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Peanuts Bioactives & Allergens

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Peanuts, the humble food with a unique flavour and taste, have been enjoyed by different cultures over the centuries. Today, whether consumed whole or processed in a myriad of ways, it remains a marked feature in many culinary dishes. Peanuts are recognized as one of the economically important oilseed and edible nut crops, being grown in 109 countries. Globally it is grown in an area of 23 million ha with a production of 40 million tonnes and an average yield of 2.1 t ha\(^{-1}\) (FAO, 2006). All of the peanut plant components are utilizable. Around 50% of world groundnut produced is used for oil extraction, 37% for confectionery use, and 12% for seed purposes.

Peanuts are also a special commodity that holds both goodness and badness in combination.

The nuts, which are rich in easily digestible protein (26–28%, and rich in sulphur containing amino acids), high quality edible oil (48–50%), vitamins (nearly half of the 13 essential vitamins, and particularly rich in the B-group vitamins), and minerals (7 of the 20 essential minerals), play an important role in human nutrition. Peanuts contain more than just macronutrients and essential micronutrients. Functional components such as bioactive peptides, arginine, fibre, antioxidants, and active principles such as resveratrol and phytosterols, may interact synergistically to provide protective functions and reduce the risk of certain diseases.

In contrast to the positive impacts, a gradual increase in the use of peanuts for food in the last 20 years coincides with an increased prevalence of peanut allergies, which is believed to have doubled in the last
20 years—1 in 200 children and 1 in 10,000 adults suffer from peanut allergy. Unlike most other food allergies where immunological reactions usually resolve themselves in childhood, only 20% of peanut allergy is resolved and most generally persist through to adulthood. Clinicians generally recommend an allergen-free diet or a total-avoidance diet as the most effective form of management. However, accidental ingestions of the food allergen remain prevalent, comprising >50% of the recurring allergic reactions, usually from hidden or undisclosed allergens in foods. More importantly, the vast majority of deaths from food allergen induced-anaphylaxis in children are due to peanut allergens.

When our publisher, Joseph Eckenrode, proposed the project of writing this book to us, our first thought was that it was very timely. This proposition arrived at a stage when our peanut research was making a turning point; from in-depth studies in agronomy for improving yields and developing appropriate management strategies through to reducing mycotoxins at both preharvest and postharvest stages, and onto breeding for functional food traits and developing strategies to reduce peanut allergy. We were not alone in this journey, however, as many of our research colleagues were also moving into these new frontiers. Numerous books already deal with mycotoxins or functional foods or food allergy, but they have generally covered a broad range of other crops. Hence, the central focus of this book aims to capture these research efforts that concentrate on peanuts alone. This book focuses on two thematic topics: bioactives and allergy; bioactives mainly in the area of phytochemicals, and allergy which is becoming important because of its increasing prevalence globally. As it was not possible to cover every aspect within these two themes, we decided to select special topics which would be of special interest to the agrifood science and food manufacturing communities. We hope that this book can serve as a reference book on peanuts as a functional food, and just as importantly, update readers on the current status regarding the latest research into peanut allergy.

The compilation of this book has been a long and dedicated journey, and we could not have made it without our friends and colleagues—their collective hard work and enthusiasm are truly commendable. More specifically, we sincerely thank our publisher Joseph Eckenrode for being particularly patient with us. We thank all of our contributors who devoted their valuable time to complete their chapters. We also thank our reviewers who made many valuable comments to improve
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CHAPTER 1

Peanut: A Friend or Foe?

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About 36 million tons of peanuts are produced annually worldwide. Peanut kernels are utilized as a major source of cooking oil in developing countries and are used in snack food industries in developed countries. Although the use of peanut kernels as a confectionary snack food is increasing rapidly due to growing trends of vegetarianism and a demand for healthy foods, aflatoxin and allergenic proteins have become major public health issues globally. The aim of Chapter 1 is to introduce the reader to “positives (bioactives)” and “negatives (allergens)” in peanuts and to provide a comprehensive and up-to-date review of both beneficial and allergenic compounds present in peanut kernels including current information about bioengineering and allergen management.

1.1. INTRODUCTION

Peanuts (Arachis hypogaea L.) are grown as an oilseed or food crop in more than 100 countries under diverse agro-climatic conditions. Peanut kernels are one of the most economically and nutritionally important commodities with an oil content of approximately 50%, protein
content of approximately 25%, and high levels of vitamins and minerals such as niacin, folate, vitamin E, magnesium, manganese, and phosphorus. Other parts of the peanut plant (stems and foliage) are also economically important as hay for livestock, while the by-products of oil extraction, i.e., shells and peanut meal, are used either as livestock feed or as fertilizer in crop production. Peanut oil derived from the crushing of kernels is a premium source of cooking oil in many Asian and African countries.

The peanut plant produces a diverse array of metabolic compounds during its growth and development, which have major benefits to human health. The peanut kernel is an important source of vegetable protein in addition to over 30 essential nutrients (including niacin, folate, fiber, magnesium, vitamin E, manganese, phosphorus, and a range of fatty acids) (Savege and Keenan 1994). The use of peanut kernels and their value-added products such as peanut butter and snack and confectionary foods is increasing rapidly due to growing trends to vegetarianism and demand for healthy foods.

Peanuts are an important source of vegetable oil for a large portion of the global population, including China, India, Africa, and many developing countries. It is a premium cooking oil with unique flavour, free from cholesterol, and has a high smoke point and well balanced lipid profile. The oil is especially rich in the monounsaturated fatty acid, oleic acid (18:1) that helps to lower low-density lipoprotein (LDL or “bad cholesterol”) and increases high-density lipoprotein (HDL or “good cholesterol”) in the blood. Recent genetic improvements in peanuts have also led to the development of “high oleic” varieties (Norden et al. 1987), which have increased kernel oleic oil content from around 50% to more than 80%, and enhanced the shelf life of many peanut products by more than 30 times. Research studies suggest that Mediterranean diets that are enriched with high levels of monounsaturated fatty acids help to prevent coronary artery disease and strokes by favoring a healthy blood lipid profile.

In addition, the peanut plant also produces an array of stilbenoids which offer protection to the plant against microbial attack via their functional role as phytoalexins. The phytoalexins also serve as functional food compounds in the human diet offering potential benefits to the immune system. In particular, the peanut stilbenoid, resveratrol, has been widely shown to have antioxidant properties. Recent studies have shown a wide genetic variation for several key functional bioactives in peanut kernels, suggesting there are good opportunities for genetic
improvement for these functional food traits. To date however, there has been little effort by peanut breeders to select varieties with high levels of these traits. With the recent development of improved analytical and genetic tools, there is huge potential to exploit these bioactive compounds via the development of new varieties with high levels of functional food traits.

Despite the multiple benefits as food for both human and animal use, peanuts are challenged by two major food safety issues: aflatoxin and allergenic proteins, both of which are recognized globally.

Aflatoxins (AF) are a group of toxins (CAST 1979) naturally produced in peanut kernels by the commonly occurring soil-borne fungi, *Aspergillus flavus*, and *Aspergillus parasiticus*, under specific moisture and temperature regimes (Doener et al. 1989). The major aflatoxins of concern are aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), and aflatoxin G₂ (AFG₂), with AFB₁ being the predominant and the most potent carcinogen. Aflatoxin production in peanut kernels can occur during the seed filling phase of crop growth or post-harvest depending on the crop growing conditions and postharvest storage practices. A number of reviews have addressed epidemiological aspects of *A. flavus* and *A. parasiticus* (Payne 1998; Scheidegger and Payne 2003) and the molecular biology of aflatoxin biosynthesis (Bhatnagar et al. 2003; Yu et al. 2004; Price and Payne 2005). The worldwide increase in the incidence of the hepatitis B and C viruses is increasing the importance of AF as a potential health risk since the toxin is implicated in predisposing people who ingest large quantities of AF to liver diseases. Hence their presence in peanuts is heavily monitored and regulated in developed countries to ensure a safe food supply. Aflatoxin contamination is most prevalent in developing countries where peanuts are cultivated in drought-prone environments and where storage practices are generally poor. Aflatoxins in peanuts are not however, discussed in this book as they are synthesized by an external organism in the kernel under specific environmental conditions. In contrast to AF, peanut allergenic proteins have been the natural constituents of peanut kernels since the domestication of crop, but have become an important public health issue in developed countries over the last decade.

A peanut allergy is typically lifelong, often severe, and potentially fatal and reactions can occur from ingestion of small amounts or even minute exposure to peanut products. In North America and the United Kingdom, prevalence rates among schoolchildren are now in excess of 1%, becoming an increasing public health concern and raising research
questions about environmental, immunologic, and genetic factors that may influence outcomes of peanut sensitization (Sicherer and Sampson 2007). In developed countries such as Australia, challenge-proven IgE-mediated food allergies now affect up to 10% of infants (Prescott and Allen 2011). Allergic disease has been linked to a more modern lifestyle, including changing dietary patterns, changing intestinal commensal bacteria, and vehicular pollution (Prescott and Allen 2011). It is not known whether the rise in food allergy is a forerunner of earlier and more severe effects of these progressive environmental changes or whether additional or unrelated lifestyle factors are implicated. In developing countries, recent reports show a rise in nonfood allergic diseases such as asthma in countries that are adopting a more “Westernised” lifestyle (Liao et al. 2009; Zar et al. 2007). This has major implications for these heavily populated regions of the world, because a second wave epidemic of food allergy may also occur in these countries because of as yet undetermined factors associated with the “modern lifestyle.”

The use and promotion of peanuts and their derivatives as a healthy food, therefore, presents a challenge for the food industry—managing the paradox of promoting a highly nutritious food versus minimising the risks of exposure to the small, but significant proportion of the allergic population.

The purpose of this book is to bring together experts to provide a comprehensive and up-to-date review of both beneficial and allergenic compounds present in peanut kernels, including aspects of chemical structure, properties, detection, prevalence, genetic variation, bioengineering, and allergen management. The book serves two purposes: first to provide a detailed understanding of the current status of research into the numerous health benefits, as well as the risks and management of peanut allergens; and second, to stimulate ideas for future work in both of these important disciplines.

1.2. REFERENCES

water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought stressed groundnut.” *Mycopathologia*, 105, 117–128.


2.3.5.4. Hexacosanoic Acid (C 26:0)

Peanuts also contain small amounts of hexacosanoic acid at levels of about 0.3% of the total fatty acids (Dean and Sanders 2009). More so than with the other VLFA, hexacosanoic acid is not able to bind easily with the protein albumin in order to be moved by the plasma between different organs. It is more likely bound by membrane proteins (Choi et al. 2002). There is concern that it may be involved in a syndrome known as X-linked adrenoleukodystrophy (ADL) because children who suffer from this have elevated levels of C 26:0; however, there has not been any link established between dietary intake of this fatty acid with elevated levels in the liver (Moser and Borel 1995).

Being able to create lipid ingredients with specific functionalities often involved hydrogenation of polyunsaturated fatty acids with the end result being the formation of harmful trans-fatty acids. These fatty acids have been found to be linked to increased rates of coronary heart disease in humans (Nelson 1998). Alternative methods of creating gels from oils have been developed which use VLFA, in particular hexacosanoic acid, using a process known as organogelation (Daniel and Rajasekharan 2003). The end products do not cause changes in the fatty acids used to make the gels without involving organic compounds that are synthetic or require long processes to produce.

2.4. SPECIFIC HEALTH ISSUES

Most fatty acids do not act singularly in biological systems. The majority of the studies in the literature have found that fatty acids combine to affect metabolism and cause syndromes such as chronic diseases. In the following sections, specific health issues and how fatty acids found in peanuts can be positively or negatively effective are presented.

2.4.1. Cancer

Although the majority of the literature reports on fatty acids, such as those found in peanut oil, have centered on circulatory and metabolism related issues, there are reports on the relationship between peanut fatty
acid consumption as related to the development, progression, and prevention of cancer. Fatty acids are thought to play a role in breast, colon, prostate, pancreatic, and stomach cancers in humans (Thiébaut et al. 2009a). In the Western Hemisphere, breast cancer is the most common type of cancer in women. The rates in Japan are also increasing (Chajès et al. 2008). Specific case studies indicate that there is a correlation between total dietary fat and incidences of breast cancer (Howe et al. 1990). Initial studies seem to indicate that high levels of monounsaturated fat were responsible for inducing cancers which would give a negative endorsement for the consumption of peanuts, especially the high oleic varieties (Trichopoulou et al. 1995; Wolk et al. 1998). However, more extensive studies have found that the association is with trans-monounsaturated fatty acids, which are not found in peanuts or peanut oil (Voorrips et al. 2002; Kim et al. 2006). In a more recent study with mice, the animals were fed diets with added walnut or peanut oils (Comba et al. 2010). Peanut oil was found to be the superior protectant against tumor proliferation and caused more cancer cell death, enabling the test animals to survive longer than those consuming the control diet or a walnut oil enriched diet. This was not the expected result, and the effect was initially attributed to the protective effect of arachadonic acid produced from the linoleic acid in the peanut oil, but the walnut oil contained higher levels of linoleic acid compared to the peanut. Although the mode of action was not clear, it was theorized that the presence of Ω-9 fatty acid in the peanut oil (that is C 20:1) allowed for more direct antitumor action due to suppression of the production of inflammation inducing prostaglandins production (PG).

One type of cancer that is becoming increasingly prevalent and has been associated with dietary fat intake is colon cancer and recent studies suggest rates of colon cancer could be meaningfully correlated to dietary fat intake and the types of dietary fat consumed (Miller 1983). Although it is impossible in this space to fully describe the biochemistry of tumor metabolism, the mode of action of dietary fats, particularly saturated and PUFA from vegetable oils, appears to be related to their ability to increase the concentration of colonic luminal secondary bile acids, which in turn, induce cell proliferation and promote carcinogenesis in the colon (Bull et al. 1983). This is thought to be because the AA in the tissues is the preferred substrate for the production of prostaglandins. These compounds act locally on cell surface receptors to mediate their pathogenic effects. Most notably, via the activation of receptors which are upregulated in tumor therefore promoting growth and re-
pressing cell apoptosis (Gupta et al. 2000). Most dietary studies of this type are conducted using corn oil as the source of PUFA, but normal oleic peanuts would have similar amounts of linoleic acid. When diets fed to experimental animals were high in fat and linoleic acid without supplementation with MUFA or Ω-3 fatty acids, such as those obtained from fish oils, the incidences of colon tumors were over 95% after carcinogen treatment. When these other fatty acids were added to the diet, the numbers of tumors were halved in most cases (Reddy 1994). Given their typical fatty acid profile, this would indicate that consumption of high oleic peanuts could have some role in regards to lowering incidences of colon cancer.

Although the occurrence of renal cell carcinoma is sporadic in the United States, it has been observed to be increasing at 2% per year and currently accounts for about 3% of the adult cases of cancer (Moore et al. 2005). The diets of 406 people who had recently been diagnosed with renal cell cancer were reviewed and compared to control patients (Brock et al. 2009). The study concluded that high dietary fat levels of all types were correlated with increases in renal cancer risk when the individual was suffering from hypertension and/or was obese.

There is little information on the relationship between pancreatic cancer and individual dietary fatty acids, but some studies indicated that high consumption of linoleic acid has been associated with decrease risk. In a large study relating intakes of fat to exocrine pancreatic cancer, the diets of 525,473 people who were members of the American Association of Retired Persons (AARP) were surveyed (Thiébaut et al. 2009b). The study found that the majority of the saturated and monounsaturated fatty acids in the diet were supplied by meat and dairy products rather than plant sources. When broken down according to individual fatty acids, incidences of pancreatic cancer were more correlated to palmitic, palmitoleic, and stearic acids. No association was seen with C 18:1, even with the trans-isomers, or with linoleic acid. When these fats and fatty acids enter the duodenum as part of the chime, that is, the mass of partially digested material, cholecystokinin is released which in turn, stimulates the release of pancreatic enzymes and possibly leads to an enlargement of the pancreas known as hyperplasia. This could allow the organ to be more susceptible to carcinogens and inflammation, although their study found no direct effect (Woutersen et al. 1999).

Like other syndromes discussed in this chapter, it is difficult to assign totally positive or negative effects on cancer to one specific fatty acid. It may be more of a balancing act where certain fatty acids, while not able
to cause an effect on their own, are able to balance the effect of others in the diet. For instance, in a study on prostate cancer risk and linoleic acid, it was the balance of linoleic to $\alpha$-linolenic acid that was found to be more important than the presence of either fatty acid (Leitzmann et al. 2004). It was seen that as the ratio of these two increased, the risk was decreased.

2.4.2. Cardiovascular Disease (CVD)

In general, discussions on CVD in humans focuses on atherosclerosis, which is a complicated course of physiological events whose final complication of clinically relevant plaques causes thrombosis, vessel occlusion, and the start of target organ ischemia (Fuster et al. 1992). In this section, CVD is used as a term to include diseases of the heart and blood vessels to include coronary heart disease, coronary artery disease, dyslipidemia (elevated levels of lipid or cholesterol in the blood), and hypertension (high blood pressure). At this stage, there is no standard method to evaluate the relationship between occurrences of CVD and specific fatty acid intake. Fatty acids from the diet have been compared to the changes in the fatty acid content of serum cholesterol fatty acids or the serum fatty acids and with specific CVD endpoints or mortality (Degirolamo and Rudel 2010). The affiliation of reduced incidence of CVD and decreased dietary intake of saturated fats and increased levels of other fatty acids, in particular polyunsaturated fatty acids (PUFA), has been reviewed by Richard et al. (2009) and Micha and Mozaffarian (2010), among others. These studies were based on the activities of the $\Omega$-3 fatty acids which are prevalent in fish oils rather than the $\Omega$-6 PUFA, which are more prevalent in seed oils such as peanut. In the peanut, linoleic acid is the most prevalent $\Omega$-6 PUFA, whereas there are really none of the $\Omega$-3 type. Instead, the effect on CVD by nuts including peanuts is primarily attributed to their MUFA content (Fraser et al. 1992; Hu et al. 1998). Based on changes in blood lipid profiles, one study found the addition of peanuts to the diet would result in small changes in CVD risk mostly due to reduction in serum triacylglycerols as a result of the substitution of MUFA and PUFA from the peanuts for saturated fatty acids in the control diet (Alper et al. 2003). The most noted study on dietary fat and CVD risk is the Nurses’ Health Study which followed 86,016 women over the course of 14 years (Hu et al. 1997). This study was concerned with the effects of the types of fat (saturated, trans-unsaturated) on CVD in women. The diets of the test
subjects were followed by the use of questionnaires and it was found that 81% of the polyunsaturated fatty acids consumed by these women were in the form of linoleic acid. This study focused on the importance of the need to reduce saturated and trans-unsaturated fatty acids in the diet as their findings indicated these were most related to relative risk of CVD, which shows the advantage of adding peanuts to the diet. There have been other studies such as the Adventist Health Study in 1976, the Iowa Women’s Health Study in 1985, the Physician’s Health Study—a 12 year study presented in 1998—and the 4-year Cholesterol and Recurrent Events Study presented in 1992 whose data has been reviewed for the effect of nut consumption on CVD (Hu and Stampfer 1999). It was found that the consumption of peanuts had similar benefits as tree nuts on prevention of CVD due to the addition of fatty acids with positive effects and the reduction of those with negative to the human diet.

Many studies on fatty acids and cardiovascular health are concerned with blood cholesterol levels and the reduction that is found to occur with consumption of nuts (Sabaté and Wien 2010; López-Miranda et al. 2006; Hu et al. 2011; Woodside and Kromhout 2005). It is accepted that lowered levels of both blood cholesterol and levels of LDL cholesterol is advantageous to health. This results in less oxidation of the plasma cholesterol which can lead to buildup of arterial plaques (Witztum and Steinberg 1991). However, it is not just the reduction of saturated fatty acids that is effective, because when saturated fatty acids are replaced in the diet with equal amounts of calories from carbohydrate, the risk factors are not reduced as is seen with the PUFA in human trials (Micha and Mozaffarian 2010). As elsewhere in the section on linoleic acid, the main PUFA in peanuts has been associated with positive effects in reducing CVD risk factors. In older studies, the effect was very significant (Leren 1970; Turpeinen et al. 1979), but was less defined in more recent studies (Bartsch et al. 1989; Hu and Willett 2002; Berry 2001; Brouwer et al. 2004). The type of PUFA, that is n-3 found in fish and walnut oils compared to n-6 (especially linoleic) found in peanuts may also have a role in the reduction of risk factors by the addition of dietary PUFA (Erkkilä et al. 2008).

The mechanism of action of the positive effects of the addition of peanuts to the diet due to reduced risk of CVD may be a result of not just the consumption of specific fatty acid from the nuts, but in reality a combination of factors. Most nuts have levels of the amino acid arginine that are higher than other foods. Since this amino acid is converted to nitric oxide which is known to be a potent vasodilator that inhibits
the adhesion of platelets and aggregation, it is possibly a major factor in the effect (Cooke et al. 1993). Nuts also provide vitamins such as folate, and minerals such as potassium that play a part in circulatory health. Various flavonoids, especially when the skins are consumed with the nuts, are being investigated for their effects and are potentially important (Ros 2009; Ros et al. 2010).

More recently, the study of risk factors for CVD has developed into other areas besides plasma lipid levels. A review of the major studies involving diets containing nuts and their effects on blood lipid levels found that the changes were greater than the effects of the fatty acids alone (Kris-Etherton et al. 2008; Higgs 2003). It is possible that the substitution of peanuts for other sources of protein and fat in the diet results not only in a replacement of energy from saturated fatty acids and the addition of PUFA, but also the addition of other bioactives not found in animal based foods (Stephens et al. 2010). These parameters are covered in other chapters in this book.

2.4.3. Diabetes

Diabetes is a group of metabolic diseases characterized by abnormally high levels of sugar in the patient’s blood due to a failure of the pancreas to produce sufficient insulin or because the blood cells are not responding to the insulin present (Polonsky 2012). Epidemiological evidence has shown a strong correlation between dietary intake of saturated fatty acids and the development of metabolic syndrome and insulin resistance (Funaki 2009; Melanson et al., 2009). High saturated fat intake has been associated with a higher risk for impaired glucose tolerance, increased insulin levels, and higher fasting glucose levels (Steyn et al. 2004). Conversely, evidence from epidemiological, dietary intervention, and animal studies have indicated that diets high in mono- and polyunsaturated fatty acids may be beneficial in minimizing the risk of the development of type 2 diabetes (Parillo and Riccardi 2004). How blood levels of fatty acid affect type 2 diabetes has been examined in humans (Kusunoki et al. 2007). It was found that by using a measure known as homoeostasis model resistance index (HOMA-R) as an indicator of insulin sensitivity, relationships between the blood levels of various fatty acids could be studied. It was found that some PUFA as well as the saturated fatty acids in the blood were positively correlated with the HOMA-R, indicating the presence of these fatty acids aggravated insulin resistance. This resistance increased when the saturated
fatty acid chain length was decreased. MUFA and most PUFA showed little effect. The researchers’ recommendation was that dietary fats need to be well balanced in diabetic patients, with saturated fatty acids levels being decreased. This would indicate that peanuts would be a good choice for such patients.

Peanuts have been specifically cited for their beneficial role in managing type 2 diabetes as well as the potential to reduce the risk for developing diabetes. In addition to the low available carbohydrates present, peanuts can potentially improve glycemic control due to their high MUFA content (Kendall et al. 2010). Diets high in MUFA, such as the Mediterranean diet, have been shown in epidemiological studies and intervention trials to improve glycemic control potentially through displacing dietary carbohydrates and thus reducing glycemic load (Kendall et al. 2010; Schwingshackl et al. 2011).

Several large studies found positive correlations between nut consumption, including peanuts, and diabetes risk in human subjects. In the Nurses’ Health Study, consumption of peanut butter and nuts had an inverse association with the risk of developing diabetes (Sabaté and Ang 2009). Study participants who consumed nuts at least five times per week had a lower risk for developing diabetes than participants who rarely consumed nuts. A significant reduced risk for developing diabetes was also associated with frequent peanut butter consumption (Jiang et al. 2002). Dietary mono- and polyunsaturated fatty acids from nuts were associated with a lower diabetes risk in this study especially when used to replace dietary sources of saturated fatty acids (Risérus et al. 2009). There was a significant reduction in diabetes risk after adjusting for age in the Iowa Women’s Health Study between the most frequent consumers of nuts (greater than or equal to five servings per week) compared to the less frequent nut consumption (less than one serving per week) (Sabaté and Ang 2009). Additionally, there was an inverse relationship between dietary substitutions of polyunsaturated fatty acids for saturated fatty acids and the risk of developing diabetes (Parillo and Riccardi 2004; Risérus et al. 2009). The Shanghai Women’s Health Study also showed an inverse relationship in pre- and postmenopausal women between peanut consumption and risk of diabetes. This inverse relationship remained significant after controlling for participant BMI and waist-to-hip ratio (Sabaté and Ang 2009). However, the majority of epidemiological studies are limited to female participants and therefore the effect of nuts on the risk for diabetes in men is not currently established (Sabaté and Ang 2009).
The types of dietary fatty acids consumed may also play a role in the prevention of type 2 diabetes. Higher consumption of oils from vegetable sources has been associated with a lower risk for developing diabetes, especially for sources high in PUFA. Evidence for the effect of MUFA on diabetes risk is currently inconsistent. In one 3-month study, the substitution of MUFA for saturated fat in healthy participants improved insulin sensitivity significantly (Steyn et al. 2004). This result was consistent with other results from intervention studies in healthy individuals where monounsaturated fatty acids were used to replace saturated fatty acids in a controlled diet (Rivellese and Lilli 2003).

Conversely, in epidemiological studies, monounsaturated and saturated fatty acids are often associated with an increased risk for developing diabetes (Salmerón et al. 2001). However, due to the conflicting evidence for monounsaturated fatty acids in intervention studies, the increased risk found in epidemiological studies may be due to shared sources of monounsaturated and saturated fat often consumed in a Western-style diet, such as meat and milk products (Steyn et al. 2004). Diets with an increased polyunsaturated to saturated fatty acid ratio have been associated with a reduced risk for diabetes in both the Cancer Norfolk Study and the Health Professionals Follow-up Study. However, while this association was independent of a variety of lifestyle factors, it was not independent of central fat distribution and obesity (Risérus et al. 2009).

In animal studies, insulin sensitivity is impaired by diets high in saturated fatty acids while diets high in polyunsaturated fatty acids improve insulin sensitivity. Diets high in monounsaturated fatty acids also improve insulin sensitivity in animals; however, they tend to have less of an effect than diets high in polyunsaturated fatty acid (McAuley and Mann 2006). By feeding rats that had been treated to induce diabetes with peanuts at a level equal to the amount consumed by the women in the Nurses’ Health Study, researchers were able to observe increases in HDL-cholesterol and decreased the atherogenic index (AI) which is the ratio of total plasma cholesterol to HDL-cholesterol (Emekli-Alturfan et al. 2008). The LDL-cholesterol remained unchanged as did other lipid parameters leading the authors to conclude that feeding peanuts resulted in cardioprotective effects in the diabetic animals. Although other components may have been at work in the animals, the main effect was attributed to oleic acid. Dietary modification recommendations to prevent insulin resistance generally focus on the reduction of saturated fat intake and replacement with mono- and polyunsaturated fatty
acids (McAuley and Mann 2006). Additionally, human intervention trials have shown that the substitution of dietary saturated fat with unsaturated fat in patients with type 2 diabetes improves glucose metabolism (Wang et al. 2003).

In the PREDIMED-Reus intervention trial, consuming a Mediterranean style diet was more effective at reducing the incidence of developing type 2 diabetes over the low fat control diet (Salas-Salvadó et al. 2011; Schwingshackl et al. 2011). This intervention trial included two experimental Mediterranean diets, one supplemented with olive oil (1L per week) and the other supplemented with nuts (30 g per day), and a low fat control diet. The risk of diabetes was reduced by 51% in the group receiving the Mediterranean diet supplemented with olive oil and by 52% in the group receiving the Mediterranean diet supplemented with nuts (Salas-Salvadó et al. 2011).

While epidemiological and intervention studies have shown the potential benefits of dietary unsaturated fatty acids, there is limited evidence that individual fatty acids have an effect on diabetes risk. When compared to the proportion of palmitic acid in cholesterol esters, a greater proportion of linoleic acid was inversely associated with diabetes incidence (Risérus et al. 2009). However, more extensive research is needed to determine if individual fatty acids have an effect on diabetes risk.

2.4.4. Obesity and Weight Management

The American Dietetic Association (ADA) recommends a diet high in fruits, vegetables, whole grains, nuts, legumes, fish, and lean protein as a way to maintain a healthy body weight and prevent chronic diseases (Kris-Etherton and Innis 2007). Nutritional metabolic and epidemiological studies have initiated focus on the potential role of tree nuts and peanuts in weight management. While nuts and peanuts are recommended as part of a healthy diet by the ADA, consumer research has indicated that nuts and peanut are typically avoided during weight loss due to concern over their high energy density (Natoli and McCoy 2007). However, a majority of epidemiological studies with a focus on body weight have found an inverse relationship between nut consumption, which includes tree nut and peanuts, and body mass index (BMI) (Iyer et al. 2006; Natoli and McCoy 2007; Martinez-Gonzalez and Bes-Rastrollo 2010).

In the California Seventh Day Adventist Health Study, higher con-
consumption of nuts had a statistically significant negative association with obesity (Iyer et al. 2006; Natoli and McCoy 2007). Study participants who ate nuts most frequently, that is greater than or equal to five servings per week, were less obese than those who infrequently ate nuts, less than one serving per week (Sabaté 2003; Mattes et al. 2008). Similarly, higher nut consumption rates in the Iowa Women’s Health Study, greater than five servings per week, were associated with a lower waist-to-hip ratio and a lower BMI compared to those who consumed less than one serving of nuts per month (Natoli and McCoy 2007).

Nut consumers also had a lower BMI than non-nut consumers for both young (6–20 years old) and adult (greater than 21 years old) participants included in the 1994–1996 Continuing Survey of Food Intakes by Individuals (Sabaté 2003). In the Nurses’ Health Study, women who consumed nuts frequently, greater or equal to five servings per week, were leaner than those who almost never consumed nuts (Natoli and McCoy 2007). Additionally, there was a negative association between frequent nut consumption and BMI (Mattes et al. 2008).

The PREDIMED study, which focused on the benefits associated with consuming a Mediterranean style diet, found an inverse relationship between nut consumption and BMI and waist circumference. Independent of other lifestyle factors, prediction models showed a decrease in BMI of 0.78 kg/m² and a decrease in waist circumference of 2.1 cm associated with every 30 g serving of nuts per day (Martinez-González and Bes-Rastrollo 2011).

Data from the The SUN cohort showed an inverse association between baseline consumption of nuts and the risk of gaining weight or average weight gain, greater than or equal to 5 kg, after a follow up period of 28 months. Additionally, the risk of developing obesity for women who were not obese at baseline was lower for those who consumed greater than or equal to two servings of nuts per week compared to those who rarely or never consumed nuts (Martinez-González and Bes-Rastrollo 2011). Conversely, the Physicians Health Study found no difference in the BMI of those who consumed nuts rarely or never and those who consumed nuts greater than or equal to two servings per week (Natoli and McCoy 2007). However, unlike the previous studies, the Physician’s Health Study had a much lower nut consumption rate for habitual nut consumers.

Evidence from epidemiological studies generally shows a reduced BMI associated with frequent nut consumption, typically greater than or equal to five servings per week. From this association, researchers
have begun to investigate the addition of tree nuts and peanuts to diets in order to determine their effect on weight loss or gain. Results from these intervention and feeding studies have produced mixed results, which can mostly be attributed to their diversity in methodology. However, adding nuts, including tree nuts and peanuts, habitually to the diet has not been shown to cause significant weight gain, especially in isocaloric and isoenergetic replacement studies (Rajaram and Sabaté 2006).

In a study conducted by Alper and Mattes (2002), 15 healthy adults without symptoms or family history of CVD had peanuts provided for an initial 8-week free feeding period followed by either peanuts in an addition diet for 3 weeks or substitution diet for 8 weeks. In the addition diet, peanuts were added to a pre-established diet and in the substitution diet peanuts were used to replace an equal amount of fat resulting in an isocaloric diet. In the free feeding portion of the study, participants gained an average of 1 kg over the 8-week period, which was below the 3.6 kg predicted based on the additional calories supplied by the peanuts provided. Similarly, during the addition diet, weight gain was again lower than predicted at 0.6 kg compared to the expected 1.4 kg. No weight gain was observed in the substitution diet. (Alper and Mattes 2002; Rajaram and Sabaté 2006).

Less than expected weight gain was also observed in a study involving peanuts as part of the diet conducted by Coelho et al. (2006). Study participants, which included 24 normal weight men with a BMI less than 25 kg/m², and 24 overweight men with a BMI between 25–30 kg/m², received peanut oil accounting for 30% of their resting energy expenditure (REE) in the form of a milk shake daily for 8 weeks. No additional dietary advice was provided to participants. Weight gain for normal and overweight participants was less than predicted, with greater weight gain occurring in the latter group. Food records showed that participants in the normal weight group compensated for 66% of the additional peanut oil caloric load in their daily dietary habits, while participants in the overweight group compensated much less for the additional caloric load, approximately 4% (Coelho et al. 2006).

The inclusion of nuts in weight loss studies was found not only to aid weight loss, but also to help prevent weight gain (Rajaram and Sabaté 2006; Natoli and McCoy 2007). In an 18-month energy-controlled weight loss intervention study, in which half of the participants were given a low fat weight loss diet (20% kcal from fat) and the other half were given a moderate fat diet (35% kcal from fat) including nuts, peanut butter, and olive oil, the moderate fat diet participants lost more
weight and experienced a greater decrease in waist circumference than the low fat diet participants. Additionally, the moderate fat participants weighed significantly less than the low fat participants 2.5 years after the 18-month intervention study (Rajaram and Sabaté 2006). In another energy-controlled weight loss study, the inclusion of peanuts resulted in a 62% increase in weight loss compared to the control diet group (Natoli and McCoy 2006).

Evidence from studies focusing on the effect of nut consumption on body weight have generally shown that adding nuts to a pre-existing diet can lead to weight gain, although the weight gain is less than predicted. In weight loss trials and intervention studies where nuts are substituted into the diet, weight gain has not been observed and there is evidence that nut consumption may be beneficial to weight loss. A proposed mechanism for the beneficial effects of peanuts in weight management suggests that the type of fatty acid consumed as well as the rate of fatty acid oxidation is responsible (Moussavi et al. 2008). Another factor contributing to less than predicted weight gain from whole peanut consumption is poor bioaccessibility as demonstrated by fecal fat measurements during controlled dietary trials (Mensink et al. 2003). A similar phenomenon has been seen in tree nuts and is attributed to the natural resistance of seed microstructure to digestion and is not observed for peanut oil alone (Moussavi et al. 2008).

2.5. CONCLUSIONS

This chapter presents a review of the effects of dietary fatty acids common to peanuts on human health. The peanut is a complicated functional food making it difficult to assign a specific effect to a single component such as one fatty acid. When doing feeding studies with peanuts there are many things that can contribute to the outcomes such as the presence of minor nutritional components and the proportion of the fatty acid themselves. This last item is especially important in peanuts since the ratio of oleic to linoleic acid varies with the variety. Designing a study to use only one type of fatty acid in order to “prove” effects of that fatty acid is not feasible nor would it be applicable to any actual life experiences. There are many metabolic interactions taking place during the course of digestion, uptake into the intestine, the transport to the liver and then to other parts of the body for metabolism, and utilization that singling out one specific fatty acid as always “good” or “healthy” is impossible. It can be stated with some certainty that different classes
This chapter focuses on risk analysis using peanuts as an example and the different risk assessment methods available to food companies. Peanuts are one of the most common allergenic foods. Peanut allergies are potentially life-threatening and the most common cause of food-allergy fatalities. The only strategy to avoid an allergic reaction is avoidance of the food. The presence of peanut in mislabeled or unlabeled packaged products has led to allergic reactions in consumers relying on clear and accurate ingredient statements. Different risk assessment methods are reviewed, including the NOAEL-based safety assessment, the benchmark dose approach, and quantitative risk assessment. Quantitative risk assessment can provide risk assessors with realistic risk profiles of allergen cross-contamination issues and help with management of product release, product recalls, and advisory labeling. Cross-contamination of peanuts in ice cream is used as a model example for the application of quantitative risk assessment methods.

8.1. INTRODUCTION

Risk analysis is a three part, interactive process that consists of a
scientific risk assessment, a risk management strategy, and an exchange of information through risk communication (Figure 8.1) (FAO/WHO 2008). All manner of risks are evaluated using this same process. Risk analysis for food allergens does not, in concept, differ from other risks associated with foods (Madsen et al. 2009).

Recognition of the importance of food allergies as a public health and food safety issue has increased considerably over the past 20 years. The prevalence of food allergies is apparently increasing in part for reasons that are not fully understood (Sicherer and Sampson 2010). The occasional severity of food allergic reactions as evidenced by the number of emergency room visits (Clark et al. 2011) and fatalities (Bock et al. 2001) has served to heighten awareness even further. As a consequence, public health authorities and the food industry have developed and implemented strategies to protect the food allergic consumer.

For public health authorities, the primary strategies have been to develop lists of priority allergenic foods and to enact regulations to assure that any ingredients derived from these foods are declared on the labels of packaged foods (Gendel 2012). Two countries have attempted to establish action levels for undeclared allergens. Switzerland has defined an action limit of 1,000 ppm for allergens. This limit states that if unavoidable, contamination above 1,000 ppm (0.1%) must be declared as an ingredient, but contamination below 1,000 ppm may be declared if desired (Kerbach et al. 2009). The use of 10 ppm protein from the allergenic source was based primarily on the current analytical capabilities of enzyme-linked immunosorbent assay (ELISA) used in Japan rather
than clinical data of allergic individuals that underwent low dose food challenges. Levels of 1,000 ppm may provide enough protein (low mg doses) to cause reactions at moderate consumption levels in multiple foods (Taylor et al. 2004). Japan has taken a stricter approach and has limited undeclared allergens to 10 ppm (0.001%) in foods (Kerbach et al. 2009). At the present time, Australia, the European Union, and the United States have not adopted legislation or regulations regarding regulatory thresholds for food allergens and there is no regulatory guidance for trace levels of allergens due to cross-contact. Allergen labeling laws (i.e., FALCPA in the United States, EU directive 2003/89/EC, Australian Food Standards 1.2.3, and 1.2.4) were implemented to address the issue of improved labeling of allergens in food when used as direct ingredients or processing aids in packaged food products (EU 2003a; FDA 2006; FSANZ 2002). A company is required to declare when an ingredient is derived from the major allergenic foods of milk, eggs, fish, crustacean shellfish, peanuts, soybeans, tree nuts, and wheat plus a few others depending on the country. Industry compliance allows consumers to identify all sources of allergens in processed food, even if the allergenic proteins from these ingredients are present at extremely low and possibly harmless levels. Thus, these labeling laws essentially create a zero tolerance for unlabeled food allergens.

For the food industry, the awareness of food allergies has led to the development and implementation of allergen control plans for manufacturing facilities, improved labeling approaches as mandated by government authorities, and a proliferation of voluntary advisory statements (e.g., “may contain” and many others) on packages. However, the no-tolerance policy for allergens used by public health authorities creates practical problems for the food industry and also the food allergic consumer because it is impossible to prove that absolutely no allergen residues are present. From an industrial cost-effectiveness perspective, the same manufacturing facility has to be used to process multiple products. Shared production facilities and manufacturing equipment for multiple products creates an opportunity for trace residues of an allergenic food to come in contact with another food. Careful production schedules and meticulous cleaning are required when products of similar nature (i.e., ice creams or chocolates) with differing allergen profiles are produced on shared equipment (Taylor et al. 2002). When companies are not able to guarantee complete avoidance of cross-contact, an advisory “may contain” statement might be placed on the product label. While advisory labeling for allergens is voluntarily used by the food
industry and not directly regulated or addressed by any of the current labeling laws in various countries, its use has increased dramatically over the past decade.

For the individual who receives a diagnosis of food allergy, the physician will typically recommend complete avoidance of the allergenic food(s). Currently, avoidance of the allergenic food is the only treatment option (Taylor et al. 1986). The onus for the implementation of a safe and effective avoidance diet falls upon the consumer causing stress and a diminution of their quality of life (Dunn-Galvin et al. 2008). The overall strategy of complete avoidance assumes that no safe level or threshold exists for allergenic foods. Food laws and regulations imposed by public health authorities have furthered this no-threshold approach by mandating source labeling of food ingredients even when the risk of an allergic reaction to food is virtually nonexistent. With no guidance on food allergen thresholds from public health authorities, the food industry has placed voluntary advisory labeling on an ever-increasing number of packaged food products in an effort to alert food-allergic consumers to the possible presence of residues of the allergenic food with no consideration of the magnitude of any risk. While this situation aims to assure the maximum extent possible the safety and well-being of the food-allergic consumer, it serves to seriously limit food choices. Consequently, evidence exists that some food-allergic consumers are ignoring precautionary allergen statements on labels (Hefle et al. 2007; Sheth et al. 2012), the exact opposite of the intent. Food-allergic individuals must try to interpret a variety of advisory labels causing confusion. Additionally, clinicians’ advice differs on whether avoidance of advisory statements is necessary for allergic consumers (Hu et al. 2008). Because of the proliferation of different forms of the wording of these voluntary advisory statements, some food-allergic consumers have the false impression that some foods with specific advisory statements (e.g., manufactured in a shared facility) are safer than foods with other statements (e.g., “may contain”) (Hefle et al. 2007). Despite the variety of statements that are used, all such statements are meant by the food industry to alert food-allergic consumers to foods possibly containing allergen residues so that they may avoid those foods. However, analytical surveys have documented, for products with advisory labels for peanut, that only a small percentage contain detectable peanut residues and that some products without advisory labels possess similar levels (Hefle et al. 2007; Pele et al. 2007; Pieretti et al. 2009).

With the use of a better approach to risk assessment for allergenic
foods and their use by industry and public health authorities to guide labeling approaches, the safety of foods for food-allergic consumers could be maintained and a greater variety of packaged foods would be available. The improved approach is predicated on the existence of safe threshold doses for allergenic foods. Reported clinical observations of confirmed peanut-allergic individuals show that doses of peanut do exist below the exposures at which they will have a reaction (Taylor et al. 2002). The past decade has witnessed an influx of allergen threshold data that has allowed risk assessors to quantitatively adapt the traditional risk analysis approach for use with food allergens. Threshold-based risk approaches are viewed favorably by public health authorities (Gendel et al. 2008) and have been endorsed in consensus conferences (Madsen et al. 2009). Similar approaches have long been used for the management of chemical and microbial hazards in food (FDA 1994, 1995, 2000; Rasekh et al. 2005). A regulatory threshold for peanut and other allergens would reduce the proliferation of advisory labeling. Additionally, regulation would help allergic consumers separate the truly risky products from the products that are safe to consume.

Thresholds can help the food industry in multiple situations including labeling decisions with new products and quality control assessment during daily manufacture. This chapter focuses on risk analysis using peanuts as an example and the different risk assessment methods available to food companies. Background methodologies are explored and an allergen cross-contamination scenario is investigated after a mistake in manufacturing is discovered.

While the risk of allergenic foods has been widely recognized, the analysis of the risks (including assessment, management, and communication) has mostly been rudimentary and based upon identification and avoidance. This chapter provides insights into an emerging, quantitative approach to risk assessment for food allergens that can inform risk management decisions in a manner that allows public health authorities and the food industry to be focused on the most serious risks.

8.2. RISK ASSESSMENT

Risk Assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards. The process consisting of four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization (FAO/WHO 2008).
8.2.1. Hazard Identification

Hazard identification is the recognition of a particular agent in foods with known or potential associated health effects (FAO/WHO 2008). In food allergies, the hazard is a protein from a specific food that can cause sensitization and allergic reactions on subsequent exposures to the protein. Sensitization can occur to multiple proteins within a single food and any of them can be the cause of an allergic reaction. Clinically, food allergies have been recognized for a long period of time and the first reports of specific oral sensitization to egg appeared 100 years ago (Schloss 1912; Schofield 1908). Food allergies affect an estimated 5–10% of children and 3–4% of adults in westernized countries (Osborne et al. 2011; Sicherer and Sampson 2010). Peanut is one of the most prevalent and severe food allergies and is a member of the “Big 8” food allergens along with milk, egg, soybean, wheat, tree nuts, fish, and crustacean shellfish (FAO 1995). These eight foods are staples in diets around the world and are responsible for close to 90% of food allergic reactions. Peanut allergy affects an approximated 0.6–1% of the population in the United States (Sicherer and Sampson 2010). Exposure to peanuts is not a risk to the majority of the population, but the peanut-allergic population must take their avoidance diets seriously as the risk of peanut consumption is potentially life-threatening. Therefore it is essential to conduct a proper and complete risk assessment of peanut as a food allergen.

8.2.2. Hazard Characterization

Hazard characterization is the qualitative and/or quantitative evaluation of the nature of the adverse effects. If data are obtainable, a dose-distribution assessment should be performed (FAO/WHO 2008).

Peanut allergies are potentially life-threatening. In the past 25 years, three studies found an average of six fatal anaphylactic reactions per year in the United States (Bock et al. 2001; Sampson et al. 1992; Yunginger et al., 1988). Restaurants and educational settings were, and still are, the most common locations of fatal allergic reactions and peanut is responsible for over 50% of food allergy related fatalities (Keet and Wood 2007). Food-allergic individuals, including peanut-allergic individuals, can experience a range of symptoms on exposure to the offending food. Not all allergic reactions are life-threatening, and some food-allergic individuals will never experience a severe reaction. Food
allergy symptoms range from very mild, such as itching and flush, to a severe drop in blood pressure and bronchospasm. The severity of an allergic reaction also depends upon the dose of exposure. Additionally, the minimal provoking dose, or threshold, varies widely across the entire population of individuals allergic to any specific food (Crevel et al. 2010). It has been reported that the Lowest Observed Adverse Effect Level (LOAEL), the lowest dose that produces an allergic effect, for peanut-allergic individuals in clinical trials spans five orders of magnitude—0.4 mg up to 30,000 mg of whole peanut (Taylor et al. 2009, 2010). Small particulates of less than 1 mg peanut can cause a reaction and are displayed in Figure 8.2 with the size of individually weighed pieces of peanut labeled from roughly half a peanut to less than 0.2 mg.

In clinical challenges, there is no significant correlation between the severity of a reaction and individual LOAELs (Taylor et al. 2010). While physicians recommend a complete avoidance of peanuts, every allergic individual is able to tolerate a dose of peanut below their personal LOAEL. Clinicians have observed these safe doses, or No Observed Adverse Effect Level (NOAEL), during low dose double-blind, placebo-controlled food challenges (DBPCFC) for peanut. Severe reactions did not occur during these low dose (low mg) trials. The DBPCFC trials can be used to derive the NOAEL and LOAEL for each allergic individual. Published DBPCFC clinical literature now exists for peanut and other major food allergens. The Food Allergen Research and Resource Program (FARRP) of the University of Nebraska has published reports on the threshold dose for peanut based on 450 peanut-allergic individuals DBPCFCs with objective symptoms (Taylor et al. 2009, 2010). Criteria for inclusion in the dataset are described in Table 8.1.

*FIGURE 8.2.* Individually weighed particulate peanut samples up to half of a peanut. The lowest MED found in DBPCFC trials is labeled. (Photo credit: Barbara Ballmer-Weber.)
The NOAELs and LOAELs from each individual were used as part of an Interval-Censoring Survival Analysis (ICSA) approach to generate a population threshold for peanut (Collett 1993). The ICSA method is appropriate as the exact dose that provokes a reaction in an individual is not known, but it is known to fall into a particular interval dependent on the dosing scheme used in the challenge (Taylor et al. 2009).

As shown in Figure 8.3, left-censoring occurs when an individual experiences an objective reaction at the first dose in a challenge trial. If left-censored, an individual’s NOAEL was set to zero (left blank in the ICSA program) with the LOAEL set as that first dose. An individual is interval-censored when they experience a reaction in the middle of a dosing scheme and that individual threshold dose is bounded by the NOAEL and LOAEL. Right-censoring occurs if an individual does not experience an objective reaction after the largest challenge dose. In such cases, the NOAEL was set to that largest challenge dose. An individual was considered as right-censored if they experienced a subjective reaction to the largest dose, or if a challenge was stopped early due to persistent subjective symptoms. The LOAEL was set to infinity (left blank in the ICSA program) for right-censored subjects as their exact threshold is unknown. The LIFEREG procedure (SAS v9.2) was used to fit cumulative probability function models to the interval-censored data. Multiple distributions were evaluated and the Log-Normal and Log-Logistic models were determined to best fit the peanut dataset at the lower end. The models were used as shown in Figure 8.4 to estimate the ED\(_{10}\), the dose predicted to provoke reactions in 10% of the peanut-allergic population (Taylor et al., 2009, 2010).

The Log-Normal ED\(_{10}\) and ED\(_{05}\) were 12.3 mg and 5.2 mg whole peanut, respectively (Table 8.2) (Taylor et al. 2010), while the Log-Logistic ED\(_{10}\) and ED\(_{05}\) were 12.6 mg and 4.5 mg whole peanut, re-

<table>
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<tbody>
<tr>
<td>Published study</td>
</tr>
<tr>
<td>Supplemented with unpublished results</td>
</tr>
<tr>
<td>Peanut allergic by history or other factors</td>
</tr>
<tr>
<td>DBPCFC for peanut</td>
</tr>
<tr>
<td>Open challenge allowed if patient is under 3 years old</td>
</tr>
<tr>
<td>Description of NOAEL and/or LOAEL (if dosing regimen provided, then can determine NOAEL from LOAEL)</td>
</tr>
<tr>
<td>Data on individual patients</td>
</tr>
<tr>
<td>Objective symptoms at doses</td>
</tr>
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</table>
respectively. By dividing the Log-Normal ED values by the Log-Logistic ED, a difference of ±2%–15% can be observed at the ED\(_{0.05}\), ED\(_{0.10}\), and ED\(_{0.50}\). If the same process is applied at the ED\(_{0.01}\), a +100% difference is observed between the Log-Normal and Log-Logistic curves. The mathematical model chosen begins to heavily influence the predicted ED value when less experimental data is available and especially from subjects with low individual thresholds.

The U.S. Food and Drug Administration (FDA) and an international

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**FIGURE 8.3.** Diagram of the Interval Censoring Survival Analysis and how it assigns censoring values. Sample dosing scheme progresses from 10 mg – 50 mg – 150 mg – 500 mg.

**FIGURE 8.4.** Log-Normal fit for the peanut population threshold from 450 DBPCFCs with objective symptoms in peanut allergic individuals (Taylor et al. 2010).
workshop of risk assessors have both indicated that statistically-based risk assessment (including statistical techniques such as dose-distribution modeling) provide the most promising approach for threshold establishment and quantitative food allergen risk assessment (Gendel et al. 2008; Madsen et al. 2009). Efforts by previous groups have shown that sufficient data exist for peanuts to set a population threshold (Taylor et al., 2009, 2010, 2014, 2013 [In prep]). The threshold curves for peanuts are stable as demonstrated when Taylor et al. (2013 [In prep]) added 300 peanut-allergic individuals to the threshold distribution reported by Taylor et al. (2010). No significant differences were found in the ED$_{01}$, ED$_{05}$, and ED$_{10}$ values of distributions based on 750 peanut-allergic individuals versus distributions based on 450 individuals. The established threshold curve could be used in quantitative risk assessment models to set regulatory and food industry action levels for peanut.

8.2.3. Exposure Assessment

Exposure assessment is the qualitative and/or quantitative evaluation of the likely intake via food and other sources if relevant (FAO/WHO 2008).

For a quantitative risk assessment, two main variables shape the exposure patterns. First, the probability of an allergic consumer purchasing a particular product will determine if there is any exposure. Second, the amount eaten by the individual will influence the outcome of the risk assessment. There is no consumption database available solely for food-allergic consumers. Risk assessors must assume that allergic and nonallergic individuals consume a product at the same rate and their reasons for nonconsumption are the same. It is well known that allergic consumers are very brand loyal and shared experiences can lead to avoidance of perceived “risky” products and product cat-

**TABLE 8.2. ED doses for Whole Peanut as Assessed By Three Statistical Probability Distribution Functions (Taylor et al., 2010).**

<table>
<thead>
<tr>
<th>Distribution</th>
<th>All Values Reported in mg Whole Peanut</th>
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<tbody>
<tr>
<td></td>
<td>ED$_1$ 95% CI</td>
</tr>
<tr>
<td>Log-Normal</td>
<td>1 0.6, 1.6</td>
</tr>
<tr>
<td>Log-Logistic</td>
<td>0.5 0.3, 0.8</td>
</tr>
<tr>
<td>Weibull</td>
<td>0.04 0.02, 0.1</td>
</tr>
</tbody>
</table>
during infancy in Israel as compared to avoidance of peanut in the diet for the first 2 years of life in the United Kingdom (Du Toit et al. 2008).

13.3. CURRENT MANAGEMENT OF A FOOD ALLERGY

There is currently no effective long-term treatment that changes the natural history of a food allergy. Management is only supportive, comprising avoidance of the food concerned, early recognition of symptoms of an allergic reaction and initiation of appropriate emergency treatment of allergic reactions, particularly anaphylaxis. This approach is fraught with difficulties, and the burden of living with a food allergy and its management is substantial. Children with a peanut allergy are reported by their parents to have significantly more disruption of their daily activities compared to children with rheumatological conditions (Primeau et al. 2000). Avoidance of food allergens is difficult to achieve, particularly with commercially prepared foods. Accidental exposures to food allergens occur in 58% of patients within 5 years and 75% within 10 years (Vander Leek et al. 2000). Furthermore, the majority of subjects who died from food anaphylaxis knew they were allergic to the food that led to their fatal anaphylaxis, and about 40–100% of deaths involved ingestion of foods catered or prepared away from home (Bock et al. 2007; Pumphrey and Gowland 2007; Liew et al. 2009).

Adrenaline is the first line of therapy for anaphylaxis. Several self-injectable devices are available which can be used to administer adrenaline for the emergency treatment of anaphylaxis following accidental exposure to food allergens. However, the use of these devices is not intuitive and requires specific training (Mehr et al. 2007). Moreover, most patients who were prescribed an EpiPen failed to use it at the time of a severe allergic reaction—only 71% of patients had their EpiPen with them, 10% of these had expired, and only 32% were able to demonstrate its correct use (Sicherer et al. 2000). Half of the food anaphylaxis deaths in the United Kingdom series involved failure to carry or use an adrenaline auto-injector correctly, highlighting the importance of patient education in the management of allergic reactions (Pumphrey and Gowland 2007). However, adrenaline may not always be sufficient to prevent fatality, since early and repeated administration of adrenaline failed to prevent death in 12–14% of anaphylaxis fatalities (Bock et al. 2007; Pumphrey and Gowland 2007). These signif-
icant limitations of current food allergy management highlight the need for alternative treatment options that can induce long-term tolerance.

13.3.1. Allergen-Specific Immunotherapy as a Treatment for Allergic Disease

Allergen-specific immunotherapy can be used for the long-term treatment of specific allergies. It is highly effective for the induction of tolerance to insect venom in patients with insect venom anaphylaxis. In addition, allergen-specific immunotherapy has been applied in asthma and allergic rhinitis to induce tolerance against specific allergen triggers that exacerbate disease. Subcutaneous immunotherapy (SCIT) has been shown to modulate the immune response to allergen (Norman 2004; Schmidt-Weber and Blaser 2004) by inducing allergen-specific CD4+CD25+ T<sub>reg</sub> cells that restore the balance of allergen-specific Th1/Th2 effector cells, leading to reduced Th2 cytokine expression (IL-4, IL-5) and in most studies increased Th1 cytokine expression (IFN-γ). These changes in Th1/Th2 cytokine responses in turn lead to reduced allergen-specific IgE and increased allergen-specific IgG4 (Norman 2004; Schmidt-Weber and Blaser 2004). Other immunological effects of SCIT include increased apoptosis of allergen-specific Th2 cells, reduced tissue mast cell numbers, and reduced serum levels of TNF-α and IL-1β (Norman 2004). Sublingual immunotherapy (SLIT) has also been shown to be effective in reducing clinical symptoms in respiratory allergy (asthma and allergic rhinitis), however immunological effects are less well characterised. Increased allergen-specific IgG4 and reduced allergen-specific IgE have been reported in some but not all studies (Norman 2004). Oral immunotherapy (OIT) has not been consistently effective when used for the treatment of respiratory allergy and has been abandoned for the treatment of these conditions. More recently, studies suggest an exciting potential for OIT as a treatment for food allergy, and there is renewed interest in the application of OIT in this setting.

13.3.2. Allergen Specific Immunotherapy for a Food Allergy

13.3.2.1. Subcutaneous Immunotherapy

Early attempts at using SCIT for food allergy showed some beneficial effects, however high rates of severe adverse reactions were ob-
served. The first placebo-controlled trial of SCIT for peanut allergy was attempted in 1992 and demonstrated decreased symptoms during food challenge in treated patients, but the study was prematurely terminated following a fatal anaphylaxis reaction (Oppenheimer et al. 1992). A subsequent double-blind placebo-controlled trial (DBPCT) evaluating aqueous peanut extract injection immunotherapy in peanut-allergic patients was conducted in 1997 (Nelson et al. 1997). The study was effective in inducing desensitization, and increasing the threshold dose required to induce a reaction from 178 mg (half a peanut) to 2,805 mg (9 peanuts) in subjects who were on maintenance therapy. However, the rate of serious systemic reactions during the maintenance phase was frequent (39%) with an average of 12.6 epinephrine injections per subject (Nelson et al. 1997). The high rate of unpredictable severe adverse reactions observed with allergen-specific subcutaneous immunotherapy has led to exploration of other alternative strategies for the long-term treatment of food allergy (Stahl and Rans 2011). Studies investigating engineered allergen proteins, that have reduced binding to allergen-specific IgE but retain relevant T cell epitopes needed for effective immunotherapy, for use in subcutaneous immunotherapy are ongoing (Nowak-Wegrzyn and Sampson 2011; Stahl and Rans 2011; Wang and Sampson 2011).

13.3.2.2. Oral Immunotherapy

Oral immunotherapy (OIT) protocols involve the daily oral administration of allergen in gradually increasing doses during the build-up phase to reach a maintenance dose that is continued for a variable period of time (usually 6 months to 2 years) (Figure 13.1) (Bauer et al. 1999; Nucera et al. 2000; Patriarca et al. 2003; Meglio et al. 2004; Buchanan et al. 2007; Morisset et al. 2007; Staden et al. 2007, 2008; Alonso et al. 2008; Longo et al. 2008; Meglio et al. 2008; Skripak et al. 2008; Zapatero et al. 2008; Clark et al. 2009; Jones et al. 2009; Narisetty et al. 2009; Blumchen et al. 2010; Itoh et al. 2010; Kurihara 2010). Study outcomes have mostly focused on achievement of desensitization (the ability to tolerate an allergen while on immunotherapy), with only a few studies evaluating the acquisition of tolerance (the long term ability to tolerate an allergen after immunotherapy is discontinued) (Buchanan et al. 2007; Staden et al. 2007) (Tang 2009). Studies of OIT for the treatment of food allergy have consistently reported successful desensitization in the majority of subjects (Bauer et al. 1999; Patriarca
et al. 2003; Meglio et al. 2004; Buchanan et al. 2007; Clark et al. 2009; Jones et al. 2009) and in most studies OIT has also been shown to induce modulation of allergen specific immune responses (Nucera et al. 2000; Patriarca et al. 2003; Meglio et al. 2008; Varshney et al. 2011). However, effective induction of long-term tolerance with OIT has yet to be demonstrated.

13.3.2.3. Oral Immunotherapy and Induction of Desensitization

The majority of early OIT studies focused on hen’s egg and cow’s milk allergies (Bauer et al. 1999; Nucera et al. 2000; Meglio et al. 2004; Alonso et al. 2008; Pajno et al. 2010; Patriarca et al. 2003). The first randomised double-blind, placebo-controlled trial (RDBPCT) of OIT was performed by Skripak et al. (2008) evaluating cow’s milk OIT. At follow-up milk challenges conducted after 13–75 weeks, 6 of 15 children tolerated 16 g without symptoms and seven children reacted at 3–16 g. Milk-specific IgE levels and SPT size were significantly decreased and milk-specific IgG4 levels increased. Adverse reactions were common and several systemic reactions occurred at previously tolerated doses, often triggered by febrile illness or exercise (Narisety et al. 2009).
Peanut OIT has now become a major focus in food allergy treatment, since severe reactions can occur following accidental peanut ingestions or inhalation. The first clinical trial of peanut OIT was conducted by Jones and colleagues (2009), in which 39 peanut-allergic children (aged 1–16 years) were enrolled in an open-label uncontrolled study involving three phases of therapy: an initial one day modified rush escalation phase (dose increased from 0.1mg to 50 mg), a build-up phase of daily doses increasing by 25 mg every 2 weeks until 300 mg was reached, and a maintenance phase for 4–22 months (dose of 300 mg peanut protein). At the end of the OIT protocol, an open oral peanut challenge was performed. During the initial day escalation, 10 (26%) subjects tolerated the highest dose of 50 mg peanut protein, 15 (38%) tolerated 25 mg, six (15%) tolerated 12 mg, five (13%) tolerated 6 mg, one (3%) tolerated 3 mg, and two (5%) tolerated 1.5 mg. Thirty-six patients (92%) experienced some symptoms during the initial escalation day with four (10%) requiring epinephrine. Twenty nine subjects completed all three phases of the study and peanut challenge, and 27 of 29 children (93%) tolerated 3.9 g of peanut during food challenge (were desensitized), with 18 of them completing the challenge without symptoms (complete desensitization). By 6 months, titrated peanut skin prick test responses and basophil activation to peanut antigen were significantly decreased. By 12–18 months, peanut-specific IgE decreased and peanut-specific IgG4 increased. Production of IL-10, IL-5, IFN-γ, and TNF-α by peanut-stimulated peripheral mononuclear cells (PBMC) increased over a period of 6–12 months. Peanut-specific FoxP3 T_{reg} cells increased until 12 months and decreased thereafter. In addition, T-cell microarray gene expression analysis showed down-regulation of genes involved in the apoptotic pathways, and production of serum inhibitory factors which blocked IgE-peanut antigen complex formation in an IgE-facilitated allergen binding assay were demonstrated. There were no severe adverse reactions during the protocol period and any symptoms were controlled with anti-histamine and albuterol alone (Jones et al. 2009). This study provided evidence for the ability of peanut OIT to induce desensitization and modulate immune regulation. It also contributed novel findings that provide insight into the mechanisms of OIT.

The efficacy and safety of a gradual dose escalation, higher dose peanut OIT was studied by Clark et al. (2009) who reported a case series of four boys aged 9–13 years with peanut allergy confirmed by DBPCFC at study entry. The investigators sought to determine the change in thresh-
old for reactivity following OIT. OIT was administered as daily doses of peanut flour increasing from 5–800 mg of protein with biweekly updosing increments. After 6 weeks, the OFC was repeated to define change in threshold while patients continued daily treatment. All patients tolerated at least 2.4 g peanut protein (complete desensitization) at the follow up challenge with a median 50-fold increase in dose threshold (Clark et al. 2009). The follow-up data of these four subjects and a further 18 children using the same protocol was evaluated by Anagnostou et al. (2011) in an uncontrolled clinical trial. Twenty-two peanut allergic children underwent initial OFC, followed by peanut flour OIT which was administered in two phases: a gradual up-dosing phase with two weekly increments (8–38 weeks) to 800 mg/day protein (approximately five to seven peanuts), followed by a 30-week maintenance phase. An open oral challenge using roasted peanut was repeated at 6 and 30 weeks of the maintenance phase. Nineteen of twenty-two (86%) children tolerated up-dosing and maintenance at 800 mg protein/day, and two tolerated up-dosing and maintenance at 200–400 mg. The majority (86%) experienced mild reactions during treatment that did not require epinephrine. After 6 weeks, 54% (12/22) completed a 2.6 g peanut protein challenge without reaction, and after 30 weeks, 64% (14/22) tolerated 6.6 g peanut protein. The median tolerated peanut dose was increased by 1,000-fold following oral immunotherapy (Anagnostou et al. 2011). This study used a higher immunotherapy maintenance dose of 800 mg peanut protein compared to 300 mg in the Jones study (Jones et al. 2009).

The first RDBCT of peanut OIT in children with peanut allergy was recently reported by Varshney et al. (2011), in which 28 children aged 1–16 years were randomised to receive high dose (4 g peanut protein) peanut flour OIT (19 subjects) or placebo (9 subjects). The OIT protocol involved an initial one-day escalation phase (from 0.1 mg to 6 mg peanut protein), followed by a build-up phase (the highest tolerated dose at initial day was increased by 50–100% every 2 weeks until 75 mg was achieved, and then by 25–33% until 4,000 mg maintenance dose was reached), and then a maintenance phase (daily maintenance dose was ingested for 1 month). At the completion of OIT, a DBPC oral peanut challenge was conducted (at week 48) with a total cumulative dose of 5 g peanut protein. Sixteen of 19 participants in the peanut OIT group completed the 1 year OIT protocol, and all 16 ingested the maximum cumulative dose of 5,000 mg peanut (approximately 20 peanuts) during the peanut challenge without reaction, whereas placebo subjects ingested a median cumulative dose of 280 mg ($p < 0.001$). The peanut
OIT group showed reductions in SPT size ($p < 0.001$), and IL-5 ($p = 0.01$) and IL-13 ($p = 0.02$) by peanut-induced PBMC, and increased in peanut-specific IgG4 ($p < 0.001$) as compared to the placebo group. An initial increase in peanut-specific IgE was evident in peanut OIT children ($p < 0.01$), but no significant changes from baseline were observed at the time of OFC. The ratio of FoxP3hi:FoxP3intermediate $T_{reg}$ cells increased at the time of OFC in peanut OIT subjects (Varshney et al. 2011). Follow up of this study will determine the ability of OIT to induce long-term clinical tolerance after discontinuing OIT.

Taken together, these findings suggest that OIT can consistently induce desensitization, allowing patients to tolerate significantly larger amounts of food than before treatment, and is also able to modulate allergen-specific immune responses in the direction of tolerance induction.

13.3.2.4. Induction of Tolerance with OIT

Although the above studies confirm the ability for OIT to induce desensitization, it remains uncertain whether current OIT protocols are effective in inducing long-term tolerance, since few studies have included a formal evaluation for tolerance by performing food challenges after immunotherapy has been discontinued for at least 2–4 weeks or more (Plaut et al. 2009), and only one study has included a control group to document the rate of natural resolution of food allergy.

In a pilot study of egg OIT involving seven children with egg allergy, Buchanan et al. (2007) used a modified rush phase (doubling of hen’s egg protein every 30 minutes until the highest tolerated dose was achieved) followed by maintenance therapy (300 mg daily dose) for 24 months. At the end of treatment, a double-blind, placebo-controlled food challenge (DBPCFC) was performed to assess for desensitization, and if this was confirmed, a second food challenge was performed 3 months after stopping OIT to assess for tolerance. All patients completed the treatment protocol, with one child experiencing hypotension during the rush induction phase, and all subjects tolerating daily doses during the home maintenance dose. All patients passed the first oral egg challenge (8 g egg protein) at 24 months without reaction indicating successful desensitization, and two of seven children (28%) tolerated the second oral egg challenge suggesting possible development of permanent tolerance. However, there was no control group, so it is possible that these two children had experienced spontaneous resolution.
of their egg allergy rather than OIT-induced tolerance. Egg OIT was associated with increased egg-specific IgG, however egg-specific IgE was unchanged.

Blumchen et al. (2010) conducted an open study of peanut OIT (maintenance dose 125–500 mg peanut protein, maintenance phase 2–22 months) in 23 children (aged 3–14 years) with severe IgE-mediated peanut allergy, confirmed by positive DBPC food challenge at study entry (Blumchen et al. 2010). A DBPC peanut challenge was then repeated 2 weeks after discontinuation of OIT and revealed acquisition of tolerance [as defined by the NIAID Workshop on Food Allergy Clinical Trials (Plaut et al. 2009)] in 4 of 23 (17%) subjects. However, once again, as there was no control group included in the study, it is not possible to confirm whether the acquisition of tolerance in the four subjects was induced by OIT or had developed spontaneously. This study highlighted the safety of long-term build-up protocol for children at high risk of peanut induced anaphylaxis (the median peanut-specific IgE level was 95.6 kU/L, 65% had asthma, and > 80% had history of allergic reaction after accidental peanut ingestion) (Blumchen et al. 2010).

A meta-analysis of SOTI in food allergic children by Fisher and colleagues (2011) included 3 RCT (Staden et al. 2007; Longo et al. 2008; Skripak et al. 2008) in their analysis, and concluded that SOTI cannot yet be recommended in routine practice for the treatment of children with IgE-mediated food allergy, and that larger, higher quality randomised control trials assessing long-term efficacy and safety of SOTI are needed. Unfortunately, the meta-analysis does not clearly distinguish desensitization versus tolerance as an outcome, as two of the studies (Longo et al. 2008; Skripak et al. 2008) did not perform a formal evaluation for tolerance by means of food challenges after discontinuing immunotherapy.

13.3.2.5. OIT with Heat-Denatured Proteins

OIT with heat-denatured proteins offers a new therapeutic direction in this field. It has been shown that children with transient milk and egg allergy possess IgE antibodies directed against conformational epitopes that are disrupted by extensive heating or food processing, whereas presence of IgE antibodies that bind sequential epitopes was a marker for persistent milk and egg allergy (Jarvinen et al. 2002, 2007). Recently, it was reported that a subgroup of children (75%) with cow’s
milk allergy who can tolerate baked milk products (e.g., muffins and waffles) but not fresh cow’s milk, demonstrated a reduction in cow’s milk SPT size and increased in cow’s milk-specific IgG4 with regular consumption of heated milk (Nowak-Wegrzyn et al. 2008), suggesting that OIT with heated cow’s milk may hasten the development of tolerance in this subgroup of children. In selecting subjects for baked milk OIT, it will be important to first evaluate the ability to tolerate baked milk products by carefully supervised food challenges, since 35% of baked milk-reactive children needed epinephrine for anaphylactic reaction during challenge with baked milk products (Nowak-Wegrzyn et al. 2008). Studies suggest that children who are able to tolerate baked milk foods have different milk-specific immune responses compared to those who cannot tolerate such foods. For example, children who were able to take heated milk had significantly lower basophil reactivity following stimulation with milk protein as compared to children who reacted to extensively heated milk (Wanich et al. 2009). In addition, a significantly higher percentage of casein-specific T_{reg} cells was evident in extensively heated milk–tolerant children compared with children who reacted to extensively heated milk (Shreffler et al. 2009). Another study demonstrated similar results in children with hen’s egg allergy—children who completed a baked egg OIT protocol and who developed tolerance demonstrated decreased SPT reactivity to egg and increased egg-specific IgG4 after challenge with baked egg products. Once again, some reactions during baked egg challenges were severe and 18% of participants who reacted during challenge needed epinephrine treatment (Lemon-Mule et al. 2008). Importantly, however, these two studies did not include a control group, so it remains uncertain whether the changes observed for the tolerant group related to the baked milk intervention or just reflecting natural resolution of food allergy.

The long-term effect of incorporating baked milk products into patient’s diet was evaluated recently by Kim et al. (2011a) which reported the outcomes of children from the Nowak-Wegrzyn et al. (2008) study who incorporated baked milk products in their diets. Eighty-eight children evaluated for tolerance to baked milk (muffin) underwent sequential food challenges to baked cheese (pizza) over a median of 37 months (range 8–75 months) followed by unheated milk challenge. In the initial baked milk challenges, 65 children passed (baked-milk tolerant) and 23 failed (baked-milk reactive). A comparison group consisting of 60 subjects who fulfilled the inclusion criteria but were not initially challenged to baked milk products were also included in the
study, which represents the current “standard of care” in the clinical setting. Among the baked-milk tolerant group, 60% (39/65) tolerated unheated milk, 28% (18/65) tolerated baked milk/baked cheese, and 12% (8/65) chose to avoid milk completely. Among the baked-milk reactive group, 9% (2/23) tolerated unheated milk, 13% (3/23) tolerated baked milk/baked cheese, and majority (78%) avoided milk strictly. For the comparison group, unheated milk challenges were performed as part of routine care. Thirteen of 60 (22%) children in the comparison group tolerated unheated milk, 22% (13/60) tolerated milk/baked cheese, and 56% (34/60) continued to avoid all milk. Children who were baked-milk tolerant were 28 times more likely to become unheated milk tolerant compared to the baked-milk reactive children (p < 0.001). Children who incorporated baked milk products in their diet were 16 times more likely than comparison group to develop tolerance to unheated milk (p < 0.001). The levels of casein IgG4 were significantly increased in the baked-milk tolerant group, but milk-specific IgE levels were unchanged (Kim et al. 2011b).

13.3.2.6. Enhancing the Tolerogenic Potential of OIT

The majority of published OIT protocols have used low maintenance doses of allergen (≤ 800 mg allergen) and short duration (usually < 1 year) of the maintenance phase. Cumulative experience from studies of immunotherapy for pollen and insect sting allergy has established that using an increased maintenance immunotherapy dose (Golden et al. 1981; Rueff et al. 2001) and longer duration of immunotherapy (Lerch and Muller 1998; Golden et al. 2000; Lang and Hawranek 2006) can improve the effectiveness of immunotherapy to induce tolerance. The use of higher maintenance dosing in OIT protocols warrants further investigation and the results of an ongoing trial of high dose peanut OIT (4 g peanut protein) will provide further insight into whether a higher maintenance dose will enhance the capacity for OIT to induce long-term tolerance (Varshney et al. 2011).

However, follow up of subjects who had achieved desensitization with cow’s milk OIT and continued with daily intake of cow’s milk for a certain period of time have reported allergic reactions to milk after daily dosing had been discontinued for a few weeks, suggesting that long-term tolerance had not been achieved despite prolonged continuation of milk OIT (Meglio et al. 2008; Narisety et al. 2009). One particular patient had successfully undergone gradual home dose escalations to
16,000 mg without reaction after 4 months of home intake, but withheld milk doses for few days during an episode of acute gastroenteritis. On reintroduction to milk, she developed symptoms suggestive of eosinophilic esophagitis that resolved only with milk avoidance (Narisety et al. 2009). Another patient who continued to consume cow’s milk regularly for 6 months after the desensitization period, had an interruption of milk consumption for 1 month due to viral diarrhoea, and then went straight back onto 100 ml of cow’s milk, but experienced urticaria and asthma (Meglio et al. 2008). These individual events suggest that oral desensitization-induced tolerance is not persistent and could decline after stopping regular consumption of the food.

Recent data from animal studies and human clinical trials has shown that use of a new class of adjuvants that act on antigen-presenting cells through toll-like receptors (TLRs)—so-called ‘immune response modifiers’—can also enhance the effectiveness of immunotherapy (Larche et al. 2006; Akdis and Akdis 2007). Compounds that can trigger TLR and control the over-expression of Th2 cytokines, skew the Th1:Th2 balance toward a Th1 profile, or induce $T_{reg}$ have been effective in murine models of allergy (Crameri and Rhyner 2006). Furthermore, based on demonstrated Th1 inducing effects in vitro and efficacy in animal studies, monophosphoryl lipid A (a derivative of bacterial LPS that triggers TLR4) and oligodeoxynucleotides containing immunostimulatory CpG motifs that trigger TLR9 were used as adjuvants with allergen immunotherapy in human clinical trials and were shown to markedly increase the effectiveness of immunotherapy for the treatment of allergic rhinitis (Creticos et al. 2006; Patel and Salapatek 2006). The application of immune modifying adjuvants for treatment or prevention of food allergy has been investigated in animal models (Nowak-Wegrzyn and Sampson 2011; Stahl and Rans 2011). Recently a randomized trial evaluating the combined administration of probiotic together with peanut oral immunotherapy for the treatment of peanut allergy was reported (Tang et al. 2015). Sixty-two children with peanut allergy were randomized to receive combined probiotic and peanut oral immunotherapy treatment or placebo. The combination therapy was found to be highly effective with 82% of children receiving the probiotic and peanut oral immunotherapy tolerating peanut after treatment was completed as compared to only 3.6% of placebo treated children. This rate of tolerance is higher than what has been reported for oral immunotherapy alone and supports the concept of using an immune modifying adjuvant to boost the effectiveness of oral immunotherapy.
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