exceeding the ADI is low for two reasons: (1) dietary patterns include few occasions of consumption of products available in a sugar free version and (2) the choice of sugar free versions is irregular. Since the intense sweeteners used are frequently modified and the market shares of sugar free products are growing, there is a need to keep the situation under control and in particular that of beverages and table top sweeteners as a source of cyclamate and saccharin.

1.8.9 Japan (Ishiwata *et al*, 1998).

This study investigates a number of food additives but only the content of Sodium Saccharin in foods was of interest. The Division of Food Chemistry, Ministry of Health and Welfare of Japan contacted 103 local governments. A number of methods were used in order to estimate daily intake of each food additive but, the results are only given here for sodium saccharin.

*(b)* Investigation methods and items studied.

*(b)* *Questionnaire* - questionnaire on the results of official inspection for food additives from April 1, 1994 to March 31, 1995 (fiscal year of 1994) was sent to 103 local governments in the country.

*(b)* *Items queried* – The names of both imported and domestic foods, the number of food samples inspected, the number of samples in which these food additives were detected, the maximum, minimum and mean concentrations of the food additives in the samples in which they were detected, and the detection limit of the analysis for each food additive was recorded. Saccharin in foods was expressed as sodium saccharin except that in chewing gums, according to the Japanese Standards for Use of Food Additives. Free saccharin is allowed to be used only in chewing gum.

*(c) Sampling of foods and methods of determining food additives* – Samples were chosen by food inspectors in markets or factories manufacturing food products according to the yearly program of each local government. Gas chromatographic methods were used for the determination of the additives.

(ii) Analyses of reports from the local governments.

Data sent from the local governments were classified according to the categories of foods in the Japanese standards for Use of Food Additives. Concentrations of these food additives were regarded as 0 mg/kg irrespective of detection limits when these food additives were not detected.

(iii) Calculation and estimation of daily intake.

The daily intakes of the food additives per person were estimated based on the mean concentrations of these food additives in foods obtained in the present study and the daily food consumption levels reported by the Japanese group and Ministry of Health and Welfare of Japan.

(iv) Estimation of daily intake per person.

Estimated daily intake was based on the analytical results of the official inspection by the local governments in fiscal year 1994. The data on the daily consumption of foods by the nation estimated by the Japanese group and the Ministry of Health and Welfare were utilised to estimate daily intakes of these food additives. The estimated daily intakes of sodium saccharin in this study were 2.9 % of the ADI.

The concentrations of the food additives including sodium saccharin might be over - or under - estimated due to the following reasons; a) most samples inspected were permitted foods for these food additives, b) although many non permissible foods for these food additives were also inspected, some of them were foods in which these food additives might be used, c) food containing excessive amounts of food additives and non permissible foods containing these additives would be excluded from markets by the local governments and d) the concentration of these food additives in samples where they were undetectable was regarded as 0 mg/kg. Factors a) and b) would most strongly affect the

results. It is therefore more likely that the results obtained are overestimated rather than underestimated.

1.8.10 The Netherlands (Hulshof and Bouman, 1995).

The use of table - top sweeteners and diet soft drinks as well as the intake of aspartame, cyclamate, saccharin and acesulfame K were assessed. Complete two - day records were provided by 6,218 individuals from the ages of 1 – 65 + years. From 6,060 individuals also information on the usual consumption of the table - top sweeteners and diet soft drinks was available from a food frequency questionnaire. Total daily intake of intense sweeteners was calculated from the sweetener content of each product (provided by manufacturers), table – top sweeteners or MPLs (diet soft drinks), and expressed in mg / kg bw / d. For users of intense sweeteners at the $90^{th}$ percentile, the intake level of these sweeteners were 5.2, 2.4, 1.0 and 0.4 mg / kg bw / d corresponding to 13 % for aspartame, 22 % for cyclamate, 40 % for saccharin and 4 % for acesulfame K of their ADIs. Three people exceeded the ADI for cyclamate and four subjects exceeded the ADI level of 2.5 mg / kg bw / d for saccharin. Compared with the two - day records, intake of intense sweeteners assessed with a food frequency questionnaire was slightly higher. For users, at the $90^{th}$ percentile these values were 5.2, 4.9, 1.0 and 0.8 mg / kg bw / d respectively, corresponding to 13 % for aspartame, 45 % cyclamate, 40 % for saccharin and 9 % for acesulfame K of their ADIs. Only one person out of 6,060 exceeded the ADI for aspartame. Based on this method, 5 and 11 subjects respectively consumed more than the ADI for cyclamate and saccharin. The present results demonstrate that even among consumers with above average consumption of intense sweeteners only a few (less than 0.5 % of the total population) had an intake above acceptable limits.

1.8.11 Norway (Bergsten, 1993).

The content of sweeteners in products is currently regulated in detail by the Norwegian approved food additives list, which is also a positive list. A positive list means that is an additive is on the list then its' use is permitted. The list is divided into various foodstuffs / groups of foodstuffs and is based on the product, which form part of the Norwegian diet. The quantity restrictions are also fixed so as to avoid people exceeding the ADI. Except for

acesulfame K, SCF has the same ADI values as JECFA. IN 1990, JECFA fixed a new ADI value for acesulfame K of 0 – 15 mg / kg bw / d while SCF retained the old ADI value, which JECFA fixed in 1983, of 0 – 9 mg / kg bw / d.

In 1992, a study on the intake of acesulfame K, aspartame, cyclamate and saccharin was carried out. 319 people completed this study, The boys and girls <19 years old completed a 3 - day record, the 19 year old women completed a 4 – day record and the men and women >19 years old completed a 48 - hour recall. The objective of the study was to study how the population's intake of these four sweeteners via their diet relates to the existing legislation. The Danish budget method was used to calculate intake quantities. The study was based on quantity restrictions for the products which were assumed to be the most common and /or which were consumed in the greatest quantities. The method provides no actual value but gives an indication of how the population's intake quantities of the additive relate to its ADI. The calculations assume large intakes of food and drink containing the additive in question. This is done so that the calculated intake also covers the intake of persons who may have a deviating diet, for example children, young people and diabetics. If the rough estimates produce a value, which is clearly, less than the ADI, it is therefore probable that the actual intake quantities of the population are also less than the ADI. If the calculated value exceed the ADI, more detailed intake studies should be carried out.

Rough estimates using the Danish Budget method, of the intake of the sweeteners in question. The sweetener levels are based on the Norwegian approved food additive list show intakes of acesulfame K, aspartame, cyclamate and saccharin to be 52 %, 42 %, 56 % and 176 % of their respective ADIs. When the sweetener levels were based on the EC's Draft Council Directive on Sweeteners for Use in Foodstuffs. Intakes of acesulfame K, aspartame, cyclamate and saccharin are 100 %, 69 %, 187 % and 204 % of their ADIs. The budget method showed that on the basis of the list currently in force, it seems as though only the intake of saccharin is high in relation to its ADI value. The possible intakes of the three other sweeteners seem to be below the fixed ADI. The results also indicate that intake may be up to two or three times greater for certain sweeteners if the EC's Draft Council Directive on Sweeteners for Use in Foodstuffs enters into force in Norway. The intake of

cyclamate and saccharin and to a certain extent acesulfame K may be high in relation to the ADI.

The method involves calculations regarding intake quantities being done in two stages. Firstly, a theoretical maximum intake is calculated. If these intake quantities are high in relation to the ADI, an estimated maximum intake is also calculated. It is important to be aware that the theoretical maximum intake and the estimated maximum intake are extreme values. The calculations show what is a possible maximum intake in the worst case scenario. An estimated average intake was also calculated in the study. An average content of the respective sweeteners in each product and an average intake of the products in question by the five different populations groups were used in the calculations.

In the theoretical maximum intake based on the Norwegian List, all groups had an intake of saccharin, which was above its ADI value, ranging from 140 – 320 % of the ADI. The groups of girls and boys exceeded the ADI values for their intake of acesulfame K (125:179 %), aspartame (111:156 %) and cyclamate (109:177 %). The groups of 19 year old women, women and men had intakes of these sweeteners, which were below the limit value. The theoretical average intake quantities were considerably lower than the theoretical maximum quantities. The intakes of the four sweeteners are well below the ADI values for all population groups.

If you look at the theoretical maximum and average intakes based on the EC's Draft Council Directive on Sweeteners for Use in Foodstuffs, the intakes were larger than the theoretical intake quantities based in the quantities indicated in the Norwegian list. The intake of all the population groups exceeded the ADI for each sweetener 150 – 359 % ADI for acesulfame K, 106 – 234 % ADI for aspartame, 156 – 420 % ADI for cyclamate and 216 – 532 % ADI for saccharin. As before, the theoretical average intakes based on EC values were well within the ADIs but higher than those expressed using the Norwegian list. When investigating the estimated maximum intake quantities based on manufacturers information on additive quantities, it was found that the intakes of cyclamate exceeded the limit value in all groups. The intake quantities of this sweetener were also greater than the equivalent theoretical maximum intake quantities. This was because the cyclamate content in one type of fizzy drink exceeded the maximum permitted content. If the fizzy drink were excluded, the intake quantities in the population groups would be below the limit value.

The intakes of the other sweeteners are below the ADI limit except for saccharin intake in the groups of girls and boys and the boy's intake of acesulfame K was up to the limit value, while the girls' was below. Again, as before, the estimated average intake of the four sweeteners were considerably below the fixed ADI values for all population groups.

The content of sweeteners in the products was based on information from 45 manufacturers. Certain manufacturers added a greater quantity of sweetener than is permitted in the Norwegian approved food additives list. However, the quantities were within the limits proposed by the EC in its Directive on Sweeteners for use in foodstuffs. However there were two exceptions; the content of cyclamate in fizzy drinks and the content of acesulfame K in one type of lozenges also exceeded the EC's proposed maximum contents.

On the basis of the Norwegian approved food additives list currently in force, the results of the intake study showed that the daily intake quantities of acesulfame K, aspartame, cyclamate and saccharin in the adult part of the population keep well within the ADI values fixed for the respective sweeteners. However, individuals who have a high consumption of table sweeteners containing a combination of cyclamate and saccharin may risk exceeding the ADI value mainly for cyclamate but also for saccharin. The daily intake quantities of the sweeteners in question by children and young people might be high in relation to the ADI value in some cases. This applies above all to their intake of saccharin. However, it seems as if the intake of the four sweeteners by most young people like the intake by the rest of the population, keeps within the fixed limits. If the quantities of sweeteners stated in the Norwegian approved food additives list are replaced by the maximum additive quantities proposed by the EC in its Draft Council Directive on Sweeteners for Use in Foodstuffs, the daily intake quantities of the sweeteners by the population will probably increase. The result of the intake study pointed to an increase risk of people with a high consumption of diet products then having an intake of the four sweeteners, which is high in relation to the fixed ADI values. Children and young people may be particularly at risk. However, it seems as though the population in general will still have quantities of the sweeteners that keep within the limit values.

1.8.12 Spain (Serra-Marjem *et al.*, 1996).

The dietary intake of cyclamate was evaluated in the north –east of Spain in 1992 in a random sample of 2,450 people aged 6 – 75 years. The dietary assessment was conducted in conjunction with an anthropometric and biochemical assessment, which consisted of combining two 24 - hour recalls and a food frequency questionnaire having 77 items. The 24 - hour recall was carried out twice, firstly in a warm season (May – July), and secondly in a cold season (November – December). Data on the sweetener content of the different products were obtained from the respective food manufacturers and from a major cyclamate producer and manufacturer. For those products for which the information was not available, the cyclamate content was estimated by analogy from corresponding products for which the precise amount of cyclamate is known. The ADI used was that allocated by the Scientific Committee for Foods (SCF) which is 11 mg / kg bw / d.

Amongst consumers, the average level of cyclamate intake among consumers was 2.44 mg / kg bw /d (22 % of the ADI), and only four people had levels above the ADI, the two highest intakes were both females aged 35 – 40 on a diet, the others were a man aged 61 and a boy aged 11. It was found that those with the highest BMI had lowest intakes of cyclamate. The statistically significant inverse relationship between sweetener intake and BMI does not support the suggestion that the intense sweeteners increase appetite and thereby lead to an increase in body weight. The results of this study reveal that hardly any of the subjects had intakes above the ADI. It also shows the patterns of cyclamate intakes in different population groups and BMI categories.

1.8.13 United Kingdom (MAFF, 1990).

This study was conducted by the Ministry for Agriculture, Fisheries and Food (MAFF) and designed to investigate the intakes of bulk and intense sweeteners. A total of 681 people completed the study aged 2 – 65 + years. An initial questionnaire was completed by all regarding demographic details, dieting history *etc.* A 7 - day diary was completed. 100 diabetics were also selected to complete a 4 - day diary (3 weekdays and 1 weekend day), after they had completed an initial questionnaire. Food manufacturers provided sweetener concentration data. However, for a number of brands of some products, generally those soft drinks, which only a few people consumed – it was necessary to make assumptions

about which sweeteners were used and at what levels. Mean sweetener concentrations were calculated from the available data for each type of product (yoghurt, lemonade, *etc.*), and these were used when actual data were unavailable. In brands of conventional soft drinks for which data was not available from manufacturers, saccharin was assumed to be present at a level of 80mg / l (statutory maximum), other intense sweeteners were not used in conventional soft drinks at the time of the study.

Although no participant exceeded the ADI for the relevant sweetener at the mean, median or maximum level. The 97.5$^{th}$ percentile of intakes were not reported due to the small number of consumers in each age group. Changes are always being carried out by manufacturers in the sweeteners they use and the quantity. There is a greater chance that as these changes continue a higher proportion of the population will become exposed to sweeteners, thus continued monitoring of intake is essential.

1.8.14 United Kingdom (Hinson and Nicol, 1992).

A demographically representative sample of the population of the United Kingdom recorded their total consumption of food and drink in and out of the home in a 9-day diary. 647 people aged 1 – 75 years were selected. Each respondent was interviewed to obtain a demographic profile including whether the respondent was on a diet and for what purpose. The consumption data were combined with sweetener concentrations for each product, using data from manufacturers or from independent analysis of the products to quantify the amount of each of the permitted intense sweeteners consumed. When looking at the population in general, the data for each sweetener was well below the ADI as it was for young children and diabetics at the 90$^{th}$ percentile of intakes. The consumption of all of the sweeteners in all of the product and population groups was within the respective ADI of each of the sweeteners. When measured, the consumption of each of the three principal intense sweeteners, acesulfame K, aspartame and saccharin was below the ADI even for high users.

1.8.15 United Kingdom (MAFF, 1995).

A survey was conducted on 940 participant diabetics aged from 2 - 65 years to investigate the intake of acesulfame K, aspartame, saccharin, thaumatin, hydrogenated glucose syrup,

isomalt, lactitol, mannitol, sorbitol and xylitol by MAFF. The consumption of foods was determined using a food frequency questionnaire. This information was then combined with the sweetener concentration data provided by manufacturers to give intakes of sweeteners.

Intense sweeteners: 7 members were found to exceed the ADI of saccharin and one for the ADI of aspartame. High level intakes of each intense sweetener as measured by the 97.5$^{th}$ percentile are all within acceptable limits, in mg /kg bw /d (% ADI), for aspartame 10.1 (25 %), acesulfame K 1.4 (16 %) and saccharin 3 (60 %). The intense sweetener thaumatin was not encountered during the survey.

Bulk sweeteners: ADIs have not been set because the polyol sweeteners are of such low toxicity that their maximum potential intake is not considered to be an identifiable hazard to health. However because the polyol sweeteners and the carbohydrate fructose may have a laxative effect it is recommended that consumers do not exceed a combined intake of 25 g per day. 14 members did consume in excess of the recommended level due to high intake of sorbitol, an average of less than 2g per day. Sorbitol was found to be the most important source of bulk sweetener occurring in diabetic jams and marmalades and chewing gums. Intakes of other bulk sweeteners are all very low with averages of less than 1g per day. 2 individuals consumed in excess of 25 g per day due to their intake of a combination of sweeteners.

The top 1 % of sweetener consumers were re-surveyed 4 months later. Of the 11 high saccharin consumers only 2 maintained a high intake of saccharin due to high intakes of table - top sweeteners. The high level aspartame consumer, a child, drastically reduced their consumption of diet carbonated drinks, thus reducing their intake of aspartame. These results indicate that whilst it is likely that there will always be a number of individuals who exceed the ADI for a given sweeteners, most individuals would not continuously do so.

## 1.9 Section D – Discussion.

To date all studies of intense sweeteners have been extremely detailed both in the estimate of the presence or absence of the target sweetener in each food and the level of sweetener used. Brand level data are required for definitive identification of the presence or absence of a sweetener. Estimates of any food chemical intake at less than brand - level data can be

criticised on the ground that consumers of specific brands could have higher intakes than consumers of the overall food category in which the brand occurs. The issue of whether or not a database is disadvantaged by not retaining brand – level data is investigated in chapter 2.

The methods used in previous intense sweetener studies have been quite detailed as already discussed. Article 4 in the 94/35/EC Directive on sweeteners states that: 'within three years of its adoption, Member States shall establish a system of consumer surveys to monitor sweetener intake'. The issue of routine monitoring is in question. Whilst the previous studies gave detailed and accurate data, the methods employed are not suitable for routine monitoring due to participant burden and also the issue of 'commercial sensitivity' for concentration data. Whether or not quick, easy to use methods of data collection can be used in place of the more burdensome yet accurate methods is of question. This issue is dealt with in chapter 3.

The focus of the previous studies on the intakes of sweeteners has been in relation to the ADI. As discussed previously, there is a debate and concern that the use of intense sweeteners may result in an increase in % energy from fat as a result of the substitution of sugar with intense sweeteners. This area of debate has not as of yet been addressed in a large cross sectional sample of diabetics – a group that are high consumers of such sweeteners due to their disease status. Chapter 4 investigates this issue in such a group of high consumers of intense sweeteners.

Thus, the three main hypotheses of this thesis are:

- That the retention of brand level data in food consumption databases is essential for accurate assessment of human exposure to food – borne chemicals.
- That a simple food frequency questionnaire, with a food list identified from a national food ingredient database as containing target intense sweeteners, would provide a reliable means of routine surveillance of sweetener intake in high users.
- That the use of high levels of intense sweeteners in potentially vulnerable groups such as Insulin Dependent Diabetics will lead to a higher percent energy from fat in their diet.

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**Table 1.1 Summary of screening methods, their strengths and limitations.**

| Type of method | Major strengths | Major Limitations |
|---|---|---|
| Danish Budget Method | Simple<br>Inexpensive | Not suitable for all additives<br>Assumes intakes at certain levels<br>Conservative method |
| Food Balance Sheet | Inexpensive<br>Not too time consuming | Not suitable for environmental contaminants<br>Conservative method<br>Reflects availability rather than consumption<br>Localised contamination not considered<br>No variations to dietary habits determined |
| Total Diet | Can be carried out several times per year<br>Monitor trends in consumption<br>More cost effective | Limited to food groups only<br>Diet of typical/average consumer<br>Not used to express intakes of critical groups of the |
| Model Diet | Simple<br>Useful when limited information is available | Hypothetical diets<br>exaggerated consumption values |
| Duplicate diet | Direct and accurate information | Very expensive<br>Labour intensive for participants |

**Table 1.2 Summary of individual dietary assessment methods, their strengths and limitations.**

| Type of method | Major strengths | Major Limitations |
|---|---|---|
| Food record | Does not rely on memory | High participation burden |
| | Easy to quantify amounts | Requires literacy |
| | Open ended | May alter intake behaviour |
| 24 hour dietary | Little respondent burden | Relies on memory |
| recall | No literacy requirement | Requires skilled interviewer |
| | Does not alter intake behaviour | Difficult to estimate amounts |
| Food Frequency | Relatively inexpensive | Relies on memory |
| Questionnaire | Preferable method for nutrients with very high day – to day variability | Requires complex calculations |
| | | Limited flexibility for describing foods |
| | Does not alter intake behaviour | |
| Diet history | No literacy requirements | relies on memory |
| | Does not alter intake behaviour | requires highly trained interviewer |
| | open-ended | difficult to estimate amounts |
| | | (FAO/WHO, 1998) |

**Table 1.3** **Methods of determining the concentration data of food additives.**

| | **Food Consumption Data** | **Levels of additive concentration** |
|---|---|---|
| Level I | Disappearance data (FAO, OECD) | Maximum permitted levels |
| | Market Survey Data | |
| Level II | Household Budget Survey | Technological levels |
| Level III | National Diet Surveys | Manufacturers levels |
| | (individual based) | |
| Level IV | Surveys of specific 'at risk' groups | Analytical data |

(Nutriscan, 1992).

**Table 1.4 Summary of sweetener studies conducted and methods employed for the acquisition of concentration data.**

| Country | Year | aces | apm | sac | cyc | neo | Concentration data |
|---|---|---|---|---|---|---|---|
| *Austria (1)* | 1996 | ✓ | ✓ | ✓ | ✓ | | Manufacturer's Information |
| *Brazil (2)* | 1990/1 | | ✓ | ✓ | ✓ | | Chemical analysis, Food labels |
| *Canada (3)* | 1987 | | ✓ | | | | Manufacturer's Information |
| *Denmark (4)* | 1991 | ✓ | ✓ | ✓ | ✓ | ✓ | Laboratory analysis, Food Labels, Manufacturer's Information |
| *Finland (5)* | 1988 | | ✓ | ✓ | ✓ | | Manufacturer's Information |
| *France (6)* | 1991/2 | ✓ | ✓ | ✓ | | | Manufacturer's Information |
| *Germany (7)* | 1988/89 | | ✓ | ✓ | ✓ | ✓ | Manufacturer's Information |
| *Italy (8)* | 1996 | ✓ | ✓ | ✓ | ✓ | | Manufacturer's Information, Food labels |
| *Netherlands (9)* | 1992 | ✓ | ✓ | ✓ | ✓ | | Maximum Permitted levels for soft drinks, Manufacturer's Information |
| *Norway (10)* | 1993 | ✓ | ✓ | ✓ | ✓ | | Manufacturer's Information, Theoretical average intake |
| *Spain (11)* | 1996 | | | | ✓ | | Manufacturer's Information |
| *United Kingdom (13)* | 1987/88 (MAFF) | ✓ | ✓ | ✓ | | | Manufacturer's Information |
| *United Kingdom (12)* | 1988 (ISA) | | ✓ | ✓ | | | Manufacturer's Information |
| *United Kingdom (14)* | 1995 (MAFF) ** | ✓ | ✓ | ✓ | | | Manufacturer's Information |

aces – acesulfame K apm – aspartame sac – saccharin cyc - cyclamic Acid neo – neohespheridine DC ISA – Intense sweeteners association MAFF – Ministry for Fisheries and Food MAFF** - also investigated thaumatin intakes.

[(1)]Elmadfa et al, 1996 [(2)]Toledo, Ioshi 1995 [(3)]Heybach, Ross 1989 [(4)]Renwick, 1999 [(5)]Virtanen et al, 1988 [(6)]CRÉDOC, 1992 [(7)]Bär, Bierman 1992 [(8)]Leclerq et al, 1996 [(9)]Hulshof, Bouman 1992 [(10)]Bergsten, 1993 [(11)]Serra-Majem et al, 1992 [(12)]MAFF 1990 [(13)] Hinson, Nicol 1992 [(14)] MAFF, 1995.

**Table 1.5 Summary of subjects and survey methods in sweetener studies conducted.**

| Country | N | Age | Healthy | Diabetic | Survey Method |
|---|---|---|---|---|---|
| *Austria(1)* | | <6-96 | | | 18 – 60 y.o. – 24 hour recall, <18 and >60 y.o. – 7 day diary |
| *Brazil (2)* | 674 | <10 – 80+ | | 35% | FFQ |
| *Canada(3)* | 5,000 | 0 - 35 | General population including diabetics, no *n* given for diabetics | | 7 day diary |
| *Denmark (4)* | 1233 | 1+ | | 76 | 7 day diary |
| *Finland (5)* | 226 | 11.5-17.5 | 74 | 152 | 2 x 48hr recall |
| *France(6) (households)* | 6194 | | General population | | Household data |
| *Germany (7)* | 2291 | <5 ->60 | General population | | 1 day diary/7 day diary for those with highest intakes |
| *Italy (8)* | 212 | 13-19 | General population | | 14 day diary |
| *Netherlands (9)* | 6218 | 1-92 | General population | | 2-day records, FFQ |
| *Norway (10)* | 319 | | General population | | Girls and boys – 3 day diary, 19 y.o. women - 4 day diary, men and women - 48 hour recall |
| *Spain (11)* | 2450 | 6-75 | General population | | 2 x 24 hour recalls |
| *UK 1987/8 (12)* | 681 | 2->65 | | 100* | 7day diary/ *4 day diary |
| *UK 1992 (13)* | 647 | 1-75 | | 35 | 9 day diary |
| *UK 1995 (14)* | 940 | 2-65 | | 940 | FFQ |

[1]Elmadfa et al, 1996 [2]Toledo, Ioshi 1995 [3]Heybach, Ross 1989 [4]Renwick, 1999 [5]Virtanen et al, 1988 [6]CRÉDOC, 1992 [7]Bär, Bierman 1992 [8]Leclerq et al, 1996 [9]Hulshof, Bouman 1992 [10]Bergsten, 1993 [11]Serra-Majem et al, 1992 [12])MAFF 1990 [13] Hinson, Nicol 1992 [14] MAFF, 1995.

# *Chapter 2*

## *An analysis of the incremental value of retaining brand-level information in food consumption databases in estimating food additive intake.*

**Food Additives and Contaminants, 1999, 16 (3), 93 – 97.**

## 2.1 Introduction

At present, member states of the European Union are required to monitor the consumption and usage of food additives (European Parliament & Council Directives 95/2/EC, 94/35/EC, 94/36/EC). To that end several consultative processes have been undertaken to consider options for the collection of such data and the advantages and disadvantages of each option (Nutriscan 1992, 1994, Commission of the European Communities 1996, Gibney and Lambe 1996). A fundamental requirement of the estimation of food additive intake is the availability of food consumption data. There is general agreement that in a cost-effective risk management approach, it is sufficient to begin with relatively crude data and proceed to more refined data only if required, as judged by the estimated intake relative to the acceptable daily intake (ADI). The closer the estimated intake to the ADI, the greater the need for more refined data either in terms of intakes of the foods in question or of the usage levels of additives in these foods. The ultimate refinement in food intake data is at actual brand-level.

Although brand-level information is routinely collected in food consumption surveys, its main use lies in assigning a suitable food code for nutrient analysis using food composition tables. The latter necessarily contain fewer codes than foods available to consumers. The rate at which new food brands enter the market are such as to preclude a detailed nutritional analysis of each to be entered onto a food composition database and hence be assigned a code. Such databases, therefore, must be confined to major foods of nutritional importance and/or to foods, which are broadly representative of others for which codes do not exist. Thus a database might contain one code and appropriate nutritional analysis for 'carbonated soft drinks containing sugar' to which all appropriate brands can be assigned. While brand level data are recorded in most food consumption studies and are used to assist in assigning an appropriate nutrition analysis code, the brand-level data are not retained in the ensuing food consumption-nutrient intake database.

Where the estimated intake of an additive is found to approach the ADI, it could be argued that while the intake from the aggregated food category is below the ADI, the intake for same brands might exceed the ADI. At present, there are no objective data available to determine whether or not such a criticism is fair in the context of protecting

consumer health. For example, if an additive used in biscuits is found to be consumed at 65% of the ADI by biscuit consumers, it could be argued that, were sub-categories of biscuits to be considered (*e.g.* sweet, savoury, filled, chocolate coated, *etc.*), one of these sub-categories might lead to an intake which exceeds this figure and comes closer to the ADI. Even if further analysis were to reveal that of the sub-categories for which intake data can be determined, some fall below 65% of the ADI and some reach 80% of the ADI, the argument can still be made that, were individual brands to be considered, some brands could lead to intakes in excess of the ADI. Without objective data to address these possible criticisms, the strength of food additive intake data may, in certain circumstances, be called into question.

In the Dietary and Nutritional Survey of British Adults (DNSBA) (ERSC Data Archives 1991) food intake data are recorded at brand-level. This provides a unique opportunity to examine this issue of the incremental value, or otherwise, of recording brand-level food intake data. It goes without saying that the incremental cost of collecting, coding, manipulating and storing such data is not insignificant. Therefore, unless it provides significant incremental benefit in helping to protect consumer health, there is little likelihood of it becoming a common feature of food consumption studies. The present study set out to examine the incremental value of brand-level data in the use of food consumption studies to estimate food additive intake. It also examined the relationship between high intakes of food categories with high intakes of food sub-categories.

## 2.2 Methods

The DNSBA database contains data on food intake of 1,087 men and 1,110 women aged 16-64 years. The survey was carried out in the late 1980s using the 7-day weighed intake method. The database contains approximately 5000 food codes for which nutritional composition data exist. In addition, independent of the nutrient intake section, the database contains data on the intake of the 5,000 foods, the description of which is detailed up to and including, for processed foods (n = 2,354), the level of brand information. The food intake data in the DNSBA, while existing at the highest level of the description of foods consumed, is also organised into the intakes of 27 food categories.

Some of these categories were not of interest in the present study given that these fresh food categories are not generally associated with food additives, *e.g.* meat, eggs, fish, vegetables, *etc.* Of the remaining categories, some were used in the present study as originally defined by the UK database managers, but changes were made to others. As an example, the DNSBA lists of food category 'yoghurts and ice-cream'. In the present study, these were considered separately as 'yoghurts' and 'ice-cream'. In all, 14 food categories were created.

Within each of these 14 food categories, sub-categories of foods were defined. Details of foods contained in each sub-category for a given food are given as appendix 2.1 to this paper. In re-constructing sub-categories, certain codes were suitable for inclusion in more than one sub-category, *e.g.* a low-fat fruit yoghurt could belong to both the sub-categories of fruit yoghurt and low-fat yoghurt. Therefore, care was taken not to compare intakes between two different sub-categories of the same food category since this could involve 'double-counting'. (Brand level data exclude intakes for 'brand recorded not in code list'/'brand name not recorded'/'recipes'). The precise number of subjects required reliably to establish food intake at the 97.5$^{th}$ percentile of the distribution of intakes was taken to be 60 (SCOOP, 1996). The reconstructed database was examined to determine the number of brands with at least 60 consumers in each food category that also had at least 60 consumers. Intakes at the 97.5$^{th}$ percentile were determined for food categories, and for those food sub-categories and brands with at least 60 consumers.

## 2.3 Results

The number of brands in each food category for which at least 60 consumers existed is given in Table 2.1. Very clearly, the numbers are too low to justify estimation of sweetener intake using brand-level food intake data. Thus of 126 food-brands within the category 'carbonated beverages', only four have data on at least 60 people. Of 63 yoghurts for which brand-level data exist, only two have data with at least 60 consumers. To some extent, this is not so surprising. If a food category is consumed by one in three people, data should exist on intakes for about 700 people. If a larger number of brands exist in that food category, then low numbers per brand are to be anticipated. Table 2.2 gives the number of sub-categories, the 97.5$^{th}$ percentile of intakes of all consumers of the category,

and the highest value found for the 97.5$^{th}$ percentile of intakes among the relevant sub-categories. In nine of the 14 food categories, the 97.5$^{th}$ percentile of intakes for the various sub-categories did not exceed that of the category itself. In only one category was there more than one sub-category where the 97.5$^{th}$ percentile exceeded that of the category.

Table 2.3 gives data on the 97.5$^{th}$ percentile intake (g/day) for 14 food categories, the value of 1.3 times that statistic and the highest recorded 97.5$^{th}$ percentile intake for brands within each food category. For nine categories of foods, the highest recorded brand level intake at the 97.5$^{th}$ percentile exceeded that of the relevant category. No brand intake at the 97.5$^{th}$ percentile exceeded 1.3 times that of the food category 97.5$^{th}$ percentile intake. The figure of 1.3 was based on the observed ratios of the brand level intake at the 97.5$^{th}$ percentile to the same statistic of the food group.

## 2.4 Discussion

Food consumption databases exist in all member states of the EU and will be used to estimate the intake of specific food additives as mandated by the relevant EU regulations in this area (Directives 95/2/EC, 94/36/EC, 94/35/EC). The results of the present study show that when estimating high intakes of foods in assessing food additive intake, variability found in the level of detail in defining food categories should not pose a problem. Estimates of food additive intakes should always begin at the crude level. Very often these crude estimates are based on published figures for food intake of populations. It is in this regard that the results of the present study apply.

Thus, for example, interest in the intake of an additive for use in specialist margarines can be accommodated with relatively crude data on spreadable fat intake. If the latter provide no basis for concern over intake, there is little point in refining the data.

The DNSBA is the largest food consumption database publicly available with brand level food intake data and in this study it can be seen that there is limited incremental value in retaining brand-level food intake data in food consumption databases to estimate intake of brands among consumers. Another important finding was that a multiple of 1.3 times the food category 97.5$^{th}$ percentile intake provides a robust worst-case analysis as no brand intake at the 97.5$^{th}$ percentile was found to exceed this factor.

In trying to understand why and therefore to predict when, a brand or sub-category intake at the 97.5$^{th}$ percentile might exceed that of the category, a number of analyses were conducted. One such analysis examined the level of brand loyalty. In effect, there was little about brand loyalty, which could be used to predict high intakes of a brand. Several other possibilities were explored including serving size and the number of consumers but no pattern emerged which would allow a reliable prediction.

The present paper focused on that approach to quantitative risk assessment, which examines intake of foods among consumers only at the 97.5$^{th}$ percentile. The findings of the present study do not apply where a complex search of a database is needed to examine true additive intake from multiple food sources. However, even where the additive is present in several foods, the sum of the high intakes from all foods might be a first line of enquiry and in those circumstances the findings of the present study would apply. On balance, food consumption databases that do not retain brand level data are not materially disadvantaged in assessing food additive intake.

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**Table 2.1** **Number of brands per food category for which the number of consumers is greater than or equal to 60.**

| | Number of consumers | | Number of brands | |
|---|---|---|---|---|
| Food Category | No. | % | Total | ≥ 60 consumers |
| Biscuits | 1725 | 79 | 286 | 15 |
| Breakfast cereals | 1040 | 47 | 22 | 8 |
| Carbonated beverages | 1190 | 54 | 126 | 4 |
| Savoury snacks | 1034 | 47 | 11 | 6 |
| Fats | 2126 | 97 | 131 | 15 |
| Fruits | 1715 | 78 | 83 | 3 |
| Fruit juices | 1093 | 50 | 152 | 5 |
| Ice creams | 750 | 34 | 54 | 3 |
| Preserves | 957 | 44 | 60 | 4 |
| Nuts and seeds | 433 | 20 | 42 | 1 |
| Pastas, rice and grains | 1273 | 58 | 99 | 5 |
| Sauces and pickles | 1891 | 86 | 211 | 11 |
| Soups | 858 | 39 | 36 | 3 |
| Yoghurts | 585 | 27 | 63 | 2 |
| Total | | | 1363 | 85 |

**Table 2.2** **Intakes (g/day) at 97.5$^{th}$ percentile for 14 food categories and the highest recorded 97.5$^{th}$ percentile intake (g/day) for relevant sub-categories of foods.**

| Food category | No. of sub-categories | Intake at the 97.5$^{th}$ percentile for a food category (g/day) | Highest intake of a sub-category at the 97.5$^{th}$ percentile of a food category (g/day) |
|---|---|---|---|
| Biscuits | 16 | 69 | 56 |
| Breakfast cereals | 3 | 81 | 103 |
| Carbonated beverages | 4 | 499 | 510 |
| Savoury snacks | 3 | 36 | 22 |
| Fats | 13 | 47 | 53 |
| Fruit | 8 | 306 | 278 |
| Fruit juices | 12 | 366 | 359 |
| Ice creams | 6 | 68 | 59 |
| Preserves | 5 | 39 | 34 |
| Nuts and seeds | 6 | 39 | 48 |
| Pastas, rice and grains | 15 | 160 | 189 |
| Sauces and pickles | 17 | 90 | 61 |
| Soups | 7 | 225 | 202 |
| Yoghurts | 7 | 143 | 142 |

**Table 2.3 Number of brands per food category for which the brand intake at the 97.5$^{th}$ percentile exceeded the 97.5$^{th}$ percentile of the food category multiplied by 1.3.**

| Food Category | No. of brands ≥ 60 consumers | 97.5$^{th}$ percentile (g/d) | 97.5$^{th}$ percentile by 1.3 | Highest intake of a brand at the 97.5$^{th}$ percentile of the food category |
|---|---|---|---|---|
| Biscuits | 15 | 69 | 90 | 56 |
| Breakfast cereals | 8 | 81 | 105 | 91 |
| Carbonated beverages | 4 | 499 | 649 | 569 |
| Savoury snacks | 6 | 36 | 47 | 37 |
| Fats | 15 | 47 | 61 | 53 |
| Fruit | 3 | 306 | 398 | 154 |
| Fruit juices | 5 | 366 | 476 | 435 |
| Ice creams | 3 | 68 | 88 | 80 |
| Preserves | 4 | 39 | 51 | 47 |
| Nuts and seeds | 1 | 39 | 51 | 25 |
| Pastas, rice and grains | 5 | 160 | 208 | 146 |
| Sauces and pickles | 11 | 90 | 94 | 76 |
| Soups | 3 | 225 | 293 | 179 |
| Yoghurts | 2 | 143 | 186 | 155 |

**Appendix 2.1 Details of foods contained in each sub-category for a given food**

| Food Category | Sub-categories |
|---|---|
| Biscuits | Chocolate coated, chocolate half coated, chocolate full coated, crackers, crispbreads, digestives, filled, flapjacks, flavoured, fruit/fruit and nut, miscellaneous, semi-sweet, shortbread, sweet, wafer, wholemeal. |
| Breakfast cereals | Puffed/extruded, flake type no fruit/nuts, muesli/flake type with fruit/nuts. |
| Carbonated beverages | Canned, bottled, diet, regular. |
| Savoury snacks | Potato crisps not low fat, puffed potato products, corn snacks. |
| Fats | Salted, unsalted, butter, slated butter, spreads, low fat spreads, yellow spreads, hard block margarines, hard block margarine others, hard block Krona margarine. |
| Fruit | Canned, canned in fruit juice, canned in syrup, dried, fresh, stewed, stewed with sugar, stewed without sugar. |
| Fruit juices | Carbonated, canned carton/bottle, sweetened or unsweetened fruit juice, apple, grapefruit, orange or pineapple juice, mixed fruit juices, fruit squash. |
| Ice cream | Dairy, non-dairy, vanilla, flavoured, hard block, soft scoop. |
| Preserves | Reduced sugar, extra fruit marmalades, edible seed, stone fruit. |
| Nuts and seeds | Peanuts, roast peanuts, peanut butter, nut kernels, salted nuts, unsalted nuts. |
| Pastas, rice and grains | Pasta: macaroni, noodles, pot noodles, spaghetti, boiled spaghetti, canned spaghetti, dumplings, milk puddings.<br>Rice: basmati, brown, white savoury, pudding, fried, boiled. |

**Appendix 2.1 Details of foods contained in each sub-category for a given food *cont.***

| Food Category | Sub-categories |
|---|---|
| Sauces and Pickles | Sauces: brown, cheese, cook in curry, gravy, mayonnaise, mint, prawn, sweet and sour, tomato, bottled tomato, white, French dressing.<br>Pickles: onion, sweet, beetroot.<br>Spreads: mustard. |
| Soups | Canned, packet, home-made, vegetable, meat, low calorie, miscellaneous. |
| Yoghurts | Creamy, low fat, fruit/flavoured, nut/muesli, natural, sweetened, unsweetened. |

# *Chapter 3.*

## *Estimation of intakes of intense sweeteners using a 3-day diary and semi – quantitative questionnaires.*

## 3.1 Introduction.

The EU permits sweeteners in a wide range of foods and beverages. Detailed conditions of use, including both the food types and the maximum levels of use (MPL), are specified in the sweetener directive (EC, 94/35/EC). The EU also allows the use of blends or mixtures of sweeteners with the only restriction being that any one sweetener may not be used above its specified use level. In practice, this blending results in lower uses of individual sweeteners thus, exposure is then lowered for consumers (Shively – Knight, 1997). The most common sweeteners in use around the world are aspartame, acesulfame K, saccharin, and cyclamates. Neohespheridine DC (NHDC) and thaumatin are widely approved but not widely used, and the newer sweeteners, sucralose and alitame are not so widely approved but are becoming more so (Knight, 1997).

Article 4 in the 94/35/EC Directive on sweeteners states that "within three years of its adoption, Member States shall establish a system of consumer surveys to monitor sweetener intake". Unfortunately no guidelines were issued with Directive 94/35/EC. As a result it had been left to each Member State to determine the appropriate method of monitoring intake. The chosen method needs to be one that is easy to administer, accurate and not too costly to sustain a continual surveillance of sweetener intakes. In Ireland as of yet, no such system has been designed or implemented. The present project set out to explore the possible use of a food frequency questionnaire to achieve this aim.

When conducting a nutritional survey, three important points need to be considered (1) the method used (2) the group studied and (3) the cost from financial and time points of view. An overall consideration in research is that the more detailed the desired data, the more expensive, time consuming and subject to error the method required (Quandt, 1987). A system of nutritional surveillance to monitor sweetener consumption must be sensitive to be able to protect particular groups of consumers and must be credible to be able to convince industry and governments of the reality of the risk detected (Verger, 1996).

Many countries around the world have conducted sweetener intake studies. The methods used for food consumption intakes varied greatly from country to country with questionnaires and diaries as the main sources of data collection used. These studies are summarised in Tables 1.4 and 1.5. It can be seen that for the sweetener usage levels, manufacturer's information or chemical analysis was used in order to provide a high level

of accuracy. Due to the market continuously changing and the composition and number of products containing sweeteners never remaining static, laboratory analysis or manufacturers' information would need to be updated on a constant basis. The cost of laboratory analysis would thus be prohibitive if a large number of foods are being investigated. Manufacturers' information, whilst very accurate, indicates that actual usage levels are usually lower than the MPL for the sweetener. Such data can be very difficult to obtain due to 'commercial sensitivity'. With such difficulties in mind, routine monitoring of this nature would prove to be very costly and cumbersome. The question arises as to which methods could be used to determine intakes that are reasonably accurate and inexpensive.

The Directives provide information regarding the MPLs of a sweetener in a food. This information is readily available and might be useful for a surveillance programme. This method would be very crude for several reasons:

i. Although the Directive may permit the use of an intense sweetener in a food category, it doesn't follow that it is actually used in the manufacture of some or even any brands within that category.
ii. The usage levels are generally well below the Directive MPL.
iii. Blends of sweeteners used in foods will almost certainly mean greatly reduced usage levels.

Using such data would assume the highest possible level of intakes. If the results showed that the intakes of the sweeteners were well below their respective acceptable daily intake (ADI), then there would be no need for concern or further investigation. If however, a problem was detected at the most crude level, then more refined data should be used in order to determine whether or not there is really a problem and if so, what sweetener and what group of the population are at risk. When conducting a survey, the study group of interest are of utmost importance. When discussing the issue of sweeteners, there are two groups within the population thought to have a higher intake of sweeteners when compared to the general population; diabetics due to their disease status and slimmers using sweeteners to aid weight loss. Slimmers and Non Insulin Dependent Diabetics (NIDDM) are likely to be overweight or restrained eaters and are known to heavily under-report

energy intakes (Lansky and Brownell, 1982, Livingstone *et al*, 1990, Mela and Aaron, 1997).

The present study set out to determine crude intakes of intense sweeteners in patients with Insulin Dependent Diabetes Mellitus (IDDM) and their age and sex matched controls using two methods of measuring food intake. The 3-day food diary record was chosen since this is typical of the approach used for large national dietary surveys and the data would be typical of that available from national survey databases. A food frequency questionnaire (FFQ) was also chosen since this is by far the easiest method of collecting food intake data and thus might be useful for a continued sweetener intake surveillance programme.

## 3.2 Subjects and Methods.

### *3.2.1 Ethical Approval.*

The protocols for this study were approved by the Joint Ethics Research Committee of St. James's Hospital and Federated Voluntary Hospitals.

### *3.2.2 Recruitment of Subjects.*

Insulin Dependent Diabetic subjects in the age range of 16 – 75 years were recruited from two outpatient departments of two diabetes clinics of a major Dublin hospital or by post from a list of insulin requiring patients held by nursing staff. These patients were diagnosed as defined by clinical criteria at least 6 months previously (in order to allow time to adapt to new regime), and had no known illness other than IDDM without major complications. Age (± 5 years) and sex matched non-diabetic controls were recruited from friends of the patients or the staff and students of Trinity College and University College Dublin. The exclusion criteria were being less than 16 or over 75 years of age, having diabetic complications such as retinopathy, nephropathy, having severe social problems, living outside the catchment area, illiteracy, pregnancy, a diagnosis of IDDM of less than 6 months and a diagnosis of NIDDM. In total, 171 IDDMs were contacted by mail or approached at the hospital. Those who were contacted by mail had an introductory letter sent explaining the study and how they were selected. A week lapsed before contact was made again, this time via telephone, where possible, to determine interest in the study.

*3.2.3 Anthropometric data.*

Each subject was measured for height (m) using a Stanley Metro measuring tape and their weight was recorded in kg using a EKS digital weighing scales. These scales were assessed in comparison to those used by the North South Ireland Food Consumption Survey field workers who had their scales calibrated. There was agreement within 0.1 kg between the scales.

*3.2.4 Materials used.*

*3.2.4.1 Semi - Quantitative Food Frequency Questionnaire.*

A 45-item questionnaire was developed using the Irish National Food Ingredient Database (INFID) to assess the intake of intense sweeteners. This database was developed in Trinity College between 1995 – 1999 (Lambe, 2000). It consists of 5,684 processed foods and the ingredient list for each food. In order to determine which foods contained the sweeteners of interest in this study, a search was run on each sweetener. This resulted in a list of foods and their brands containing the sweetener in question. This list was then re-organised into a 45-item list of food categories for the FFQ. In some cases, more general groups could be formed *e.g.* diet carbonated beverages, diet yoghurts *etc.* The portion sizes in the questionnaire corresponded to natural units *e.g.* a glass of..., a stick of ..., *etc.* These were based on average portion sizes (Crawley, 1993). Participants reported the frequency of consumption of each food by selecting one of ten frequency categories, ranging from "rarely or never" to "6 or more times per day". Mid - point values were assigned to frequency alternatives *e.g.* 4 - 5 times per day was taken as 4.5 times a day. An example of the questionnaire is shown in Appendix 3.1.

*3.2.4.2 The 3- day diary.*

The diary used was one designed and used in the North–South Ireland Food Consumption Survey of Irish Adults in 1998-1999 by the Irish Universities Nutrition Alliance (Harrington *et al*, 2000). Subjects were required to complete the diary for 2-week days and 1 weekend day. Controls completed the diary for the same week and weekend days as their corresponding subjects.

The diary layout enabled details of every eating occasion to be recorded: date, day, time, location, meal/snack, main ingredients of home-made recipes, precise name (brand/flavour/type) of manufactured foods, cooking methods and leftovers. An example of the diary used is given in Appendix 3.2.

### *3.2.5 Survey Method.*

The fieldwork of the study was carried out from March 1998 to April 1999. This ensured that the data collected was spread out evenly over the seasons. No fieldwork was conducted during public holiday periods *e.g.* Christmas, Easter *etc.* On average, a two-week lapse in time was allowed between each part of the survey to reduce influence of memory on answers given (*i.e.* on average there was a 6-week gap between completing the first and last questionnaire –shown in Table 3.1). Over the whole sample, the beginning times for the respondents were spread out equally over the seven days of the week and over seasons. All participants signed a consent form and an identification number was assigned to each participant to ensure confidentiality.

The 45-item semi-quantitative questionnaire was sent to each participant by post who agreed to take part in the study. It was completed and returned to the researcher using the stamped addressed envelope that was provided. From here on, it shall be referred to as the self - administered food frequency questionnaire or SAFFQ. Each food was assigned a quantity and entered into the database with the identification number of each subject/control.

Seven to ten days after receiving the questionnaire from the participant, contact was made again by telephone. An appointment was made to visit the participant in their homes or a place of convenience at a set time the day before they were due to begin recording their food and beverage intake. This appointment took place 2 - 3 weeks after the subject had returned the SAFFQ. At this meeting, the respondent was given their 3-day diary. Instructions on how to complete the diary and on how to express the quantity of foods and beverages with standard household measures or standard portions were given verbally and as written guidelines. Respondents were asked to enter records in the diary after each eating occasion and to maintain their usual eating habits.

After completing the diary, a second meeting was arranged. This meeting entailed the researcher going through the previous three days entries with the subject, checking food description, brands, ingredients of recipes and quantities. Queries were made to identify possible omissions such as between meal eating occasions (beverage, confectionery, chewing gums, snacks *etc.*) or the use of sweeteners or condiments.

Each single food or beverage reported in a diary was coded with the quantity, the unit of measurement (grams (g) or millilitres (ml)) and a food code (of either single food or recipe). On occasion, no food code was available in the nutritional analysis database for the food/cooking method *etc.* When this occurred, the nearest cooking method/food with nutritional information in the database per 100g and per portion size was compared to that of the information on the product packet of the food consumed. In the case of a completely a new product, a new food code was created for it using the nutritional information provided on the packaging. Two weeks after the diary was collected, the subject's weight in kilograms (kg) and height in metres (m) were measured by the researcher.

Approximately 6 weeks after completing the SAFFQ, subjects again completed the same 45-item FFQ. This time the researcher was present at the completion of the questionnaire. The subject completed the questionnaire with some help from the researcher (if asked) and after its completion, the researcher went through the answers given with the subject. From this point on, it will be referred to as the interviewer present food frequency questionnaire or the IPFFQ. Again each food was assigned a quantity and entered into the database with the identification number of each subject/control.

### *3.2.6 Quality Control.*

Rigorous quality control of the procedure of coding of foods and data entry was performed. Each item was entered twice in order to limit the level of errors found in the database. A random 25% selection was then made of the diaries, and questionnaires (*i.e.* 60 diaries, 60 SAFFQs and 60 IPFFQs) and these again were checked for inaccuracies. From these, a level of error ranging from 0 - 3% was found, that being only one food code or weight/portion size being incorrect in one or more of the diaries/questionnaires. This level of error was deemed satisfactory and analysis could be proceeded with.

For snacks, meals purchased away from the home and other foods that could not be weighed, verbal descriptions of portions were obtained in terms of familiar volumes, dimensions and purchasing units. Ingredients and packaging were also described. If possible, similar items were purchased by the researcher and weighed.

### *3.2.7 Sweeteners.*

Food intake data of this study were analysed to assess the intake of nutrients and artificial sweeteners. In the present study the Acceptable Daily Intakes (ADIs) used are established by the Scientific Committee for Food for the intense sweeteners. These are 5 mg/kg bw/d for saccharin, 9 mg/kg bw/d for acesulfame K, 11 mg/kg bw/d for cyclamate and 40 mg/kg bw/d for aspartame, 5 mg/kg bw/d for Neohespheridine DC (Commission for the European Communities 1985, 1989, 1992, European Commission, 1997). The other intense sweetener authorised in the European Union is Thaumatin E957 does not have an ADI.

The consumption data collected from the subjects was converted from portion sizes into grams per day (g/day) or millilitres per day (ml/day) as appropriate. This information was then combined with the MPLs to give intakes of sweeteners in mg/d for each participant in the survey. To facilitate comparison to the acceptable daily intake (ADI), sweetener intakes were expressed in mg/kg bw/d of each respondent. The use of tabletop sweeteners was evaluated by enquiring after the addition of sweeteners to tea, coffee, other beverages and yoghurt/desserts for week and weekend days. Enquiries related to the form of sweetener (tablets/powder/liquid) and the quantity added to each cup or bowl were made. Subjects also reported the type of sweeteners they commonly used (trade name).

The 3 - day diary is considered the 'gold standard' for the estimation of consumption of foods containing intense sweeteners in comparison to the FFQs.

### *3.2.8 Statistical Analysis.*

The age, weight, height and body mass index (BMI) of the diabetic and control groups were analysed using an independent t - test and the employment and education levels were investigated using a chi-squared ($X^2$) test.

When investigating the intakes of the intense sweeteners, the intake data from the diary and questionnaires were expressed on a 'total population' and 'consumer only' basis

in order to estimate more precisely the intake among those actually consuming sweeteners. 'Total population' was defined as all individuals in each group (*i.e.* diabetic and control), who completed the diary for the 3 days, SAFFQ and the IPFFQ irrespective of whether or not they consumed foods or beverages containing sweeteners. 'Consumers only' were defined as individuals in either the diabetic or control group who consumed any product containing a sweetener on at least one of the three survey days in the diary or within the month period in the FFQs.

Due to the fact that the data were non - parametric, the Kruskall Wallis method was used to compare the data of the SAFFQ, IPFFQ and the 3-day diary for consumer only intakes for each sweetener. The Mann Whitney test was used to compare the consumer only intakes of each sweetener in diabetics versus controls.

The estimated sweetener intake for total population intakes using the 3-day diary and the IPFFQ were also compared, using the Bland and Altman method (Bland and Altman, 1986). Total population intakes were used due to that fact that there is a high number of consumers of the intense sweeteners in the two groups, 93% and 86% respectively for diabetics and controls. Bland and Altman plots are the average intakes of both methods plotted against the difference in intakes between the methods.

### 3.3 Results.

Of the 171 IDDM patients contacted, 123 agreed to participate (72% response rate). In all, 119 patients completed all aspects of the study. Four subjects did not complete all aspects of the study due to time constraints.

The age, weight, height and BMI of subjects are given in Table 3.2. There are no significant differences in age, weight, and height. The diabetics had a significantly higher BMI in comparison to the controls (26.8 v 25.2, $p = 0.04$). The level of education and employment are described in Table 3.3. Whilst education level is quite different between groups ($p = 0.001$), employment is quite similar and not significant.

The mean intakes of sweeteners amongst the total population of the diabetic and control groups can be seen in Figure 3.1 for each of the intense sweeteners and for the three methods used in this study. It can be seen that the diabetics have a higher level of

usage of the sweeteners for each method. Since the present study is concerned with the protection of consumers' health, the subsequent analysis will focus on consumers only.

Table 3.4 looks at the data for the consumers only of diabetics and controls for each of the sweeteners and methods used. It can be seen that in the case of acesulfame K, the 97.5$^{th}$ percentile of intake for the diabetic consumer only group exceeded the ADI in both questionnaires (104% and 103% of the ADI for IPFFQ and SAFFQ respectively). No other sweetener with the exception of cyclamate exceeded or even met the ADI. For cyclamate, intakes in both questionnaires were quite high at the 97.5$^{th}$ percentile level of intake for the diabetic group (92% and 89% of the ADI for IPFFQ and SAFFQ respectively).

Table 3.5 summarises the results of the Kruskall Wallis comparison across the 3 methods for each subject group. Only in the case of thaumatin were there no differences between methods. The 3-day diary and the IPFFQ were not significantly different in their estimates of the intakes of saccharin and cyclamic acid for controls. Otherwise all comparisons were significantly different ($p$<0.001 – $p$ = 0.05). Table 3.6 summarises the results of the Mann Whitney comparisons of intense sweetener intake of diabetics versus controls for each method. In all instances, there were significant differences between the two groups with diabetics showing higher intakes of all intense sweeteners ($p$ <0.001 – $p$ = 0.05). Figures 3.2-3.7 show the Bland and Altman plots comparing the 3-day diary and the IPFFQ for the total population. In all instances, a triangular shape was observed indicating significant divergence between the methods in estimates of individual intense sweetener intake.

## 3.4 Discussion.

Previous studies have recorded intense sweetener intake based on (a) a precise knowledge of the presence of a sweetener in the brand-food being consumed and (b) precise knowledge of the true concentration of the intense sweetener in the brand-food. The present study grossly over-estimates the exposure to intense sweetener intake for two reasons. Firstly, the presence of any sweetener in brand foods within a food category used in the food frequency questionnaire was assumed positive based on the observed presence of a given sweetener in any brand in that category using the INFID. For example, acesulfame K is permitted for use in 'energy reduced jams, jellies and marmalades' and

according to the INFID is present in at least one brand of 'energy reduced jams, jellies and marmalades'. In this study it was assumed that all exposure to 'energy reduced jams, jellies and marmalades' is exposure to 'energy reduced jams, jellies and marmalades' containing acesulfame K. However, of the 29 brands of 'energy reduced jams, jellies and marmalades' present in the INFID, only 5 (17%), actually contain acesulfame K. The levels of acesulfame K used in this study are the maximum permitted levels set out in the Directives. Industry was approached for data on usage levels but none was made available. Of the 5,684 brands in the INFID database, only 78 (1.4%), brands of foods were found to contain acesulfame K. Table 3.7 shows the percentage of brands within the 45-foods/food groups of the FFQ that contained acesulfame K in comparison to the number of brands within that food group.

Aspartame is the one intense sweetener common to both previous sweetener intake studies and the present study. Table 3.8 shows the comparison of consumer only intakes of aspartame between European countries expressed as a percentage of the ADI (40mg/kg bw/d). In the present study, crude methods of sweetener intake estimation were used. Despite using such crude methods of assessing sweetener intake in comparison to the refined methods employed by the other countries, the estimates of intakes are not dramatically different. Whilst the findings of this study are generally higher than the other studies, the intakes are not that dissimilar. It can be seen that the intakes of of 4.9 mg/kg bw/d of the control group in this study, fall within the intakes of the general population in the Netherlands, Austria and Norway, mean intakes vary from 3.3 – 6.8 mg/kg bw/d. At the 97.5$^{th}$ percentile level of intakes for diabetic groups, the range of higher intakes were between 15.6 and 25.3 mg/kg bw/d. In the present study intakes at the 97.5$^{th}$ percentile are at 28.4mg/kg bw/d for the diabetics and are broadly comparable to the other studies.

The two questionnaires yielded higher average estimates of intakes of all sweeteners. This can be seen in Figure 3.8 where the ratio for total population intakes of both questionnaires to the 3-day diary is shown. There are several reasons why the diary would produce a lower value. Firstly, the diary covered a 3-day period whereas the questionnaire covered intakes for a month; it is possible that the FFQ detected irregular use of foods that the diary missed. Secondly, there is the issue of portion size as in the questionnaire the portion size was pre-determined to the administration of the

questionnaires, thus the participants were not offered the opportunity to select or report the portion size most representative of their usual intake. Thirdly, the issue of food preference, as ' people might be more likely to report a high frequency of consumption for a preferred than for a non-preferred food. Thus, memory of foods consumed is biased by food likes and dislikes' (Drenowski and Hann, 1999). It is possible that subjects in the present study recorded a greater frequency of consumption in the questionnaire than was reported in the diary.

The Bland and Altman plots (Figures 3.2 – 3.7), clearly show that the methods differ in estimates and that the scale of the difference increases with estimated sweetener intakes. Although the plots show a triangular nature, there is a clear tendency for the differences of the two estimates in intakes to be negative as the average of the intake increases. The most probable explanation, also supported by the data in Figure 3.8, is that the FFQ led to high estimates for some individuals, which would increase both the average intake of the two estimates and also the difference in intakes between the two methods. Thus if the diary gave a value of X and the FFQ gave a value of 5X, the mean would be 3X and the difference would be –4X. Clearly the FFQ did give a large estimate of intake for very many individuals.

For reasons of applicability for 'routine' surveillance, the most obvious option is to use a food frequency questionnaire due to the many advantages with using this method. Is it then possible that the present questionnaire could be used to estimate exposure to intense sweeteners? The answer is probably not, although there are possibly a number of ways that the present approach can be adapted. One such possibility would be to decrease the cut off value for high consumers *i.e.* the $90^{th}$ percentile due to the fact that the FFQ overestimates intakes in comparison to the 3-day diary. This would then take account of such overestimates and still be participant friendly. Another would be to use the FFQ to determine which foods were consumed the most in relation to the sweetener of concern or the foods in which the sweetener is widely used. These prime foods could then be investigated thoroughly. Another possibility would be to incorporate stochastic modelling or probabilistic analysis. There are a number of steps in this analysis. Step one, the probability of being a consumer is calculated from the data collection method *e.g.* food frequency questionnaire. Step two is that the food intake is entered into the model as a

histogram of total population/consumer only intakes. A value is then drawn from the distribution of intakes in a series of iterations (hypothetical people). The intakes with a high probability of occurrence are selected more often than intakes with a low probability of occurrence. Step three is then to look at the probability of a sweetener occurring in the food. For this, INFID is used. The number of brands of the food(s) being investigated in which the additive/sweetener occurs is determined; this is called the probability of occurrence. Step four is to multiply the probability of being a consumer, the intake selected and the probability of occurrence with either the MPL or actual usage data. The final value given is the probability of consuming the additive. There are many potential methods with which the present questionnaire in this study could be refined, it is a matter of finding out which is most accurate and participant friendly without being too expensive, as the key to it all is routine monitoring.

In conclusion, the results of the present study indicate that currently FFQs are not an appropriate method for routine monitoring. Further work as discussed earlier in the text needs to be done such as lowering the cut off point for estimating high intakes and introducing the use of stochastic modelling.

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**Table 3.1 Summary of methods and time scale of study.**

| Time | Method |
|---|---|
| Week 0 | SAFFQ |
| Week 2 - 3 | Diary |
| Week 4 - 7 | Weight, Height & IPFFQ |

**Table 3.2 Sex, Age, Weight, Height and BMI of participants.**

| | **Diabetics (n = 119)** | **Controls ( n = 119)** | |
|---|---|---|---|
| | Mean (± SD) | Mean (± SD) | *p* value = |
| Men | 65 | 65 | |
| Women | 54 | 54 | |
| Age (years) | 36.7 ± 15.2 | 36.6 ± 14.5 | NS |
| Weight (Kg) | 74.9 ± 13.8 | 72.2 ± 13.2 | NS |
| Height (m) | 1.67 ± 0.1 | 1.69 ± .01 | NS |
| BMI ($kg/m^2$) | 26.8 ± 4.5 | 25.2 ± 4.0 | 0.04 |

**Table 3.3 Number of Participants per Education and Employment level.**

| **Group** | **Education** | | | | **Employment** | | | |
|---|---|---|---|---|---|---|---|---|
| ***Level*** | ***1*** | ***2*** | ***3*** | ***4*** | ***1*** | ***2*** | ***3*** | ***4*** |
| **All** | 52 | 80 | 67 | 39 | 179 | 19 | 14 | 26 |
| **IDDM** | 36 | 54 | 28 | 1 | 86 | 13 | 8 | 12 |
| **Control** | 16 | 26 | 39 | 38 | 93 | 6 | 6 | 14 |

Education 1 = Primary, 2 = Secondary, 3 = Tertiary, 4 = Postgraduate

Employment 1 = Employed, 2 = Unemployed, 3 = Retired, 4 = Student

**Table 3.4** **Consumer only intakes of intense sweeteners for diabetics (D; n = 119) and controls (C;n= 119) for diary, interviewer present food frequency questionnaire (Ipffq) and self-administered food frequency questionnaire (Saffq) in mg/kg bw/d at the mean, median, 95$^{th}$ and 97.5$^{th}$ percentiles and the ADI in mg/kg bw/d for each sweetener.**

| | | | | *Consumer population* | | | *High intakes* | | *ADI* |
|---|---|---|---|---|---|---|---|---|---|
| | *Method* | *Group* | *N* | *Median* | *Mean* | *SD* | *95th* | *97.5th* | |
| ***aces.*** | *Diary* | D | 113 | 0.96 | 1.72 | 1.74 | 5.11 | 5.96 | 9 |
| | | C | 100 | 0.81 | 1.03 | 1.15 | 3.52 | 4.05 | |
| | *Ipffq* | D | 117 | 1.85 | 2.75 | 2.64 | 7.63 | 9.40 | |
| | | C | 119 | 0.86 | 1.27 | 1.30 | 4.6 | 5.46 | |
| | *Saffq* | D | 119 | 1.94 | 2.99 | 2.64 | 7.95 | 9.31 | |
| | | C | 118 | 1.23 | 1.72 | 1.65 | 5.57 | 7.29 | |
| **apm.** | *Diary* | D | 113 | 2.06 | 3.26 | 3.13 | 9.04 | 11.34 | 40 |
| | | C | 100 | 1.52 | 1.96 | 2.06 | 6.01 | 6.84 | |
| | *Ipffq* | D | 117 | 3.80 | 5.36 | 5.18 | 14.12 | 17.19 | |
| | | C | 119 | 1.64 | 2.43 | 2.37 | 8.63 | 9.57 | |
| | *Saffq* | D | 119 | 4.04 | 5.83 | 4.99 | 14.94 | 18.89 | |
| | | C | 118 | 2.37 | 3.26 | 3.25 | 9.77 | 13.57 | |
| **sac.** | *Diary* | D | 113 | 0.34 | 0.63 | 0.65 | 2.11 | 2.42 | 5 |
| | | C | 100 | 0.24 | 0.45 | 0.60 | 1.89 | 2.69 | |
| | *Ipffq* | D | 117 | 0.64 | 0.88 | 0.84 | 2.45 | 2.87 | |
| | | C | 119 | 0.29 | 0.49 | 0.69 | 1.29 | 2.57 | |
| | *Saffq* | D | 119 | 0.73 | 0.99 | 0.89 | 3.24 | 3.61 | |
| | | C | 118 | 0.43 | 0.60 | 0.59 | 2.03 | 2.40 | |
| **cyc.** | *Diary* | D | 103 | 1.12 | 1.96 | 1.95 | 5.92 | 6.83 | 11 |
| | | C | 87 | 0.86 | 1.24 | 1.32 | 3.78 | 4.52 | |
| | *Ipffq* | D | 117 | 1.66 | 2.71 | 2.77 | 7.83 | 10.10 | |
| | | C | 119 | 0.78 | 1.24 | 1.35 | 4.30 | 5.29 | |
| | *Saffq* | D | 119 | 1.89 | 2.96 | 2.74 | 8.22 | 9.74 | |
| | | C | 117 | 1.22 | 1.69 | 1.73 | 6.28 | 7.78 | |
| **neo.** | *Diary* | D | 112 | 0.13 | 0.18 | 0.16 | 0.49 | 0.58 | 5 |
| | | C | 96 | 0.08 | 0.11 | 0.11 | 0.31 | 0.39 | |
| | *Ipffq* | D | 117 | 0.23 | 0.30 | 0.28 | 0.81 | 0.89 | |
| | | C | 119 | 0.10 | 0.14 | 0.14 | 0.48 | 0.65 | |
| | *Saffq* | D | 119 | 0.24 | 0.33 | 0.28 | 0.89 | 1.05 | |
| | | C | 118 | 0.14 | 0.19 | 0.18 | 0.54 | 0.74 | |
| **th.** | *Diary* | D | 42 | 0.01 | 0.01 | 0.02 | 0.10 | 0.10 | NSA |
| | | C | 20 | 0.003 | 0.01 | 0.01 | 0.03 | 0.03 | |
| | *Ipffq* | D | 105 | 0.004 | 0.02 | 0.02 | 0.08 | 0.1 | |
| | | C | 73 | 0.002 | 0.01 | 0.01 | 0.03 | 0.04 | |
| | *Saffq* | D | 107 | 0.003 | 0.01 | 0.02 | 0.04 | 0.09 | |
| | | C | 80 | 0.001 | 0.004 | 0.006 | 0.02 | 0.03 | |

aces = acesulfame K cyc. = cyclamic Acid apm.= aspartame sac. = saccharin neo. = Neohespheridine DC
th. = thaumatin NSA – no specified ADI

**Table 3.5** **Kruskall Wallis analysis comparing the intakes of each intense sweetener in mg/kg bw/d within diabetic consumer only and within control consumer only groups for the three methods.**

| Sweetener | Group | Diary v IPFFQ | Diary v SAFFQ | IPFFQ v SAFFQ |
|---|---|---|---|---|
| acesulfame K | Diabetics | *** | *** | * |
| | Controls | *** | *** | *** |
| aspartame | Diabetics | *** | *** | ** |
| | Controls | ** | *** | *** |
| saccharin | Diabetics | *** | *** | ** |
| | Controls | NS | ** | *** |
| cyclamic Acid | Diabetics | *** | *** | * |
| | Controls | NS | *** | *** |
| neohespheridine DC | Diabetics | *** | *** | ** |
| | Controls | ** | *** | *** |
| thaumatin | Diabetics | NS | NS | NS |
| | Controls | NS | NS | NS |

*** $p < 0.001$ ** $p = 0.01$ *$p = 0.05$ NS – non significant

**Table 3.6** **Mann Whitney statistical analysis test comparing consumers only groups of diabetics and controls for each intense sweetener and each method.**

| Sweetener | Group | Diary | SAFFQ | IPFFQ |
|---|---|---|---|---|
| acesulfame K | Diabetics v Controls | ** | *** | *** |
| aspartame | Diabetics v Controls | *** | *** | *** |
| saccharin | Diabetics v Controls | ** | *** | *** |
| cyclamic Acid | Diabetics v Controls | * | *** | *** |
| neohespheridine DC | Diabetics v Controls | *** | *** | *** |
| thaumatin | Diabetics v Controls | * | ** | ** |

*** $p < 0.001$ ** $p = 0.01$ *$p = 0.05$ NS – non significant

**Table 3.7** **Number of brands within the 45 food groups of the FFQ that contained acesulfame K (aces) in comparison to the number of brands present in INFID for the food group.**

| Food/food group | Database brands | aces in brands | % |
|---|---|---|---|
| Diet fizzy drink | 11 | 8 | 73 |
| Cola flavoured fizzy drink, not diet | 4 | 0 | 0 |
| Fruit flavoured fizzy drink, not diet | 75 | 1 | 1 |
| Diet Soda Stream | 2 | 2 | 100 |
| Soda stream, not diet | 9 | 1 | 11 |
| Diet/low sugar squash | 14 | 4 | 29 |
| Squash, not diet | 32 | 2 | 6 |
| Sports drink | 12 | 4 | 25 |
| Flavoured water | 22 | 14 | 64 |
| Tonic water | 5 | 2 | 40 |
| Light drinking chocolate | 9 | 9 | 100 |
| Diabetic biscuits | 12 | 0 | 0 |
| Mini cakes | 10 | 1 | 10 |
| Diabetic chocolate | 8 | 0 | 0 |
| Sugar free mr freeze | 1 | 0 | 0 |
| Tinned spaghetti | 3 | 1 | 33 |
| Angel delight, no sugar added | 2 | 2 | 100 |
| Low calorie chocolate mousse | 2 | 1 | 50 |
| Sugar free jelly | 6 | 4 | 67 |
| Jelly, not sugar free | 10 | 0 | 0 |
| Canned rice pudding, low fat | 1 | 1 | 100 |
| Low calorie ketchup | 1 | 0 | 0 |
| Light pasta sauce (not homemade) | 2 | 0 | 0 |
| Low fat dressings | 22 | 3 | 14 |
| Diabetic fruit bonbons | 2 | 0 | 0 |
| Fruit bonbons | 2 | 0 | 0 |
| Sugar free mints | 1 | 0 | 0 |

**Table 3.7 Number of brands in INFID database containing acesulfame K cont'd.**

| Food/food group | Database brands | aces in brands | % |
|---|---|---|---|
| Prawn cocktail flavoured snacks | 2 | 0 | 0 |
| Diabetic jam | 7 | 0 | 0 |
| Diabetic marmalade | 2 | 0 | 0 |
| Reduced sugar jam | 25 | 4 | 16 |
| Reduced sugar marmalade | 6 | 1 | 17 |
| Artificial sweetener | 6 | 2 | 33 |
| Pickled beetroot | 12 | 0 | 0 |
| Sugar free processed peas | 1 | 0 | 0 |
| Processed peas | 2 | 0 | 0 |
| Sugar free baked beans | 2 | 0 | 0 |
| Sweet pickled onions | 6 | 0 | 0 |
| Tinned vegetables | 69 | 0 | 0 |
| Diet yoghurt | 57 | 12 | 21 |
| Regular yoghurt | 106 | 0 | 0 |
| Jelly toppings | 11 | 1 | 9 |
| Sugar free chewing gum | 5 | 3 | 60 |
| Non sugar free chewing gum | 2 | 0 | 0 |
| Slimfast | 7 | 0 | 0 |

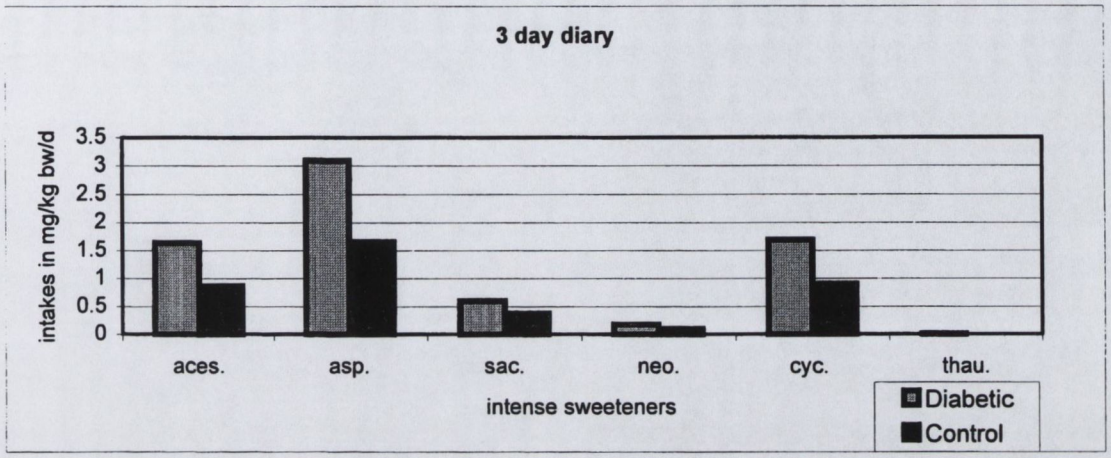
**Table 3.8** **Summary of consumer only intakes of aspartame mg/kg bw/d across Europe at the mean, median, 90th, 95th and 97.5th percentiles as a percent of the ADI (40mg/kg bw/d).**

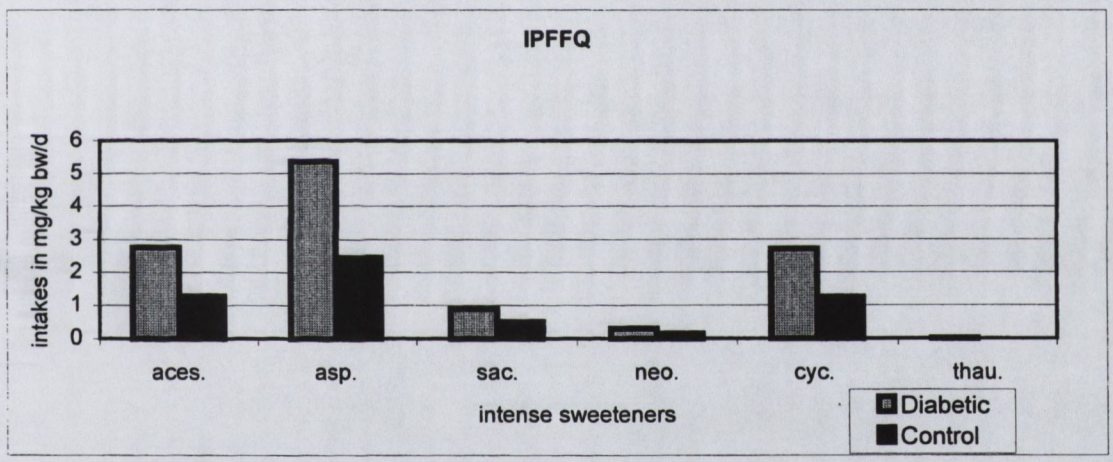
| | Intakes as % of ADI | | | Percentile intakes as % of the ADI | | |
|---|---|---|---|---|---|---|
| **Country** | **Group** | **Mean** | **Median** | **90th** | **95th** | **97.5th** |
| ***This study**** | Diabetics | 8.2 | 5.2 | NC | 22.6 | 28.4 |
| | Controls | 4.9 | 3.8 | NC | 15.0 | 17.1 |
| ***Austria*** | Gen. Pop. | 3.3 | NC | NC | 23.5 | NC |
| ***Denmark*** | Gen. Pop. | 1.1 | NC | NC | NC | 15.3 |
| | Diabetics | 3.0 | NC | NC | NC | 15.6 |
| ***Finland*** | Diabetics | 2.9 | NC | NC | NC | NC |
| ***France*** | Gen. Pop. | 0.5 | NC | NC | 2.5 | NC |
| ***Germany*** | Gen. Pop. | 3.0 | NC | 6.9 | NC | NC |
| | Diabetics | 0.3 | NC | NC | NC | NC |
| ***Italy***** | Gen. Pop. | 0.1 | 0.3 | NC | 0.3 | NC |
| ***Netherlands**** | Gen. Pop. | 4.8 | 3 | 13 | 18.8 | NC |
| ***Norway*** | Gen. Pop. | 6.8 | NC | NC | NC | NC |
| ***UK -1987/8*** | Gen. Pop | NC | 1.2 | NC | NC | NC |
| | Diabetics | NC | 7.5 | NC | NC | NC |
| ***UK - 1992*** | Gen. Pop. | 1.0 | 1.0 | 4 | NC | 6 |
| | Diabetics | NC | NC | 6 | NC | NC |
| ***UK - 1995*** | Diabetics | NC | NC | NC | NC | 25.3 |

* based on 3-day diary ** based on a 2-day diary *** based on 14 day diary

NC – not calculated/given Gen.Pop. - General Population

**Figure 3.1** **Mean total population intakes of intense sweeteners mg/kg bw/d for diabetic (n = 119) and control (n=119) groups for each method.**



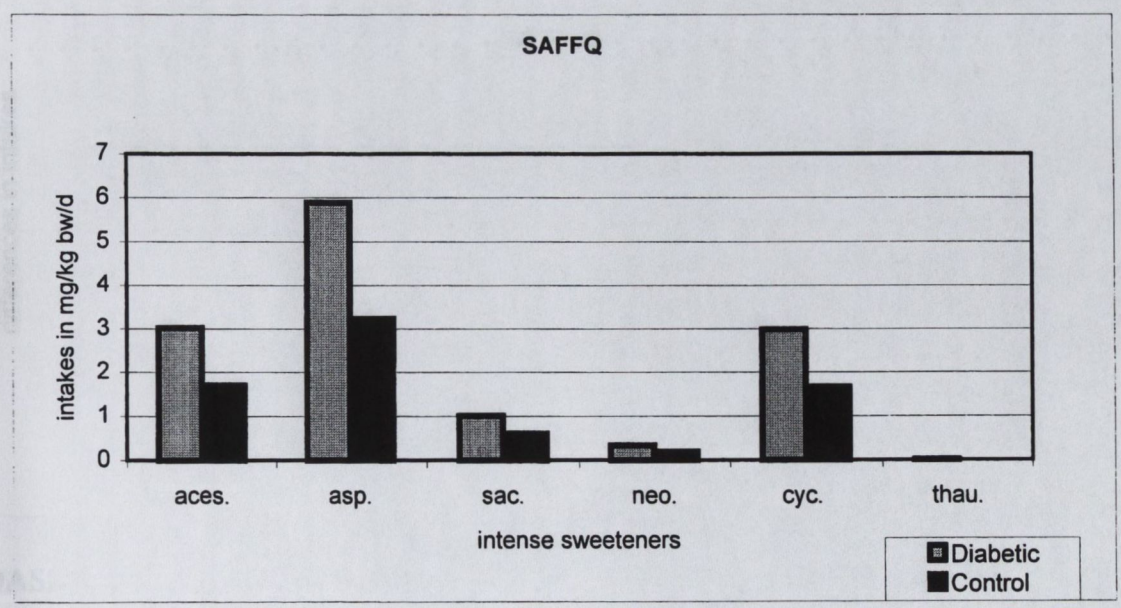



**Figure 3.2** **Bland and Altman plot of average intakes of acesulfame K mg/kg bw/d against differences in intakes for the diary and the IPFFQ.**

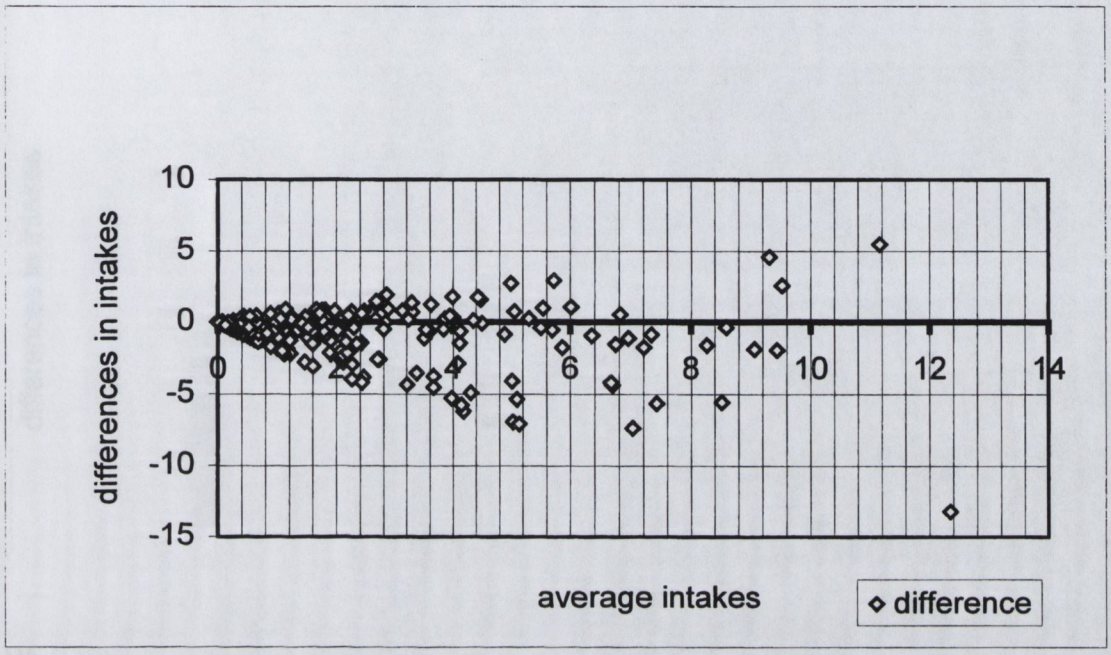
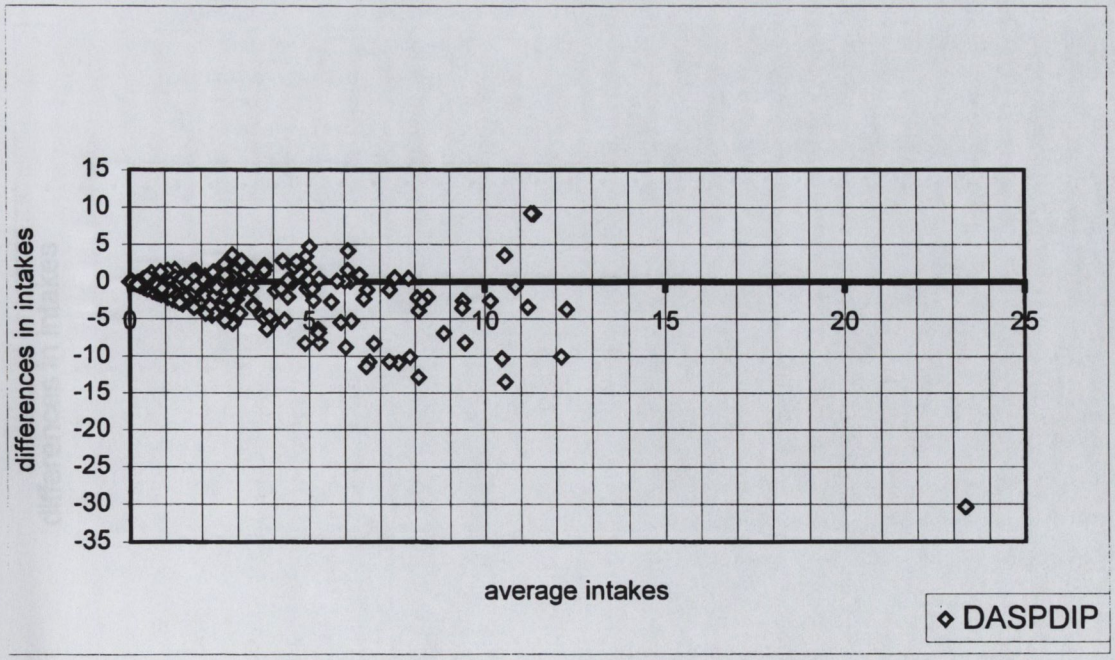
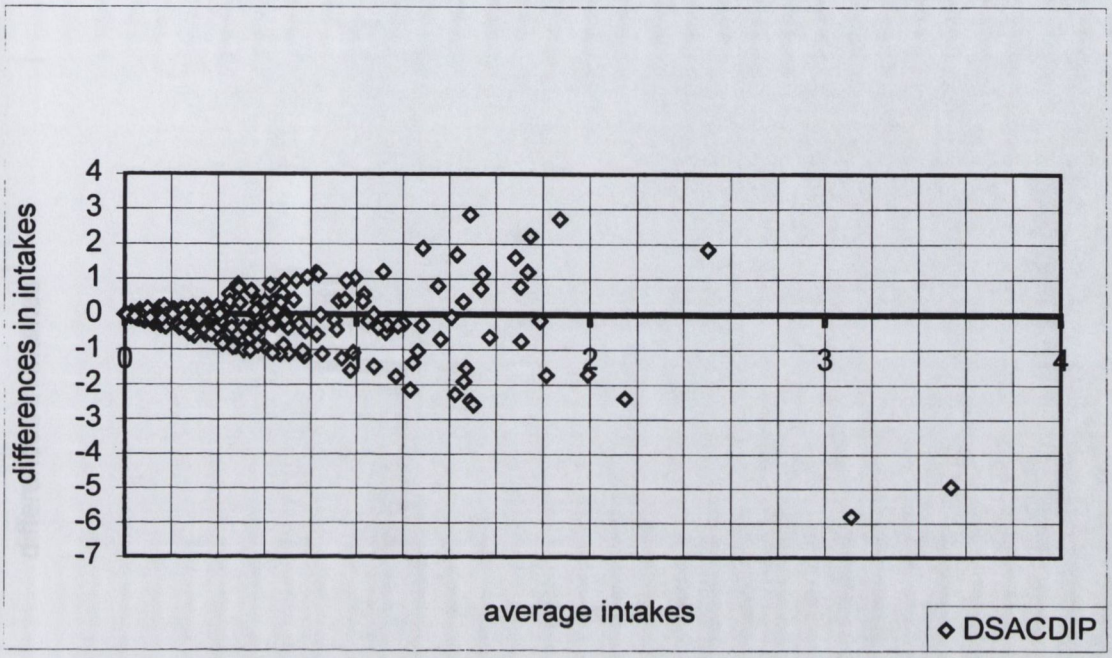



**Figure 3.3** **Bland and Altman plot of average intakes of aspartame mg/kg bw/d against differences in intakes between diary and IPFFQ.**



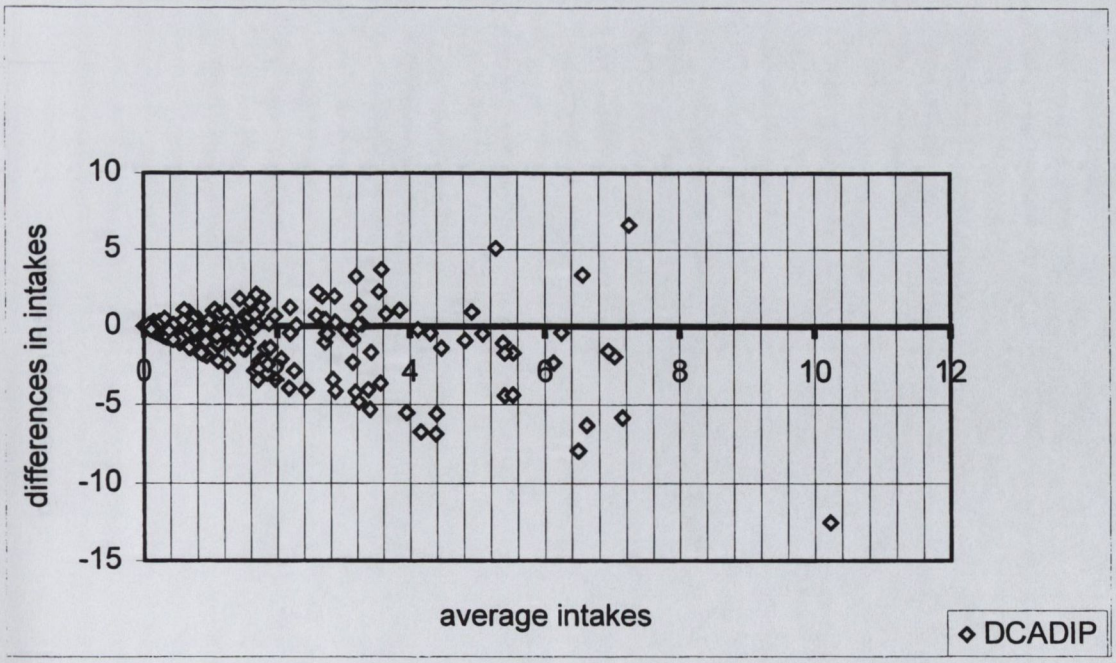

DASPDIP - differences in aspartame intake between the diary and the IPFFQ

**Figure 3.4** **Bland and Altman plot of average intakes of saccharin mg/kg bw/d against differences in intakes between diary and IPFFQ.**



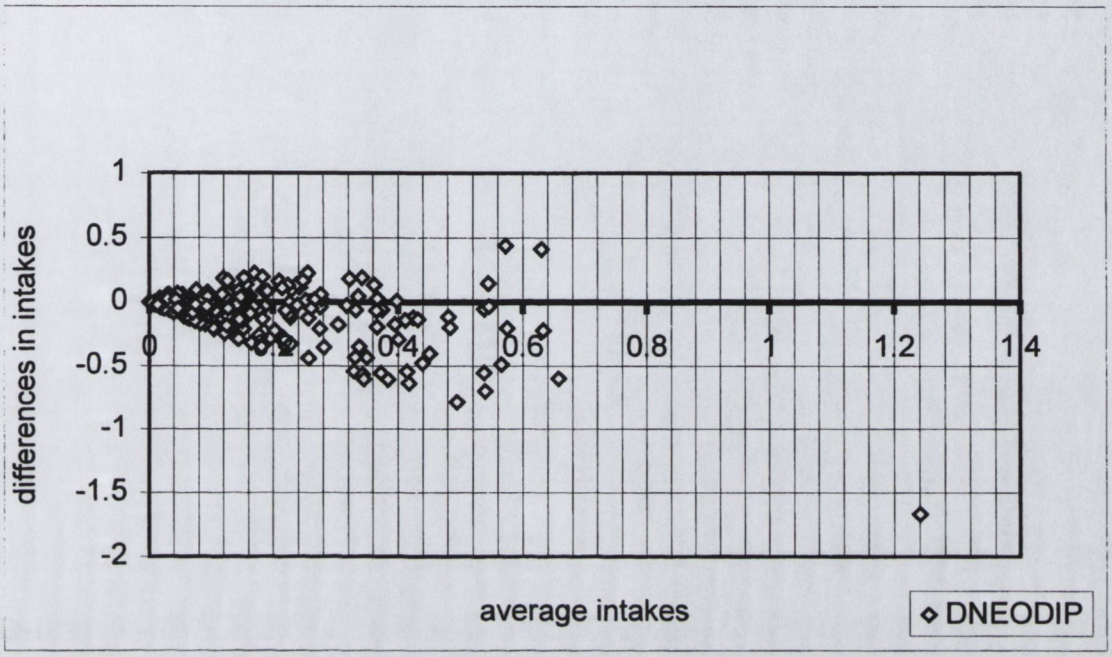

**DSACDIP**- differences in saccharin intake between the diary and the IPFFQ

**Figure 3.5** **Bland and Altman plot of average intakes of cyclamate mg/kg bw/d against differences in intakes between diary and IPFFQ.**



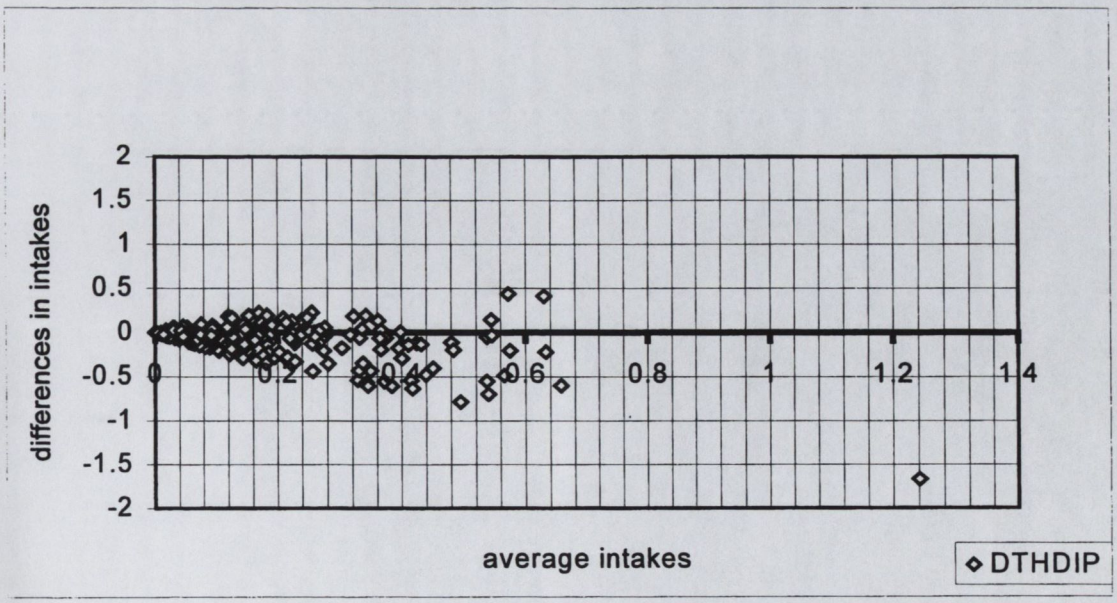

**DCADIP**- differences in cyclamate intake between the diary and the IPFFQ

**Figure 3.6** **Bland and Altman plot of average intakes of neohespheridine DC mg/kg bw/d against differences in intakes between diary and IPFFQ.**



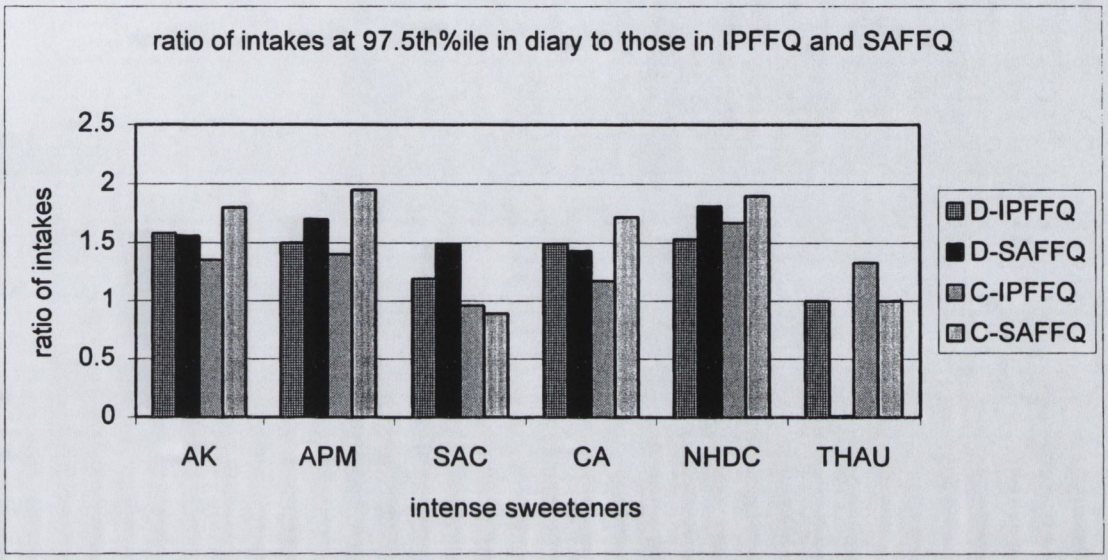

DNEODIP- **differences in neohespheridine DC intake between the diary and IPFFQ**

**Figure 3.7** **Bland and Altman plot of average intakes of Thaumatin mg/kg bw/d against differences in intakes between diary and IPFFQ.**




**DTHDIP**- differences in thaumatin intake between the diary and the IPFFQ

**Figure 3.8 Ratio of diary total population intakes of intense sweeteners at the 97.5$^{th}$ percentile to IPFFQ and SAFFQ.**




D-IPFFQ – intakes by diabetics in IPFFQ D-SAFFQ– intakes by diabetics in SAFFQ

C-IPFFQ – intakes by controls in IPFFQ C-SAFFQ– intakes by controls in SAFFQ

**Appendix 3.1** **45- item Semi-Quantitative Food Frequency Questionnaire.**

**Name :** _____ **Date :** _____ **ID No. :** _____

For each of the foods listed below, please tick the box indicating how often on average you would currently eat these foods.

| | 6+ per day | 4-5 per day | 2-3 per day | once per day | 5-6 per week | 2-4 per week | once per week | 1 or more days a fortnight | Monthly | Rarely or never |
|---|---|---|---|---|---|---|---|---|---|---|
| **Beverages** | | | | | | | | | | |
| A glass of diet fizzy drink | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Cola flavoured fizzy drink (not diet) | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Fruit flavoured fizzy drink *e.g* orange, lemon (not diet) | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Diet Soda Stream drinks | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Soda stream drinks (not diet) | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Diet / low sugar squash | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Squash (not diet) | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A bottle of Sports drink *e.g.* Lucozade sport | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A bottle of Flavoured Water | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Tonic water | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

**Appendix 3.1** **45- item Semi-Quantitative Food Frequency Questionnaire Cont'd.**

| | 6+ per day | 4-5 per day | 2 -3 per day | once per day | 5 -6 per week | 2 - 4 per week | once per week | 1 or more days a fortnight | Monthly | Rarely or never |
|---|---|---|---|---|---|---|---|---|---|---|
| A mug of Light drinking chocolate | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Biscuits** | | | | | | | | | | |
| Diabetic biscuits | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Cakes** | | | | | | | | | | |
| A Mini cake *e.g.* mini battenburg | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Confectionery** | | | | | | | | | | |
| Diabetic chocolate | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Ice Lollies** | | | | | | | | | | |
| Sugar free Mr Freeze | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Pasta, rice & grains** | | | | | | | | | | |
| Tinned spaghetti | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Puddings and chilled desserts** | | | | | | | | | | |
| A bowl of Angel Delight, no sugar added | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A carton of low calorie Chocolate mousse | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A bowl of Sugar free jelly | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A bowl of Jelly (not sugar free) | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A bowl of canned Rice pudding *e.g.* weight watchers | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

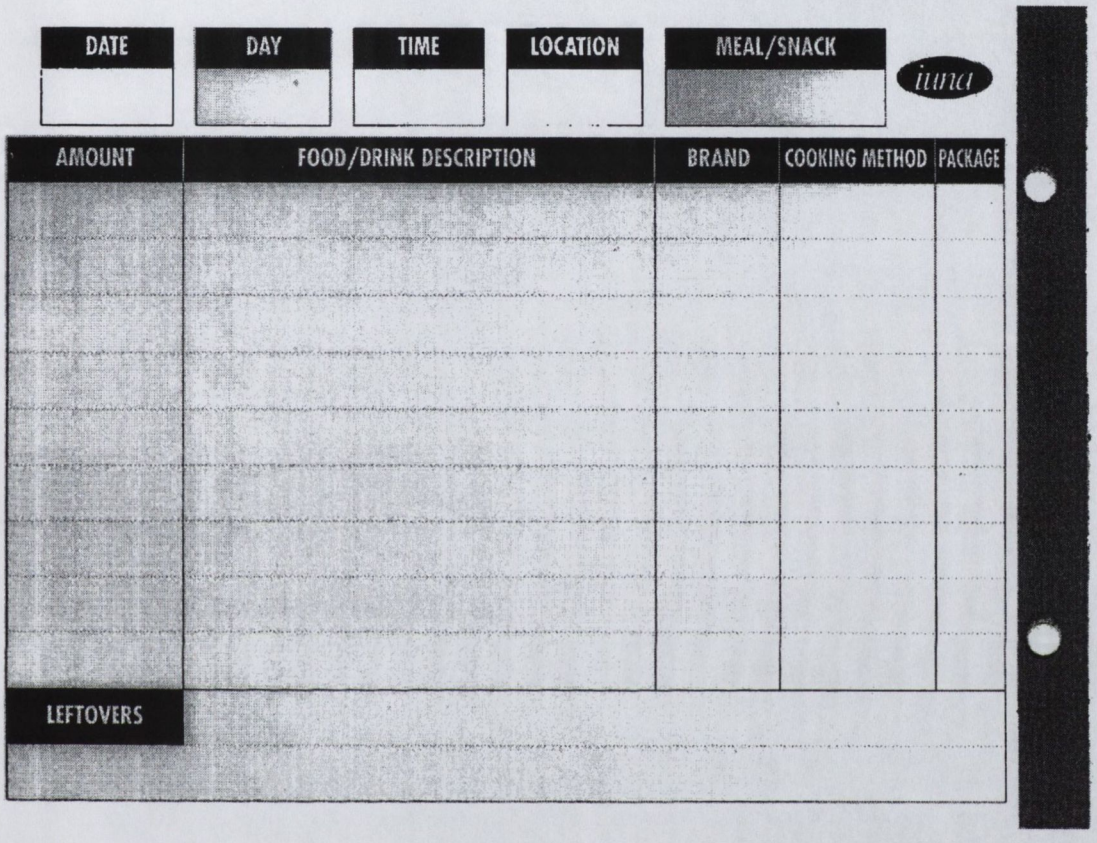
**Appendix 3.1** **45- item Semi-Quantitative Food Frequency Questionnaire Cont'd.**

| | 6+ per day | 4-5 per day | 2 -3 per day | once per day | 5 -6 per week | 2 - 4 per week | once per week | 1 or more days a fortnight | Monthly | Rarely or never |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sauces** | | | | | | | | | | |
| Low calorie Ketchup | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Light pasta sauce (not homemade) | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Low fat dressings *e.g.* Heinz weight watchers | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Savoury snacks and sweets** | | | | | | | | | | |
| Diabetic Fruit Bonbons | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Fruit bonbons | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sugar free mints | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A bag of prawn cocktail flavoured snacks *e.g.* KP Skips | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Sweeteners and preserves** | | | | | | | | | | |
| Diabetic jam | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Diabetic marmalade | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Reduced sugar jam *e.g.* Fruit field waistline | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Reduced sugar marmalades *e.g.* Fruit field waistline | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

| Appendix 3.1 | 45- item Semi-Quantitative Food Frequency Questionnaire Cont'd. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6+ per day | 4-5 per day | 2 -3 per day | once per day | 5 -6 per week | 2 - 4 per week | once per week | 1 or more days a fortnight | Monthly | Rarely or never |
| Artificial sweetener *e.g.* Canderel, Flix | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Vegetables** | | | | | | | | | | |
| Pickled Beetroot | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sugar free processed peas | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Processed peas | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sugar free baked beans | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sweet pickled onions | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Tinned vegetables | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Yoghurts** | | | | | | | | | | |
| A carton of Diet yoghurt | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A carton of Regular yoghurt (not diet/low fat) | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **Miscellaneous** | | | | | | | | | | |
| Jelly toppings for buns | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Sugar free chewing gum | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Non sugar free chewing gum | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| A glass of Slimfast | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

**Thank you for your co-operation.**

**Appendix 3.2** **Sample page of the 3-day diary.**



| DATE | DAY | TIME | LOCATION | MEAL/SNACK |
|---|---|---|---|---|
| | | | | |

| AMOUNT | FOOD/DRINK DESCRIPTION | BRAND | COOKING METHOD | PACKAGE |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

| LEFTOVERS | |
|---|---|
| | |

# *Chapter 4*

## *The influence of intense sweetener intake on dietary composition in Insulin Dependent Diabetics and their age and sex matched controls.*

## 4.1 Introduction.

Intense sweetener intake has been widely studied in both the diabetic and general populations (Leclerq *et al*, 1999, Renwick, 1999, Ishiwata *et al*, 1998, Elmadfa, Zarfl and König, 1996, Toledo and Ioshi, 1995, MAFF, 1995, CRÉDOC, 1994, Bergsten, 1993, Hinson and Nicol, 1992, Bär and Bierman, 1992, Hulshof and Bouman, 1992, Serra-Majem *et al*, 1992, MAFF, 1990, Heybach and Ross, 1989, Virtanen *et al*, 1988). There is no evidence in any of these studies that individuals exceed the Acceptable Daily Intake (ADI), of one or more intense sweeteners on a long-term basis. Indeed the 97.5$^{th}$ percentile of intakes tends to be in the order of 2 – 4 % of the ADI.

The public health issue of intense sweetener usage is always related to the ADI, which is based on detailed toxicological evaluation. However, it is possible that the use of intense sweeteners, displacing sugar in the diet, may lead to distortion of dietary macronutrient intakes. Fat and sugar, as a % of energy, have been shown in several studies to be inversely related, the so-called 'fat – sugar seesaw' (Flynn *et al*, 1996, Naismith *et al*, 1995, Gibney, 1995, Moloney 1993, Gibney, 1993, Lewis *et al*, 1992, Gibney, 1990). Several acute studies have been conducted to examine the effects of intense sweeteners on ratings of hunger and most have found decreased or unchanged ratings of hunger (Blackburn *et al*, 1997, Anderson, 1995). Other acute studies have examined the effects of intense sweetener intake on subsequent food intake and have reported either no change (Drewnowski, 1995, Rodin, 1990, Kanders *et al*, 1988), or a reduction in food intake (Drewnowski, 1994, Rolls, Hetherington, Laster, 1988).

A number of intervention studies have been conducted on intense sweetener and macronutrient intakes. One study has investigated the longer-term effects of covert substitution of aspartame for sugar and *vice versa* on patterns of nutrient intake in free-living subjects (Naismith and Rhodes, 1995). The covert removal of 500 kcal of sugar and its replacement by an equal level of sweetness of aspartame over 10 days among free living subjects led to an 8 % fall in energy intake and an 11 % rise in total fat intake. The covert substitution of aspartame with sugar increased energy intake by 8 % and decreased total fat intake by 5 %. In contrast, a study by Gatenby *et al* (1997), investigated the extended use of foods modified in fat and sugar content and the nutritional implications in 49 free living non-obese females aged 18 – 50 years old for 10 weeks. The data suggests a

reduction in fat intake by using reduced fat foods, particularly among subjects who had high fat intakes initially with little change seen among those with initial intakes < 35 % of energy. Subjects maintained energy balance when consuming reduced fat foods under these free-living conditions. The use of reduced sugar (RS), foods led to significant reductions in sucrose intake but not in intake of total sugars. Overall, these results suggest that use of RS foods may have a modest effect on intake of major macronutrient classes in the absence of other wilful efforts to control overall diet. This suggests the likelihood that many reduced sugar foods in practice are simply added to a pre-existing diet rather than specifically used to replace sugar-containing foods. Because many common reduced sugar products have a minimal macronutrient or energy content, the effect of intense sweeteners on sugar intake or indeed of any macronutrients could thus appear negligible. Neither approach led to identifiable changes in total energy intakes or body weights. However, the authors do show that reduced fat food used and associated with reductions in fat intakes were not related to consistent directional changes in sucrose intakes in any subgroup examined thus far. For subjects starting from a low fat intake, use of reduced fat foods prompted a marked increase in percent energy from fat. When subjects were classified by initial sucrose intake, high sucrose consumers changing to the use of reduced sugar foods were found to increase their fat intake. Analyses based on subjects' initial diets showed no significant difference in changes in total energy intake or body weights (Mela, 1997). A study by Chen and Parham (1991), corroborate these findings as in a group of 135 healthy young people which included a few serious dieters, scant evidence was found that the use of high intensity sweeteners conferred any nutritional benefits. They do however note that these findings may not apply to persons with diabetes and others who are committed to a restricted intake of sugars. Anderson and Leiter (1996), also state that 'there is no evidence that present intense sweetener availability and use has had an impact on the macronutrient contents of diets and therefore consumption of high intensity sweeteners is not associated with the development of obesity.

Thus, there is still debate as to whether or not the use of intense sweeteners can increase % energy from fat intake by displacing sugar, if it is the case, this phenomenon could also be as important as the ADI in evaluating the public health significance of widespread use of intense sweeteners. Since looking at intense sweeteners, a group of food

additives, it is advisable to investigate those individuals considered to be high consumers of the additive in question. In this cross sectional study, a small number of insulin dependent diabetic patients, who are generally recognised as high users of intense sweeteners due to their disease status were recruited and an age- and sex-matched control group.

## 4.2 Methods.

### *4.2.1 Subject Selection.*

The protocols for this study were approved by the Joint Ethics Committee of St. James's Hospital and Federated Voluntary Hospitals. One hundred and nineteen Insulin dependent diabetics in the age range of 16 – 75 years were recruited from outpatients' clinics of St. James's Hospital. A similar number of age (± 5 years), and sex matched controls who were non-diabetic were recruited from either friends of the patients or from the staff and students of Trinity College Dublin and University College Dublin.

### *4.2.2 Dietary Survey.*

A 3 - day diary used. This diary was designed for and used in the North / South Ireland Food Consumption Survey 2000 by the Irish Universities Nutrition Alliance (IUNA), (Harrington *et al*, 2000a). Subjects were required to complete the diary for two week - days and 1 weekend day. The beginning times for the respondents were spread equally over the seven days of the week and over all seasons, over the entire sample. Controls completed the diary for the same week and weekend days as their corresponding subjects.

Verbal and written instructions on how to complete the diary were given to all subjects as were the instruction on how to express the quantity of foods and beverages consumed with household measures or standard portions. For snacks or meals purchased away from home and other foods that could not be weighed, verbal description of portions were obtained in terms of familiar volumes, dimensions (*i.e.* household measures), and purchasing units. Ingredients and packaging were also described. Where possible, similar items were purchased by the researcher and weighed. Respondents were asked to fill in the diary after each eating occasion and to maintain their usual eating habits. The diary layout enables details of every eating occasion to be recorded: date, day, time, location,

meal/snack, description of food or main ingredients of home made recipes, precise name (brand/flavour/type), of manufactured foods, cooking methods and leftovers.

After completing the diary, the researcher met with the participant to go through the previous three days entries, checking food description, brands, ingredients of recipes and quantities. Questions were asked of subjects, to identify possible omissions such as between meal eating occasions (beverages, confectionery, chewing gums, snacks *etc.*), or the use of tabletop sweeteners by inquiring after the addition of sweeteners to tea, coffee, other beverages and yoghurt / desserts for the week and weekend days. The form of the sweetener (tablets / powder / liquid), and the quantity added to each bowl or cup was also assessed. Participants reported the type of sweetener they commonly used (trade name).

In some cases published average portion weights were used (Crawley, 1993). Foods were coded according to the McCance and Widdowson Food Composition Tables or relevant supplements (Chan *et al*, 1996, Holland, Welch and Buss, 1996, Chan *et al*, 1995, Chan, Brown and Buss, 1994, Holland, Brown and Buss, 1993, Holland *et al*, 1992, Holland, Unwin and Buss, 1992, Holland, Unwin and Buss, 1991, Holland, Unwin and Buss, 1989, Holland, Unwin and Buss, 1988). Nutrient intakes were determined using the computerized programme, WISP version 1.27 (Tinuviel© Software, 1998). The subject's weight (kg), and height (m), were taken following completion of the diary.

Rigorous quality control of the coding and data entry procedures was performed. Each item was entered twice in order to limit the number of errors found in the database. Then a random 25% selection of diaries was made and these again were checked for inaccuracies.

### *4.2.3 Intense sweetener intakes.*

The Irish National Food Ingredient Database (INFID), (Lambe, 2000), was used to determine the foods on the Irish market containing intense sweeteners. This database consists of approximately 5,000 processed foods and the ingredient list for each food. In order to find out which foods contained the sweeteners of interest in this study, a search using the name of the sweetener was conducted. The intense sweeteners of interest in this study were acesulfame K, aspartame, saccharin, cyclamate, neohespheridine DC and thaumatin. The outcome of this search was a list of foods and their brands containing the

sweetener in question. This list was then re-organised into a 45 - item list of food categories for the FFQ. In some cases, more general groups could be formed *e.g.* diet carbonated beverages, diet yoghurts *etc.*

Food consumption data collected from the subjects using the 3 - day diary was combined with sweetener concentration data for each of the 45 foods derived from the Maximum Permitted Levels (MPLs), given in the European Union Directive 94/35/EC (European Commission, 1994). This gives intakes of intense sweeteners in mg / kg bw / day for each participant in the survey.

Since different intense sweeteners have different intensities of sweetness, an index was devised for each participant for 'intense sweetness' intake based on the sweetness index of each sweetener in comparison to sugar (International Sweeteners Association, 2000). This index (the Intense Sweetness Intake Index), was the sum of the quotient of each intake of an intense sweetener and the respective sweetness.

### *4.2.4 Statistical analysis.*

Nutrient intakes, consumer only total intense sweetness intake index, consumer only intakes of each intense sweetener and consumer only total intense sweetener intakes in the diabetic and control groups were compared using an independent t-test. A Pearson correlation was then conducted to examine the relationship between each intense sweetener and the intense sweetness intake index and also the relationship between each of the intense sweeteners to each other. The participants in each of the groups were then divided into tertiles of % energy from fat. Since both groups were found to have similar intakes (no significant differences found), a two way ANOVA was used to examine the independent and interactive effects of group and tertiles of % energy from fat on nutrient, consumer only intense sweetness intake index, consumer only intense sweetener intakes and consumer only total intense sweetener intakes. The groups were then similarly divided into consumer only tertiles of the three indices of sweetener intake: intense sweetness intake index, total intense sweetener intakes and aspartame intakes. Due to the fact that in each of these cases, the control group was found to have a significantly lower range of intakes, a one way ANOVA was conducted separately for each group. These statistical analyses were carried out using SPSS® version 8.0 (SPSS Inc., 1998).

## 4.3 Results.

The results of this study are given in Tables 4.1 – 4.8. In Table 4.1, the ratio of males to females, mean values with standard deviations (± SD), for age (years), weight (kg), height (m), and Body Mass Index (BMI, $kg/m^2$), for the diabetic and control groups. It was found that diabetics had a significantly higher BMI than controls (26.8 v 25.2, $p = 0.04$).

Mean values with standard deviations (± SD), for daily nutrient intakes are given in Table 4.2. Significant differences between diabetics and controls were found for % energy from starch (28.6 v 24.3, $p < 0.001$), % energy from sugars (15.6 v 20.8, $p < 0.001$), the ratio of the % energy from starch to % energy from sugars (2.4 v 1.4, $p < 0.001$), fibre (25.0g v 20.0g, $p = 0.0002$), vitamin D (4μg v 3μg, $p = 0.005$), retinol (675.1μg v 476.0μg, $p = 0.03$) and copper (1.4mg v 1.2mg, $p = 0.007$).

The mean daily intakes with standard deviations (± SD), of macronutrients, selected micronutrients, consumer only intense sweetness intake index, consumers only intakes of each intense sweetener, and consumer only intakes of intense sweetener intakes, for diabetics and controls, classified according to tertiles of % energy from fat, are given in Table 4.3. Significant differences across tertiles were found for energy (MJ), ($p = 0.010$), % energy from fat ($p < 0.001$), % energy from carbohydrate ($p = 0.001$), % energy from sugars ($p < 0.001$), % energy from alcohol ($p = 0.02$), the ratio of the % energy from starch to % energy from sugars ($p = 0.007$), calcium ($p = 0.05$), zinc ($p = 0.01$), vitamin E ($p < 0.001$), folic acid ($p = 0.04$), vitamin C ($p = 0.0001$) and retinol ($p = 0.001$). Significant differences across groups were found for BMI ($p = 0.003$), % energy from protein ($p < 0.001$), % energy from starch ($p < 0.001$), % energy from sugars ($p < 0.001$), % energy from alcohol ($p = 0.034$), the ratio of the % energy from starch to % energy from sugars ($p < 0.001$), fibre ($p< 0.001$), iron ($p = 0.014$), vitamin D ($p = 0.007$), copper ($p = 0.009$), retinol ($p = 0.048$), consumer only intense sweetness intake index ($p < 0.001$), consumer only intakes of acesulfame K ($p < 0.001$), consumer only intakes of aspartame ($p < 0.001$), consumer only intakes of saccharin ($p < 0.001$), consumer only intakes of cyclamic acid ($p < 0.001$), consumer only intakes of neohespheridine DC ($p < 0.001$), consumer only intakes of thaumatin ($p = 0.017$) and consumer only intense sweeteners intake ($p < 0.001$). A significant interaction (group by tertiles of % energy from fat), was found for % energy from sugars ($p = 0.05$).

The consumer only mean values with standard deviations (± SD), of each intense sweetener, intense sweetness intake index and intense sweetener intakes for the diabetic and control groups are given in Table 4.4. A significant difference between groups was found for acesulfame K (1.7 v 1.0 mg/kg bw/d, $p < 0.001$), aspartame (3.3 v 2.0 mg/kg bw /d, $p < 0.001$), saccharin (0.6 v 0.5 mg/kg bw /d, $p = 0.01$) cyclamic acid (2.0 v 1.2 mg/kg bw/d, $p < 0.001$), neohespheridine DC (0.2 v 0.1 mg/kg bw/d, $p < 0.001$), thaumatin (0.01 v 0.01 mg/kg bw/d, $p < 0.001$), intense sweetness intake index (2.6 v 1.6, $p < 0.001$) and intense sweetener intake (7.6 v 4.6 mg/kg bw/d, $p = 0.001$).

Table 4.5a shows the significantly positive relationship between consumer only intense sweetness intake index and intakes of the 6 intense sweeteners for the total study population (n = 238), diabetic and control group populations. The $r^2$ and $p$ values for the sweeteners ranged from ($r^2 = 0.35 - 0.94$, $p < 0.001$). Table 4.5b gives the values ($r^2$ and $p$) for the correlation of each intense sweetener to each other again for the total study population, diabetic and control groups. A significantly positive correlation was found for each sweetener although with the exception of saccharin and thaumatin. These two sweeteners were not as strongly positively correlated to the each of the other 4 intense sweeteners.

Table 4.6 shows the mean daily intakes with standard deviations (± SD), of nutrients, consumer only intakes of each of the 6 intense sweeteners and consumer only intakes of intense sweeteners classified according to consumer only tertiles of intense sweetness intake index. In the diabetic group, a significant difference across tertiles was found for age ($p < 0.001$), BMI ($p = 0.03$), retinol ($p = 0.04$), consumer only intakes of acesuflame K ($p < 0.001$), consumer only intakes of aspartame ($p < 0.001$), consumer only intakes of saccharin ($p < 0.001$), consumer only intakes of cyclamic acid ($p < 0.001$), consumer only intakes of nesohespheridine DC ($p < 0.001$) and consumer only intense sweetener intakes ($p < 0.001$). For the control group, a significant difference was found for age ($p = 0.02$), weight ($p = 0.05$), BMI ($p = 0.04$), % energy from protein ($p = 0.04$), consumer only intakes of acesulfame K ($p < 0.001$), consumer only intakes of aspartame ($p < 0.001$), consumer only intakes of saccharin ($p < 0.001$), consumer only intakes of cyclamic acid ($p < 0.001$), consumer only intakes of neohespheridine DC ($p < 0.001$), and consumer only intense sweetener intakes ($p < 0.001$).

The mean daily intakes with standard deviations (± SD), of nutrients, consumer only intense sweetness intake index and intense sweeteners classified according to consumer only tertiles of intense sweetener intake are given in Table 4.7. In the diabetic group, significant differences were found across tertiles for age ($p < 0.001$), BMI ($p = 0.012$), retinol ($p = 0.05$), consumer only intense sweetness intake index ($p < 0.001$), consumer only intakes of acesulfame K ($p < 0.001$), consumer only intakes of aspartame ($p < 0.001$), consumer only intakes of saccharin ($p < 0.001$), consumer only intakes of cyclamic acid ($p < 0.001$) and consumer only intakes of neohespheridne DC ($p < 0.001$). The control group was found to have significant differences across tertiles for age ($p = 0.004$), weight ($p < 0.001$), BMI ($p = 0.002$), % energy from protein ($p = 0.04$), consumer only intakes of acesulfame K ($p < 0.001$), consumer only intakes of aspartame ($p < 0.001$), consumer only intakes of saccharin ($p < 0.001$), consumer only intakes of cyclamic acid ($p < 0.001$), consumer only intakes of neohespheridine DC ($p < 0.001$) and consumer only intakes of intense sweeteners ($p < 0.001$).

Table 4.8 shows mean daily intakes with standard deviations (± SD) of nutrients, consumer only intense sweetness intake index, consumer only intakes of each intense sweetener and consumer only intakes of intense sweeteners classified according to consumer only intakes of aspartame. In the diabetic group, a significant difference was found for age ($p < 0.001$), weight ($p = 0.05$), BMI ($p = 0.008$), retinol ($p = 0.05$), consumer only intakes of intense sweetness intake index ($p < 0.001$), consumer only intakes of acesulfame K ($p < 0.001$), consumer only intakes of aspartame ($p < 0.001$), consumer only intakes of saccharin ($p < 0.001$), consumer only intakes of cyclamic acid ($p < 0.001$), consumer only intakes of neohespheridine DC ($p < 0.001$) and consumer only intakes of intense sweeteners ($p < 0.001$). In the control group, significant differences were found for age ($p = 0.02$), weight ($p < 0.001$), BMI ($p = 0.002$), % energy from protein ($p = 0.02$), % energy from sugar ($p = 0.05$), consumer only intense sweetness intake index ($p<0.001$), consumer only intakes of acesulfame K ($p < 0.001$), consumer only intakes of aspartame ($p < 0.001$), consumer only intakes of saccharin ($p < 0.001$), consumer only intakes of cyclamic acid ($p < 0.001$), consumer only intakes of neohespheridine DC ($p < 0.001$) and consumer only intakes of intense sweeteners ($p < 0.001$).

The consumer only intake of the 6 intense sweeteners was also correlated with BMI and a significantly negative correlation was found for the diabetic and control groups in general. The exception being the control group for aspartame and thaumatin for both the diabetic and control groups. For the sweeteners that were significantly negatively correlated the $r^2$ values range from 0.02 – 0.12 and $p$ was < 0.001 for each sweetener.

The % energy from fat was correlated with the % energy from sugars for the diabetic and control groups. A significantly negative correlation was found for each, diabetics ($p < 0.001$, $r^2 = 0.1489$) and controls ($p < 0.001$, $r^2 = 0.3052$), thus clearly showing that the 'fat – sugar seesaw' is present in this study although at a lower level in the diabetic group compared to the controls.

### 4.4 Discussion.

The patterns of nutrient intakes in the present study are very similar to those reported for a representative sample of 1,379 adult subjects in the North and South of Ireland (Harrington *et al*, 2000b). The pattern of nutrient intakes of the insulin dependent diabetics in the present study were very close to those reported by Toeller *et al* (1996), in the Irish cohort of the multi-centre EURODIAB study for similar number of diabetics (n=118) with a comparable ratio of males to females (65:53). In both studies, the mean macronutrient intakes did not meet conventional goals for diabetics, indicating that more intensive nutritional counselling of diabetics may be needed, (Nutrition Subcommittee of the British Diabetic Association (BDA), (1991). In the present study the ratio of mean energy intakes (EI), to estimated basal metabolic rates (BMR), indicated that energy under-reporting was at an acceptable level in the present study as it is above the cut off level of 1.2 (Goldberg *et al*, 1991). However, the present study shows that some areas of dietary importance to diabetics are more favourably achieved compared to controls. The diabetic group had significantly higher intakes of dietary fibre (25 v 20g/d), lower intakes of sugar (16 v 21 % energy) and higher intakes of starch (29 v 24 % energy) than the control group. When the tertiles of intense sweetness were investigated it could be seen that whilst the energy intakes (MJ) in the diabetic group increased although not significantly, their BMIs decreased with increasing intense sweetener usage. The energy increase can be accounted for by the increase in %energy from carbohydrates and % energy from starch. The BMI

was significantly negatively correlated for both groups with each sweetener, this agrees with the result of the Serra -Majem *et al* study (1996) for BMI and consumer only intakes of cyclamate.

Notwithstanding these more favourable patterns in diabetics, the fat tertile analysis clearly shows that the frequently observed inverse relationship between fat and sugar as a % of energy exists both in diabetics and controls. This is illustrated in Figure 4.1. The inverse relationship of sugar to fat energy exists in the two groups but at a lower level in diabetics. Furthermore, whereas there is a linear decline in % energy from sugars within the controls with increasing tertiles of % energy from fat, most of the difference in the diabetics is between tertile 1 and the other two tertiles. This is reflected in the interactive effect in the two way ANOVA. The ratio of % energy from sugar to % energy from starch is also more favourable in the diabetic group compared to the controls. Based on tertiles of % en from fat, the diabetics' intake of sugar lowers and the intake of starch increases, whereas in the control the starch intakes remain the same whilst the sugar intakes lower. This can be expected due to the dietary advice the diabetics receive. A significant difference was found in the intakes of certain micronutrients when tertiles of % en from fat were examined. Since Vitamin E is a fat-soluble vitamin it can be expected that levels of intake would increase with increasing tertiles. Calcium intakes also increased probably due to a greater consumption of high fat dairy products. Zinc also increased, as meat is a major source of this nutrient. Vitamin C and Folic acid intakes decreased with increasing % en fat tertiles probably due to the decreased consumption of fruit, fruit juices and vegetables.

The present study was not designed to provide definitive data on intense sweetener usage. Several studies have undertaken this task, which requires very specialised methods *i.e.* receiving manufacturers information and chemical analysis of the food product to ascertain the presence of and the concentration of sweeteners. A similar patter of nutrient intakes was found in the diabetic and control groups when intakes based on the tertiles of the three indices of sweetener intake. Each investigation showed that the diabetics had a much higher usage level of intense sweeteners in comparison to the controls. The direct comparison of the diets of the diabetics and controls across the tertiles of the three indices of sweetener intake showed no differences in fat percent energy even though the diabetics had double the intake of the three indices of sweetener intake. Even within groups, across

tertiles of the three indices of sweetener intake, no evidence was found in this study that macronutrient intakes are significantly influenced apart from % energy from protein for the control group and % energy from sugars in the aspartame investigation. In the diabetic group as the three indices of sweetener intake increased, the % en from sugars decreased and the % en from starch increased but not significantly. In contrast, in the control group as the three indices of sweetener intake increased, the % en from sugars and % en from carbohydrates increased. The higher use of intense sweeteners in controls represents a tendency towards the consumption of foods of a sweeter nature. It must be kept in mind that in this study it was assumed that if a sweetener use was permitted in a product, that it was used at the MPL. Hence the % consumers in both the diabetic and control groups are high - this is illustrated in Table 4.4 where it can be seen that the % consumers in both groups are generally in the range of 80 - 95%, although for sweeteners of limited permitted use the % consumers is a lot lower.

Thus the present cross - sectional study therefore (a) confirms the fat-sugar seesaw is present even among insulin dependent diabetics and (b) it shows no evidence that the use of intense sweeteners leads to higher fat diets. Previous cross sectional and intervention studies show conflicting results (as previously discussed), in this regard and thus a more detained consideration of these papers is warranted. The group numbers in the study by Naismith and Rhodes, (1995) were 10 and 4, and the study was carried out for 10 days x 2 and a 3 - day run in period. The study by Gatenby *et al,* (1997), had between 13 - 19 subjects in each group and they were studied for 10 weeks with their diets assessed on 4 occasions. However, in sub-group analyses of the Gatenby *et al,* (1997), data starting from an initial low-fat intake, the use of reduced sugar food prompted a marked increase in percent energy from fat. It was also found that when subjects were classified by initial sucrose intake, high sucrose consumers changing to the use of reduced sugar foods were found to increase their fat intake (Mela, 1997). Thus, whereas the main study of Gatenby *et al,* (1997), differs from that of Naismith and Rhodes, (1995), a sub analysis of high sugar consumers would agree with Naismith and Rhodes, (1995). In a cross – sectional study by Chen and Parham, (1991), investigating whether the use of high intensity sweeteners effectively reduced sugar intake among 280 healthy college students, there was no evidence that high intensity sweeteners were associated with a reduction in sugar intakes.

Whilst, this corroborates what has been found in the present study and by Gatenby *et al*, (1997), the data are difficult to interpret for a number of reasons. Firstly, the nutrient intakes were presented in g/d and in order to determine whether or not there is a difference between consumer and non-consumer intakes, the energy intakes have to be calculated. The percent energy from protein for users and non-users for each sex were (men, 18 % v 19 %, women, 16 % v 15 %), for percent energy from fat, (men, 33 % v 36 %, women, 38 % v 38 %) and for percent energy from carbohydrate, (men, 49 % v 45 %, women, 46 % v 47 %) respectively. The percent energy from alcohol could not be calculated, as it was not presented in the data. The actual levels of sweetener intake were also not given and weights were not taken even though a high percentage of the users had a desire to lose 15lbs in comparison to the non-users of either sex.

The present study, a more comprehensive study in the comparison to the Chen and Parma, (1991), such data was collected and examined. No evidence has been produced in the present study to show that in a free living population on a self-selected diet, intense sweetener intake influences macronutrient balance.

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**Table 4.1.** **Mean values (with standard deviations: SD) for age, weight, height and body mass index (BMI) for the diabetic and control subjects.**

| | **Diabetics (n = 119)** | **Controls (n = 119)** | |
|---|---|---|---|
| | Mean (± SD) | Mean (± SD) | *p* value = |
| Male:Female | 65:54 | 65:54 | |
| Age (years) | 36.5 ± 15.2 | 36.6 ± 14.5 | NS |
| Weight (kg) | 74.9 ± 13.8 | 72.4 ± 13.5 | NS |
| Height (m) | 1.7 ± 0.1 | 1.7 ± .01 | NS |
| BMI ($kg/m^2$) | 26.8 ± 4.5 | 25.2 ± 4.0 | 0.04 |

**Table 4.2. Mean Daily Intakes (with standard deviations:±SD) of macronutrients and selected micronutrients in Insulin Dependent Diabetics and age- and sex- matched Controls.**

| Nutrients | Diabetics | Controls | |
|---|---|---|---|
| | Mean (± SD) | Mean (± SD) | *p* = |
| Energy (MJ/d) | 9.8 ± 3.4 | 10.1 ±3.5 | NS |
| EI:BMR | 1.4 ± 0.4 | 1.5 ± 0.5 | NS |
| | | | |
| % en fat | 35.6 ± 6.5 | 35.2 ± 6.5 | NS |
| % en pro | 16.5 ± 4.0 | 14.6 ± 3.3 | NS |
| % en cho | 44.4 ± 7.5 | 44.5 ± 9.1 | NS |
| % en starch | 28.6 ± 6.4 | 24.3 ± 5.7 | <0.001 |
| % en sugars | 15.6 ± 7.5 | 20.8 ± 8.7 | <0.001 |
| % en alcohol | 3.9 ± 6.4 | 5.9 ± 7.5 | NS |
| % en starch:sugars | 2.4 ± 1.4 | 1.4 ± 0.8 | <0.001 |
| | | | |
| Fibre (g) | 25.0 ± 9.7 | 20.0 ± 7.8 | 0.00002 |
| Calcium (mg) | 955.0 ± 448.0 | 919.0 ± 644.0 | NS |
| Iron (mg) | 15.0 ± 7.4 | 12.9 ± 5.9 | NS |
| Zinc (mg) | 10.5 ± 4.3 | 9.3 ± 4.8 | NS |
| Folic acid (μg) | 366.0 ± 297.0 | 314.0 ± 135.0 | NS |
| Vitamin C (mg) | 143.0 ± 160.0 | 183.0 ± 189.0 | NS |
| Vitamin D (μg) | 4.05 ± 2.67 | 3.17 ± 2.02 | 0.005 |
| Vitamin E (mg) | 7.9 ± 3.6 | 7.8 ± 3.6 | NS |
| Retinol (μg) | 675.1 ± 902.4 | 476.0 ± 421.9 | 0.03 |
| Carotene (μg) | 2008.8 ± 1684.9 | 1987.4 ± 1456.7 | NS |
| Copper (mg) | 1.39 ±0.84 | 1.16 ± 0.43 | 0.007 |

**Table 4.3.** **Mean Daily Intakes (with standard deviations: SD) of macronutrients and selected micronutrients in insulin dependent diabetics and age- and sex- matched Controls based on tertiles of percent energy from fat (%Enfat).**

| | Diabetic group | | | Control group | | | Two way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tertiles % en fat** | **Low** | **Medium** | **High** | **Low** | **Medium** | **High** | **Group** | **Tertiles** | **Group by tertile** |
| Age (years) | 38.6 ± 17.5 | 36.0 ± 12.1 | 35.8 ± 15.4 | 34.5 ± 13.5 | 37.2 ± 16.1 | 38.3 ± 13.8 | NS | NS | NS |
| Weight (kg) | 73.3 ± 15.6 | 74.7 ± 10.6 | 76.7 ± 14.8 | 71.6 ± 12.1 | 73.4 ± 14.4 | 71.6 ± 13.2 | NS | NS | NS |
| Height (m) | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | NS | NS | NS |
| BMI ($kg/m^2$) | 26.6 ± 5.0 | 27.3 ± 4.8 | 26.5 ± 3.8 | 25.1 ± 3.8 | 25.4 ± 3.8 | 24.8 ± 4.4 | 0.003 | NS | NS |
| Energy (MJ/d) | 8.7 ± 4.0 | 10.5 ± 2.7 | 10.2 ± 3.0 | 9.6 ± 3.0 | 9.4 ± 2.7 | 11.2 ± 4.4 | NS | 0.010 | NS |
| EI:BMR | 1.3 ± 0.5 | 1.5 ± 0.4 | 1.5 ± 0.4 | 1.4 ± 0.4 | 1.4 ± 0.4 | 1.7 ± 0.6 | NS | 0.028 | NS |
| % en fat | 28.4 ± 2.9 | 35.8 ± 1.6 | 42.7 ± 3.2 | 28.0 ± 2.8 | 35.2 ± 1.6 | 42.3 ± 3.7 | NS | 0.0001 | NS |
| % en pro | 17.7 ± 5.1 | 16.4 ± 2.9 | 15.5 ± 3.6 | 14.4 ± 3.2 | 14.8 ± 3.7 | 14.6 ± 3.0 | <0.001 | NS | NS |

**Table 4.3 continued**

| | Diabetic group | | | Control group | | | Two way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | **Low** | **Medium** | **High** | **Group** | **Tertiles** | **Group by tertile** |
| **% en fat** | | | | | | | | | |
| % en cho | 49.2 ± 8.7 | 43.9 ± 5.3 | 39.9 ± 5.0 | 51.0 ± 9.4 | 43.1 ± 7.4 | 39.3 ± 5.7 | NS | 0.0001 | NS |
| % en starch | 29.9 ± 7.8 | 29.3 ± 6.0 | 26.6 ± 4.3 | 24.9 ± 6.4 | 24.0 v 6.2 | 23.9 ± 4.2 | <0.001 | NS | NS |
| % en sugars | 18.9 ± 8.9 | 14.5 ± 6.7 | 13.3 ± 5.3 | 27.0 ± 9.8 | 19.4 ± 6.3 | 15.8 ± 5.2 | <0.001 | 0.0001 | 0.05 |
| % en alcohol | 5.4 ± 9.2 | 4.2 ± 5.0 | 1.9 ± 2.9 | 6.7 ± 10.0 | 7.2 ± 6.7 | 3.9 ± 4.6 | 0.034 | 0.02 | NS |
| % en Starch:Sugars | 1.6 ± 1.1 | 2.0 ± 1.4 | 2.1 ± 1.2 | 1.1 ± 0.8 | 1.4 ± 0.8 | 1.7 ± 0.7 | <0.001 | 0.007 | NS |
| | | | | | | | | | |
| Fibre (g) | 23.4 ± 9.7 | 27.4 ± 9.5 | 24.1 ± 9.6 | 19.8 ± 8.5 | 19.3 ± 7.3 | 20.8 ± 7.7 | <0.001 | NS | NS |
| Calcium (mg) | 830.2 ± 394.0 | 1041 ± 447.4 | 993.6 ± 482.1 | 862.3 ± 506.5 | 849.4 ± 324.4 | 1040.8 ± 934.3 | NS | 0.05 | NS |
| Iron (mg) | 15.3 ± 9.2 | 15.1 ± 5.0 | 14.7 ± 7.4 | 12.4 ± 4.9 | 13.5 ± 7.7 | 12.7 ± 5.0 | 0.014 | NS | NS |
| Zinc (mg) | 9.6 ± 4.3 | 11.1 ± 4.0 | 10.7 ± 4.7 | 8.4 ± 3.9 | 8.3 ± 3.5 | 11.1 ± 6.2 | NS | 0.01 | NS |

**Table 4.3 continued**

| | Diabetic group | | | Control group | | | Two way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tertiles % en fat** | **Low** | **Medium** | **High** | **Low** | **Medium** | **High** | **Group** | **Tertiles** | **Group by tertile** |
| Folic acid (mg) | 408.3 ± 432.5 | 378.9 ± 219.7 | 309.0 ± 162.0 | 370.5 ± 145.5 | 280.5 ± 117.5 | 288.9 ± 125.0 | NS | 0.04 | NS |
| Vitamin C (mg) | 168.7 ± 196.4 | 138.9 ± 150.2 | 121.3 ± 125.3 | 284.8 ± 242.3 | 131.7 ± 127.3 | 132.7 ± 136.5 | NS | 0.0001 | NS |
| Vitamin D (μg) | 3.4 ± 2.5 | 3.7 ± 2.5 | 3.8 ± 2.2 | 3 ± 2.3 | 3.1 ± 1.5 | 3.5 ± 2.1 | 0.007 | NS | NS |
| Vitamin E (mg) | 6.4 ± 2.9 | 8.1 ± 2.9 | 9.4 ± 4.4 | 6.8 ± 3.0 | 7.3 ± 3.0 | 9.4 ± 4.1 | NS | 0.0001 | NS |
| Retinol (μg) | 572.1 ± 981.7 | 510.6 ± 200.8 | 913.0 ± 1154.6 | 305.8 ± 159.9 | 427.6 ± 174.5 | 729.7 ± 657.0 | 0.048 | 0.001 | NS |
| Carotene (μg) | 1913.5 ± 1937.2 | 2171.1 ± 1803.4 | 1943.7 ± 1334.1 | 1738.6 ± 1167.9 | 2158.2 ± 1705.4 | 2083.2 ± 1454.2 | NS | NS | NS |
| Copper (mg) | 1.3 ± 0.7 | 1.2 ± 0.4 | 1.4 ± 0.9 | 1.2 ± 0.4 | 1.1 ± 0.4 | 1.2 ± 0.5 | 0.009 | NS | NS |

**Table 4.3 continued**

| | Diabetic group | | | Control group | | | Two way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tertiles % en fat** | **Low** | **Medium** | **High** | **Low** | **Medium** | **High** | **Group** | **Tertiles** | **Group by tertile** |
| Total Intense sweetness | 2.6 ± 2.2 | 2.4 ± 2.8 | 2.9 ± 2.5 | 1.8 ± 1.8 | 1.7 ± 1.5 | 1.2 ± 1.2 | <0.001 | NS | NS |
| Acesulfame K | 1.8 ± 1.7 | 1.6 ± 1.9 | 1.8 ± 1.6 | 1.2 ± 1.5 | 1.1 ± 1.0 | 0.8 ± 0.8 | 0.001 | NS | NS |
| Aspartame | 3.5 ± 3.3 | 3.0 ± 3.4 | 3.3 ± 2.8 | 2.3 ± 2.6 | 2.1 ± 1.8 | 1.4 ± 1.5 | <0.001 | NS | NS |
| Saccharin | 0.5 ± 0.5 | 0.5 ± 0.6 | 0.8 ± 0.8 | 0.5 ± 0.7 | 0.5 ± 0.5 | 0.4 ± 0.7 | 0.042 | NS | NS |
| Cyclamic acid | 2.0 ± 1.9 | 1.8 ± 2.2 | 2.0 ± 1.8 | 1.4 ± 1.7 | 1.3 ± 1.0 | 0.9 ± 1.0 | 0.004 | NS | NS |
| Neohespheridine DC | 0.2 ± 0.2 | 0.2 ± 0.2 | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | <0.001 | NS | NS |
| Thaumatin | 0.01 ± 0.01 | 0.02 ± 0.02 | 0.02 ± 0.02 | 0.003 ± 0.002 | 0.01 ± 0.01 | 0.004 ± 0.003 | 0.017 | NS | NS |
| Total Intense sweetener | 7.2 ± 7.4 | 6.8 ± 8.0 | 7.6 ± 6.9 | 4.7 ± 6.0 | 4.1 ± 4.1 | 2.8 ± 3.5 | <0.001 | NS | NS |

**Table 4.4** **Consumer only intakes of intense sweeteners for diabetics (n = 119) and controls (n= 119) for diary in mg/kg bw at the mean ± SD and percent consumers (%cons) and total intense sweetness intake (ISI) index.**

| Sweetener | Diabetic | n | %cons | Control | n | %cons | p |
|---|---|---|---|---|---|---|---|
| *Intense sweetness intake* | 2.6 ± 2.5 | 113 | 95 | 1.6 ± 1.5 | 100 | 84 | <0.001 |
| *AcesulfameK* | 1.7 ± 1.7 | 113 | 95 | 1.0 ± 1.2 | 100 | 84 | <0.001 |
| *Aspartame* | 3.3 ± 3.1 | 113 | 95 | 2.0 ± 2.1 | 100 | 84 | <0.001 |
| *Saccharin* | 0.6 ± 0.7 | 113 | 95 | 0.5 ± 0.6 | 100 | 84 | 0.01 |
| *Cyclamate* | 2.0 ± 2.0 | 103 | 87 | 1.2 ± 1.3 | 87 | 73 | <0.001 |
| *Neo dc* | 0.2 ± 0.2 | 112 | 94 | 0.1 ± 0.1 | 96 | 81 | <0.001 |
| *Thaumatin* | 0.01 ± 0.02 | 42 | 35 | 0.01 ± 0.01 | 20 | 17 | <0.001 |
| *Intense sweetener intake* | 7.6 ± 7.4 | 113 | 95 | 4.6 ± 4.9 | 100 | 84 | 0.001 |

**Table 4.5a** **Pearson correlation of total population and diabetic and control groups for each intense sweetener against total intense sweetness index, significant at 0.01 level**

| | Total pop | | Diabetics | | Controls | |
|---|---|---|---|---|---|---|
| | *p* | $R^2$ | *p* | $R^2$ | *p* | $R^2$ |
| *Aces* | <0.001 | 0.93 | <0.001 | 0.94 | <0.001 | 0.89 |
| *Asp* | <0.001 | 0.91 | <0.001 | 0.91 | <0.001 | 0.89 |
| *Sac* | <0.001 | 0.43 | <0.001 | 0.47 | <0.001 | 0.35 |
| *Ca* | <0.001 | 0.91 | <0.001 | 0.93 | <0.001 | 0.86 |
| *Neo* | <0.001 | 0.89 | <0.001 | 0.89 | <0.001 | 0.88 |
| *Thau* | 0.003 | 0.04 | NS | 0.02 | 0.04 | 0.04 |

**Table 4.5b** **Pearson correlation of each intense sweetener against another for total population and the diabetic and control groups, significance at 0.01 level (two tailed).**

| | Total pop | | Diabetics | | Controls | |
|---|---|---|---|---|---|---|
| | *p* | $R^2$ | *p* | $R^2$ | *p* | $R^2$ |
| *Aces v asp* | <0.001 | 0.96 | <0.001 | 0.98 | <0.001 | 0.92 |
| *Aces v ca* | <0.001 | 0.99 | <0.001 | 0.99 | <0.001 | 0.99 |
| *Aces v sac* | <0.001 | 0.37 | <0.001 | 0.44 | <0.001 | 0.24 |
| *Aces v neo* | <0.001 | 0.94 | <0.001 | 0.94 | <0.001 | 0.91 |
| *Aces v th* | 0.002 | 0.04 | NS | 0.02 | 0.03 | 0.04 |
| *Asp v ca* | <0.001 | 0.94 | <0.001 | 0.96 | <0.001 | 0.90 |
| *Asp v sac* | <0.001 | 0.39 | <0.001 | 0.46 | <0.001 | 0.26 |
| *Asp v neo* | <0.001 | 0.93 | <0.001 | 0.96 | <0.001 | 0.86 |
| *Asp v th* | 0.002 | 0.04 | NS | 0.02 | 0.022 | 0.04 |
| *Ca v sac* | <0.001 | 0.32 | <0.001 | 0.39 | <0.001 | 0.19 |
| *Ca v neo* | <0.001 | 0.89 | <0.001 | 0.90 | <0.001 | 0.86 |
| *Ca v th* | 0.008 | 0.03 | NS | 0.01 | 0.38 | 0.04 |
| *Sac v neo* | <0.001 | 0.55 | <0.001 | 0.59 | <0.001 | 0.47 |
| *Sac v th* | <0.001 | 0.06 | 0.003 | 0.07 | NS | 0.01 |
| *Neo v th* | 0.003 | 0.04 | NS | 0.02 | 0.03 | 0.04 |

Aces– acesulfame k  Asp – aspartame  Sac – saccharin

Ca – cyclamic acid  Neo – neohespheridine DC  Th – thaumatin

**Table 4.6** **Mean Daily Intakes (with standard deviations: SD) of macronutrients, micronutrients, consumer only intense sweetener intakes and consumer only total intense sweetener intakes in Insulin Dependent Diabetics and age- and sex- matched controls based on consumer only tertiles of intense sweetness intake index.**

| | Diabetic consumers only of intense sweetness | | | | Control consumers only of intense sweetness | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| Intense sweetness | 0.6 ±0.3 | 1.8 ± 0.6 | 5.5 ± 2.2 | | 0.3 ± 0.2 | 1.2 ± 0.4 | 3.3 ± 1.4 | |
| Age(years) | 42.9 ± 16.1 | 38.7 ± 13.4 | 27.5 ± 10.4 | < 0.001 | 41.0 ± 12.4 | 36.1 ± 16.0 | 31.1 ± 14.1 | 0.023 |
| Weight (kg) | 75.1 ± 12.7 | 76.1 ± 14.9 | 72.0 ± 13.2 | NS | 76.8 ± 13.1 | 71.8 ± 13.9 | 68.8 ± 12.0 | 0.05 |
| Height (m) | 1.7 ± 0.01 | 1.7 ± 0.1 | 1.7 ± 0.1 | NS | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | NS |
| BMI ($kg/m^2$) | 26.8 ± 4.3 | 27.6 ± 4.6 | 25.1 ± 3.4 | 0.032 | 26.7 ± 4.4 | 25.0 ± 4.3 | 24.1 ± 3.2 | 0.04 |
| Energy MJ | 9.4 ± 2.4 | 9.7 ± 3.0 | 10.6 ± 4.3 | NS | 10.1 ± 4.6 | 10.0 ± 3.5 | 10.2 ± 2.9 | NS |
| EI: BMR | 1.4 ± 0.3 | 1.4 ± 0.4 | 1.5 ± 0.5 | NS | 1.4 ± 0.6 | 1.5 ± 0.8 | 1.2 ± 0.5 | NS |

**Table 4.6 continued**

| | Diabetic consumers only of intense sweetness | | | | Control consumers only of intense sweetness | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| % en fat | 35.9 ± 6.8 | 34.9 ± 5.5 | 36.5 ± 6.7 | NS | 35.8 ± 8.2 | 35.6 ± 5.4 | 33.7 ± 5.7 | NS |
| % en pro | 17.3 ± 3.8 | 16.8 ± 4.1 | 15.7 ± 4.0 | NS | 15.6 ± 4.1 | 14.4 ± 2.2 | 13.6 ± 3.1 | 0.04 |
| % en cho | 43.5 ± 8.7 | 44.5 ± 7.1 | 44.8 ± 6.5 | NS | 43.7 ± 9.3 | 44.5 ± 8.4 | 47.8 ± 8.3 | NS |
| % en starch | 28.5 ± 7.3 | 28.8 ± 6.3 | 29.3 ± 5.0 | NS | 24.0 ± 5.8 | 25.0 ± 5.4 | 25.2 ± 5.2 | NS |
| % en sugars | 15.2 ± 7.7 | 15.7 ± 7.4 | 14.8 ± 6.2 | NS | 20.5 ± 7.7 | 19.9 ± 8.1 | 22.9 ± 8.2 | NS |
| % en alcohol | 4.1 ± 6.5 | 4.0 ± 6.2 | 3.0 ± 4.6 | NS | 5.2 ± 6.3 | 5.5 ± 5.9 | 5.0 ± 5.1 | NS |
| % en starch:sugars | 2.4 ± 1.3 | 2.3 ± 1.5 | 2.5 ± 1.6 | NS | 1.4 ± 0.7 | 1.5 ± 0.8 | 1.6 ± 0.4 | NS |
| Fibre (g) | 24.3 ± 8.2 | 26.9 ± 11.6 | 24.7 ± 9.4 | NS | 19.8 ± 8.5 | 20.9 ± 8.0 | 20.6 ± 8.1 | NS |
| Calcium (mg) | 923.0 ± 406.2 | 970.3 ± 496.6 | 1003.3 ± 446.3 | NS | 1031.9 ± 1015.6 | 859.0 ± 382.8 | 846.9 ± 321.3 | NS |
| Iron (mg) | 14.3 ± 6.6 | 15.8 ± 6.6 | 15.3 ± 9.1 | NS | 13.7 ± 6.9 | 13.9 ± 7.0 | 11.8 ± 4.9 | NS |

**Table 4.6 continued**

| | Diabetic consumers only of intense sweetness | | | | Control consumers only of intense sweetness | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| Zinc (mg) | 11.3 ± 4.2 | 10.3 ± 4.2 | 10.2 ± 4.7 | NS | 10.1 ± 6.1 | 9.2 ± 4.7 | 8.3 ± 3.6 | NS |
| Folic acid (mg) | 355.5 ± 154.5 | 365.0 ± 230.6 | 389.3 ± 447.8 | NS | 291.4 ± 139.2 | 323.9 ± 140.1 | 317.0 ± 128.0 | NS |
| Vitamin C (mg) | 152.8 ± 172.6 | 146.1 ± 173.5 | 120.6 ± 123.8 | NS | 153.5 ± 167.6 | 183.4 ± 184.5 | 215.1 ± 203.0 | NS |
| Vitamin D (µg) | 4.0 ± 2.3 | 4.0 ± 2.9 | 4.1 ± 2.9 | NS | 3.1 ± 2.1 | 3.5 ± 2.0 | 3.1 ±2.1 | NS |
| Vitamin E (mg) | 8.0 ± 3.4 | 7.8 ± 3.5 | 8.2 ± 4.0 | NS | 7.2 ± 3.6 | 8.1 ± 4.3 | 8.0 ± 3.1 | NS |
| Retinol (µg) | 1004.1 ± 1470.1 | 559.5 ± 505.3 | 514.1 ± 269.2 | 0.039 | 605.0 ± 689.1 | 472.0 ± 310.3 | 382.5 ± 188.6 | NS |
| Carotene (µg) | 2172.4 ± 1645.6 | 2030.7 ± 2021.2 | 1856.0 ± 1441.8 | NS | 2173.8 ± 1760.5 | 1900.1 ± 1188.7 | 2090.8 ± 1529.3 | NS |
| Copper (mg) | 1.6 ± 1.2 | 1.4 ± 0.7 | 1.3 ± 0.6 | NS | 1.2 ± 0.5 | 1.1 ± 0.4 | 1.2 ± 0.5 | NS |

**Table 4.6 continued**

| | Diabetic consumers only of intense sweetness | | | | Control consumers only of intense sweetness | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| Acesulfame K | 0.3 ± 0.2 | 1.1 ± 0.4 | 3.7 ± 1.6 | <0.001 | 0.2 ± 0.1 | 0.8 ± 0.3 | 2.2 ± 1.3 | <0.001 |
| Aspartame | 0.8 ± 0.5 | 2.2 ± 0.9 | 6.8 ± 2.9 | <0.001 | 0.3 ± 0.3 | 1.5 ± 0.5 | 4.1 ± 2.3 | <0.001 |
| Saccharin | 0.2 ± 0.2 | 0.6 ± 0.6 | 1.1 ± 0.6 | <0.001 | 0.2 ± 0.3 | 0.4 ± 0.6 | 0.8 ± 0.7 | <0.001 |
| Cyclamic acid | 0.3 ± 0.3 | 1.0 ± 0.5 | 4.0 ± 1.8 | <0.001 | 0.1 ± 0.1 | 0.8 ± 0.4 | 2.3 ± 1.6 | <0.001 |
| Neo dc | 0.1 ± 0.03 | 0.1 ± 0.1 | 0.4 ± 0.1 | <0.001 | 0.03 ± 0.2 | 0.1 ± 0.004 | 0.2 ± 0.1 | <0.001 |
| thaumatin | 0.003 ± 0.01 | 0.01 ± .0.01 | 0.1 ± 0.2 | NS | 0.001 ± 0.003 | 0.0003 ± 0.0001 | 0.002 ± 0.01 | NS |
| Total intense sweetener | 1.6 ± 1.0 | 5.0 ± 1.9 | 16.0 ± 6.7 | <0.001 | 0.8 ± 0.6 | 3.5 ± 1.3 | 9.7 ± 5.3 | <0.001 |

**Table 4.7** **Mean Daily Intakes (with standard deviations : SD) of macronutrients, micronutrients, consumer only intense sweetness intakes and consumer only intense sweetener intakes in Insulin Dependent Diabetics and age- and sex- matched controls based on consumer only tertiles of Intense sweetener intakes.**

| | Diabetic consumers only of intense sweeteners | | | | Control consumers only of intense sweeteners | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| Intense sweeteners | 1.6 ± 1.0 | 5.0 ± 1.9 | 16.0 ± 6.7 | | 0.8 ± 0.6 | 3.5 ± 1.3 | 9.7 ± 5.3 | |
| Age | 42.3 ± 16.2 | 38.0 ± 12.8 | 28.3 ± 11.4 | <0.001 | 41.6 ± 13.5 | 36.8 ± 15.1 | 29.9 ± 13.2 | 0.004 |
| Weight (kg) | 77.0 ± 13.2 | 76.2 ± 13.8 | 70.6 ± 13.2 | NS | 78.0 ± 13.3 | 74.8 ± 13.9 | 64.6 ± 8.5 | <0.001 |
| Height (m) | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | NS | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | NS |
| BMI ($kg/m^2$) | 27.3 ± 4.6 | 27.5 ± 4.5 | 24.9 ± 3.1 | NS | 26.8 ± 4.3 | 25.7 ± 4.4 | 23.3 ± 2.7 | NS |
| Energy MJ | 9.3 ± 2.5 | 10.0 ± 2.8 | 10.3 ± 4.4 | NS | 10.1 ± 4.7 | 10.2 ± 3.5 | 10.1 ± 2.7 | NS |
| EI:BMR | 1.4 ± 0.4 | 1.4 ± 0.3 | 1.5 ± 0.5 | NS | 1.4 ± 0.6 | 1.5 ± 0.6 | 1.6 ± 0.3 | NS |

**Table 4.7 continued**

| | Diabetic consumers only of intense sweeteners | | | | Control consumers only of intense sweeteners | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| % en fat | 35.8 ± 6.5 | 35.3 ± 5.5 | 36.4 ± 7.0 | NS | 35.6 ± 7.4 | 35.8 ± 6.5 | 33.7 ± 5.4 | NS |
| % en pro | 17.4 ± 4.6 | 16.5 ± 3.2 | 15.7 ± 4.0 | NS | 15.6 ± 4.1 | 14.4 ± 2.3 | 13.6 ± 3.0 | 0.042 |
| % en cho | 43.3 ± 8.8 | 44.6 ± 7.0 | 44.7 ± 6.5 | NS | 43.9 ± 9.5 | 44.6 ± 8.4 | 47.4 ± 8.1 | NS |
| % en starch | 28.1 ± 7.2 | 29.5 ± 6.3 | 28.8 ± 5.2 | NS | 23.6 ± 5.8 | 25.6 ± 5.5 | 24.7 ± 5.1 | NS |
| % en sugars | 15.3 ± 7.8 | 15.1 ± 7.2 | 15.3 ± 0.6 | NS | 21.1 ± 8.5 | 19.5 ± 7.1 | 22.9 ± 8.2 | NS |
| % en alcohol | 4.1 ± 6.6 | 3.9 ± 2.9 | 3.2 ± 4.8 | NS | 5.2 ± 6.5 | 5.2 ± 5.4 | 5.3 ± 5.2 | NS |
| % en starch:sugars | 2.4 ± 1.2 | 2.5 ± 1.5 | 2.4 ± 1.7 | NS | 1.3 ± 0.7 | 1.5 ± 0.8 | 1.2 ± 0.5 | NS |
| | | | | | | | | |
| Fibre (g) | 23.9 ± 8.5 | 27.5 ± 11.3 | 24.5 ± 9.3 | NS | 19.2 ± 8.4 | 22.2 ± 8.5 | 20.0 ± 7.3 | NS |
| Calcium (mg) | 926.2 ± 410.1 | 978.7 ± 494.6 | 994.0 ± 450.6 | NS | 1016.2 ± 1002.5 | 861.6 ± 374.7 | 855.1 ± 329.2 | NS |
| Iron (mg) | 14.4 ± 6.7 | 15.6 ± 6.6 | 15.5 ± 9.0 | NS | 13.9 ± 7.2 | 13.9 ± 6.8 | 11.6 ± 4.6 | NS |

**Table 4.7 continued**

| | Diabetic consumers only of intense sweeteners | | | | Control consumers only of intense sweeteners | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| Zinc (mg) | 11.3 ± 4.3 | 10.7 ± 4.2 | 9.9 ± 4.7 | NS | 10.0 ± 6.1 | 9.8 ± 5.1 | 8.0 ± 3.1 | NS |
| Folic Acid (mg) | 357.1 ± 156.4 | 364.9 ± 231.5 | 391.6 ± 446.9 | NS | 293.0 ± 129.8 | 319.9 ± 137.9 | 322.3 ± 138.4 | NS |
| Vitamin C (mg) | 157.8 ± 173.8 | 140.8 ± 175.0 | 123.6 ± 122.6 | NS | 168.9 ± 173.9 | 162.9 ± 168.6 | 229.9 ± 213.8 | NS |
| Vitamin D (µg) | 4.0 ± 2.3 | 3.9 ± 2.9 | 4.2 ± 2.9 | NS | 3.0 ± 1.9 | 3.9 ± 2.4 | 2.9 ± 1.8 | NS |
| Vitamin E (mg) | 8.0 ± 3.6 | 7.8 ± 3.4 | 8.2 ± 4.0 | NS | 7.0 ± 3.3 | 8.2 ± 4.5 | 8.1 ± 3.0 | NS |
| Retinol (µg) | 1004.3 ± 1494.1 | 561.5 ± 503.8 | 519.5 ± 265.1 | 0.045 | 583.2 ± 680.5 | 493.6 ± 307.2 | 375.9 ± 194.4 | NS |
| Carotene (µg) | 2202.8 ± 1759.8 | 1960.4 ± 1896.5 | 1886.6 ± 1503.4 | NS | 2044.7 ± 1622.6 | 2070.0 ± 1514.1 | 2088.8 ± 1385.0 | NS |
| Copper (mg) | 1.6 ± 1.2 | 1.4 ± 0.6 | 1.3 ± 0.6 | NS | 1.2 ± 0.5 | 1.2 ± 0.4 | 1.2 ± 0.4 | NS |

**Table 4.7 continued**

| | Diabetic consumers only of intense sweeteners | | | | Control consumers only of intense sweeteners | | | |
|---|---|---|---|---|---|---|---|---|
| **Tertiles** | **Low** | **Medium** | **High** | ***p* =** | **Low** | **Medium** | **High** | ***p* =** |
| intense sweetness | 0.6 ± 0.4 | 1.8 ± 0.7 | 5.5 ± 2.3 | <0.001 | 0.3 ± 0.3 | 1.3 ± 0.6 | 3.2 ± 1.5 | <0.001 |
| Acesulfame K | 0.3 ± 0.2 | 1.1 ± 0.4 | 3.7 ± 1.6 | <0.001 | 0.2 ± 0.1 | 0.7 ± 0.3 | 2.2 ± 1.3 | <0.001 |
| Aspartame | 0.7 ± 0.4 | 2.2 ± 0.8 | 6.8 ± 2.9 | <0.001 | 0.3 ± 0.2 | 1.4 ± 0.5 | 4.2 ± 2.2 | <0.001 |
| Saccharin | 0.2 ± 0.2 | 0.6 ± 0.7 | 1.1 ± 0.6 | <0.001 | 0.2 ± 0.3 | 0.3 ± 0.6 | 0.9 ± 0.7 | <0.001 |
| Cyclamic acid | 0.4 ± 0.2 | 1.0 ± 0.4 | 4.0 ± 1.8 | <0.001 | 0.2 ± 0.1 | 0.8 ± 0.3 | 2.5 ± 1.5 | <0.001 |
| Neo DC | 0.1 ± 0.02 | 0.1 ± 0.1 | 0.4 ± 0.1 | <0.001 | 0.03 ± 0.02 | 0.1 ± 0.04 | 0.01 ± 0.01 | <0.001 |
| Thaumatin | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.02 | NS | 0.004 ± 0.004 | 0.003 ± 0.004 | 0.01 ± 0.01 | NS |

**Table 4.8** **Mean Daily Intakes (with standard deviations: SD) of macronutrients, micronutrients, consumer only intakes of intense sweetness intake index, each intense sweetener based on consumer only intakes of the diabetic and control groups for aspartame.**

| | Diabetic | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|
| Tertiles | **Low** | **Medium** | **High** | **p =** | **Low** | **Medium** | **High** | p = |
| Aspartame | 0.7 ± 0.4 | 2.2 ± 0.7 | 6.8 ± 2.8 | | 0.3 ± 0.2 | 1.4 ± 0.4 | 4.2 ± 2.1 | |
| Age (years) | 44.7 ± 16.7 | 35.3 ± 11.5 | 28.9 ± 11.5 | <0.001 | 40.8 ± 13.7 | 36.8 ± 15.6 | 30.6 ± 13.2 | 0.016 |
| Weight (kg) | 76.8 ± 12.1 | 76.5 ± 4.7 | 70.0 ± 13.4 | 0.049 | 78.6 ± 14.2 | 73.4 ± 13.5 | 65.4 ± 8.4 | <0.001 |
| Height (m) | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | NS | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | NS |
| BMI ($kg/m^2$) | 27.4 ± 4.4 | 27.3 ± 4.7 | 24.8 ± 3.0 | 0.008 | 26.9 ± 4.5 | 25.6 ± 4.2 | 23.3 ± 2.8 | 0.002 |
| Energy MJ | 9.4 ± 2.4 | 9.7 ± 3.1 | 10.5 ± 4.3 | NS | 9.8 ± 4.7 | 10.3 ± 3.6 | 10.1 ± 2.6 | NS |
| EI:BMR | 1.4 ± 0.3 | 1.4 ± 0.4 | 1.5 ± 0.5 | NS | 1.4 ± 0.6 | 1.5 ± 0.6 | 1.2 ± 0.5 | NS |

**Table 4.8 continued**

| | Diabetic | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|
| Tertiles | **Low** | **Medium** | **High** | **p =** | **Low** | **Medium** | **High** | p = |
| % en fat | 35.3 ± 6.7 | 36.1 ± 5.3 | 35.9 ± 7.0 | NS | 35.2 ± 7.6 | 36.5 ± 6.2 | 33.4 ± 5.4 | NS |
| % en pro | 17.1 ± 3.7 | 16.9 ± 4.2 | 15.7 ± 4.0 | NS | 15.8 ± 4.1 | 14.4 ± 2.2 | 13.5 ± 3.0 | 0.017 |
| % en cho | 44.1 ± 9.1 | 43.5 ± 6.4 | 45.2 ± 6.5 | NS | 43.9 ± 9.4 | 43.9 ± 8.5 | 48.1 ± 7.9 | NS |
| % en starch | 27.9 ± 7.3 | 29.9 ± 6.0 | 28.8 ± 5.2 | NS | 24.1 ± 5.7 | 25.3 ± 5.5 | 24.7 ± 5.2 | NS |
| % en sugars | 16.1 ± 9.0 | 13.7 ± 5.2 | 15.8 ± 6.2 | NS | 20.6 ± 5.7 | 19.0 ± 6.7 | 23.7 ± 8.2 | 0.046 |
| % en alcohol | 4.1 ± 6.6 | 3.8 ± 6.0 | 3.2 ± 4.8 | NS | 5.4 ± 6.7 | 5.4 ± 5.5 | 4.0 ± 5.0 | NS |
| % en starch:sug | 2.3 ± 1.2 | 2.7 ± 1.8 | 2.2 ± 1.3 | NS | 1.4 ± 0.7 | 1.5 ± 0.8 | 1.2 ± 0.5 | NS |
| Fibre (g) | 24.5 ± 8.2 | 26.0 ± 11.7 | 25.5 ± 9.5 | NS | 19.3 ± 8.8 | 21.9 ± 8.4 | 20.0 ± 7.2 | NS |
| Calcium (mg) | 916.3 ± 399.2 | 966.8 ± 515.6 | 1014.7 ± 432.0 | NS | 1014.0 ± 1021.0 | 895.8 ± 408.5 | 826.3 ± 274.2 | NS |

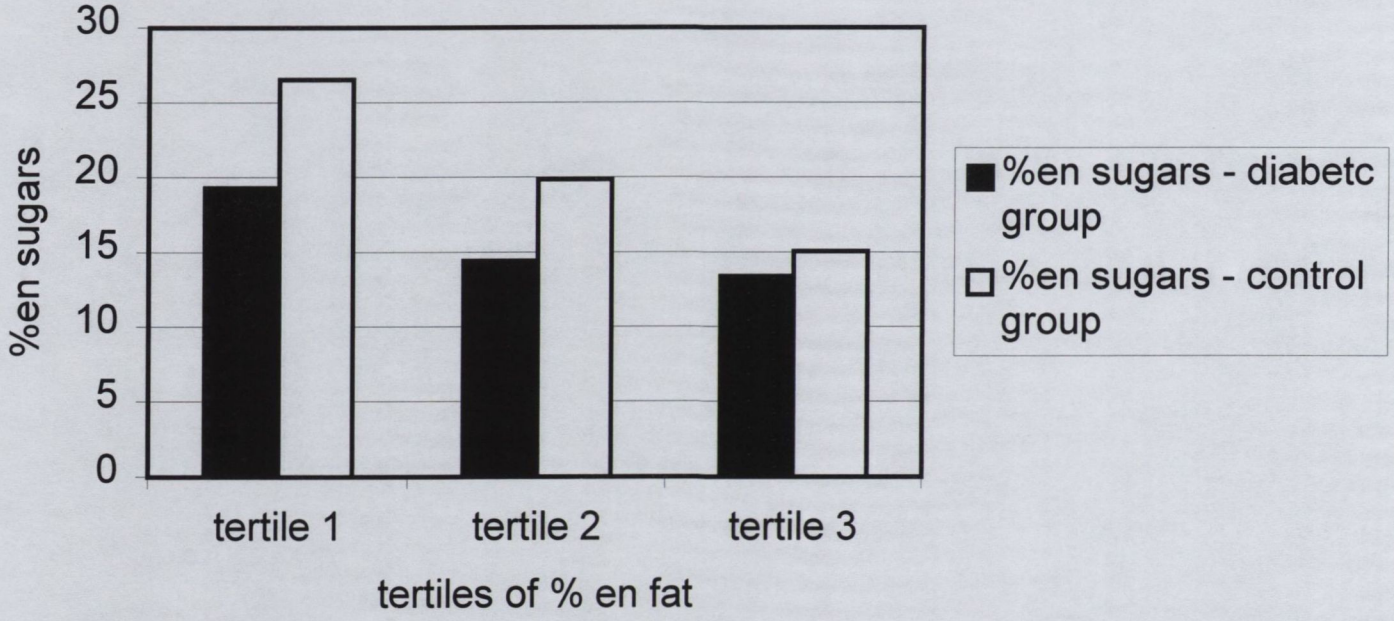
**Table 4.8 continued**

| | Diabetic | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|
| Tertiles | **Low** | **Medium** | **High** | **p =** | **Low** | **Medium** | **High** | p = |
| Iron (mg) | 14.9 ± 7.0 | 14.2 ± 5.6 | 16.3 ± 9.3 | NS | 13.7 ± 7.3 | 14.0 ± 6.9 | 11.7 ± 4.5 | NS |
| Zinc (mg) | 11.3 ± 4.7 | 10.2 ± 3.7 | 10.3 ± 4.7 | NS | 10.1 ± 6.2 | 9.7 ± 5.0 | 7.9 ± 2.9 | NS |
| Folic acid (mg) | 370.1 ± 156.9 | 333.0 ± 230.8 | 407.2 ± 445.3 | NS | 286.3 ± 137.4 | 314.4 ± 128.6 | 331.9 ± 139.9 | NS |
| Vitamin C (mg) | 172.3 ± 193.6 | 108.2 ± 128.1 | 137.8 ± 139.5 | NS | 147.4 ± 163.2 | 159.9 ± 154.7 | 245.2 ± 222.6 | NS |
| Vitamin D (µg) | 4.1 ± 2.3 | 3.7 ± 2.9 | 4.3 ± 2.8 | NS | 3.0 ± 1.8 | 3.6 ± 2.3 | 3.14 ± 2.0 | NS |
| Vitamin E (mg) | 7.9 ± 3.5 | 7.6 ± 3.5 | 8.4 ± 3.9 | NS | 6.8 ± 3.3 | 8.4 ± 4.5 | 8.0 ± 3.0 | NS |
| Retinol (µg) | 982.4 ± 1456.6 | 481.5 ± 232.1 | 600.0 ± 514.6 | 0.047 | 507.5 ± 470.0 | 574.5 ± 590.9 | 371.4 ± 154.8 | NS |
| Carotene (µg) | 2153.5 ± 1781.5 | 1964.5 ± 1888.7 | 1935.6 ± 1475.3 | NS | 2128.1 ± 1786.3 | 1907.4 ± 1234.8 | 2127.6 ± 1465.2 | NS |
| Copper (mg) | 1.6 ± 1.2 | 1.3 ± 0.6 | 1.4 ± 0.7 | NS | 1.2 ± 0.5 | 1.2 ± 0.4 | 1.2 ± 0.4 | NS |

**Table 4.8 continued**

| | Diabetic | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|
| Tertiles | **Low** | **Medium** | **High** | **p =** | **Low** | **Medium** | **High** | p = |
| Total intense sweetness | 0.6 ± 0.4 | 1.9 ± 0.8 | 5.4 ± 2.3 | <0.001 | 0.3 ± 0.3 | 1.3 ± 0.6 | 3.2 ± 1.5 | <0.001 |
| Acesulfame K | 0.4 ± 0.2 | 1.1 ± 0.4 | 3.7 ± 1.6 | <0.001 | 0.2 ± 0.1 | 0.7 ± 0.3 | 2.2 ± 1.3 | <0.001 |
| Saccharin | 0.2 ± 0.3 | 0.6 ± 0.6 | 1.1 ± 0.6 | <0.001 | 0.2 ± 0.3 | 0.5 ± 0.8 | 0.7 ± 0.5 | <0.001 |
| Cyclamic acid | 0.3 ± 0.3 | 1.0 ± 0.5 | 4.0 ± 1.8 | <0.001 | 0.1 ± 0.2 | 0.7 ± 0.3 | 2.4 ± 1.5 | <0.001 |
| Neo DC | 0.1 ± 0.3 | 0.1 ± 0.1 | 0.4 ± 0.1 | <0.001 | 0.03 ± 0.02 | 0.1 ± 05 | 0.2 ± 0.1 | <0.001 |
| Thaumatin | 0.004 ± 0.001 | 0.004 ± 0.001 | 0.01 ± 0.02 | NS | 0.001 ± 0.002 | 0.004 ± 0.02 | 0.002 ± 0.005 | NS |
| Total intense sweeteners | 1.6 ± 1.0 | 5.0 ± 1.9 | 16.0 ± 6.7 | <0.001 | 0.8 ± 0.5 | 3.4 ± 1.2 | 9.7 ± 5.2 | <0.001 |

**Figure 4.1. Mean intakes of percent energy from sugars for diabetic and control groups based on tertiles of percent energy from fat.**



# *Chapter 5*

# *General Discussion.*

## 5.1 CONCLUSIONS AND RECOMMENDATIONS

At present, member states of the European Union are required to monitor the consumption and usage of food additives (European Parliament / Council Directives 95/2/EC, 94/35/EC, 94/36/EC). The work reported in this thesis was done primarily to investigate the assessment of exposure to food additives in Ireland, with a particular interest in intense sweeteners. Several hypotheses were tested.

### 5.2 The use of brand-level data in assessing intake of food additives

A fundamental requirement for the estimation of food additive intake is the availability of food consumption data. The ultimate refinement of food intake data is at actual brand - level. Although brand - level information is routinely collected in food consumption surveys, its main use lies in assigning a suitable food code for nutrient analysis using food composition tables. In the Dietary and National Survey of British Adults (DNSBA), (ERSC Data Archives, 1991), food intake data are recorded at brand - level. This provides a unique opportunity to examine this issue of the incremental value, or otherwise, of recording brand - level food intake data. The DNSBA is the largest food consumption database publicly available with brand level food intake data.

The first hypothesis was that the retention of brand level data in food consumption databases is essential for accurate assessment of human exposure to food – borne chemicals. Using the DNSBA database in the present study, it was found that there is limited incremental value in retaining brand-level food intake data in food consumption databases to estimate intake of brands among consumers. Another important finding was that a multiple of 1.3 times the food category 97.5th percentile intake provides a robust worst-case analysis, as no brand intake at the 97.5th percentile was found to exceed this factor. The findings of the present study do not apply where a complex search of a database is needed to examine true additive intake from multiple food sources. In order to examine true additive intake, brand level data is required. Currently, the retention of brand - level data is expensive, but this cost could possibly be reduced using less labour-intensive data capture. For instance, brand - level data could be collected and maintained quite easily by using the data derived from scanning

the bar codes of foods purchased and consumed. This method of data capture could be investigated for use in post marketing surveillance.

## 5.3 The food frequency questionnaire in assessing intake of artificial sweeteners

The EU permits the use of sweeteners in a wide range of foods and beverages. Detailed conditions of use, including both the food types and the maximum levels of use, are specified in the sweetener directive (EC, 94/35/EC). Although there is a wealth of data on intense sweetener intake internationally, prior to this investigation no comprehensive data existed on the intake of intense sweeteners in Ireland. Due to the disease status and dietary advice received by diabetics, they are considered to be a high risk group for exposure to intense sweeteners. Many countries around the world have conducted sweetener intake studies. The methods used for consumption intakes varied greatly with questionnaires and diaries as the main sources of data collection used.

The second hypothesis tested in this thesis was that a simple food frequency questionnaire, with a food list identified from a national food ingredient database as containing target intense sweeteners, would provide a reliable means of routine surveillance of sweetener intake in high users. In order to determine food chemical concentration and occurrence, food composition data is needed from industry. The main issue in acquiring such data from industry is the fact that it is commercially sensitive. One possibility of over-coming this problem is that legislation would enforce the provision of such data when required. An average level of occurrence and concentration could then be created through the contributions of each company involved. This would eliminate the issue of commercial sensitivity. Despite using such crude methods of assessing sweetener intake in comparison to the refined methods employed by the other countries, the estimates of intakes are not dramatically different. Currently as it stands, the FFQ could not be used as a method of routine surveillance, because it tended to over-estimate intakes in comparison to the diary. One way to overcome this problem would be to decrease the cut off value for high consumers (i.e. the 90th percentile). This would take counteract the over-estimation and make it possible to continue using the FFQ as a participant-friendly data collection tool. Another possibility would be to use the FFQ as a screening step to identify which foods were consumed the most in relation

to the sweetener of concern, or the foods in which the sweetener is widely used. These prime foods could then be investigated thoroughly using a more accurate method in follow-up studies. Since exposure assessment is an increasing part of risk assessment, probabilistic exposure assessment may be the method for the future. This would greatly reduce the cost of maintaining food consumption databases and would also give a relatively accurate estimation of intakes of the brand and/or additive. It would provide a worst - case scenario as the intakes with a high probability of occurrence are selected more often than intakes with a low probability of occurrence.

### 5.4 The effect of artificial sweeteners on fat intake

Food additives can have an impact on the diets of the general public due to their extensive use in foods. The third and final hypothesis of this thesis was that the use of high levels of intense sweeteners in potentially vulnerable groups, such as Insulin Dependent Diabetics, will lead to a higher percent energy from fat in their diet. The present cross - sectional study confirmed that the fat-sugar seesaw is present even among insulin-dependent diabetics, and it showed no evidence that the use of intense sweeteners leads to higher fat diets. Another group considered to be 'at risk' in relation to food additives are children, because of their tendency to consume more food and beverages expressed as grams per kilogram body weight. This is due to the demands of growth and development, thus their level of exposure is potentially higher to sweeteners and other food additives. Other potential groups at risk are the elderly and the pregnant. Using crude methods, the present study has shown that diabetic adults are not at risk of exceeding the ADI of a sweetener if actual usage data were used. The data are crude but the results do provide assurance that the general adult population is not at risk of exceeding the ADI. The question remains as to whether or not this is the case for children.

Another area to be investigated in the future is that of macronutrient substitutes. The issue of the nutritional composition of the diet is becoming increasingly more important with the growing availability on the market of foods containing macronutrient substitutes. For example, what will be the impact on the composition of the diet if one is consuming reduced fat foods or if there is a combination of reduced sugar and reduced fat foods in the diet?

## 5.5 References

ERSC DATA ARCHIVES, 1991. Dietary and Nutritional Survey of British Adults, 1986 – 1987, Volume 2 (Colchester: Office of Population, Consensus and Surveys, Social Survey Division).

European Parliament and Council Directive 95/2/EC on food additives, other than colours and sweeteners for use in foodstuffs.

European Parliament and Council Directive 94/35/EC on sweeteners for use in foodstuffs.

European Parliament and Council Directive 94/36/EC on colours for use in foodstuffs.