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Mid Day Meal Menace in Bihar: The Public Health Concerns of the Tragedy

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Abstract Discussions regarding the “Mid Day Meal” programme have many times been an issue of public health professionals in many different ways. Most frequently it is related to the issues of policy, implementation and impact of the same in different communities but discussions in the name of MDM tragedy or disaster may not be much. The issue in Bihar is really distressing in many different ways. It is an issue of great socio-political and public health concern which requires attention from either one of them. Death of 23 children after a Mid Day Meal contaminated with pesticide in Gandaman-Dharmasati Primary school of Chhapra District of Bihar is of great concern. This is a case of point source or common source epidemic as per the classical epidemiological terminology with high case fatality ratio (51.11, n=100) and high attack rate (45, n=100). Institution of proper food and nutritional surveillance system and strengthening the primary health system is of paramount importance in handling situations like this. In this article an attempt has been made to discuss the public health concerns and the associated socio-political issue on the basis of data obtained from the published news articles of various national dailies.
Keywords *Bihar; Menace; Mid Day Meal; Tragedy*

1. Introduction

The idea of supplementary nutritional support to the school children in the form of a social welfare concept roots back to 1925 when the Madras Corporation lunched it for the under privileged children. It was providing cooked food during that time and was introduced in a larger scale in 1960. Post independent Gujarat is the first state in India to start school lunch programme in 1984. However, it was only in 1995 that the National Programme of Nutritional Support to Primary Education (NP-NSPE) was launched at the national level [1]. Subsequently the Mid Day Meal programme became the part of the Minimum Needs Programme in the fifth five year plan [2]. Later on, the programme was revised and was called as Mid Day Meal Scheme in 2004. The main objective of the programme was to give boost to universalization of primary education and to impact the nutritional intake of students in primary classes [3]. Broadly speaking MDM is a developmental intervention and it encompasses two vital components of development one is primary education and the second is the nutrition as well as

health of school children for that matter. This is also linked with Millennium Development goals more specially the goal I (Eradicate extreme Poverty and Hunger), goal II (Achieve Universal Primary Education), goal IV (Reduce Child Mortality). Again nutrition is one of the most important indicators of health and plays a vital role in health from womb to tomb. This makes MDM an important topic for public health and is described as a nutritional intervention programme. The National Institute of Nutrition has developed a model menu for the preparation of school meals suitable for north and south Indians. Given the importance of MDM it is imperative that the programme should be implemented in proper manner to meet its desired goal. But the recent tragedy happened in Bihar draws attention from many segments including public health. Apart from the socio-political concerns the public health concerns are many in this context which have been attempted to delineate in the following sections. The broad objectives of this paper are to uncover the public health concerns and few recommendations to get rid of that.

2. Methodology

The data pertaining to this tragic incident were collected from news papers which published the news during the period of the incident. The data were primarily collected from the e-portals of the news papers. The news dailies used for this purpose were NDTV, The Hindu, Z News, Live Mint and The Wall Street Journal.

3. Discussion

Bhopal gas tragedy in India and Minamata disease in Japan are classical examples of point source or common source epidemic in public health text books but for a small village, in a primary school, death of 23 children following the consumption of contaminated Mid Day Meal is probably going to be the next example of point source epidemic. This is really a tragic case with high epidemiological indices, both the attack rate and the case fatality ratio. In one of the news paper article it has been mentioned that there were 100 students on 26 July 2013 [4] when the incident happened out of which 23 died and 22 got hospitalized so the case fatality turns to be 51.11 (n=100) and the attack rate is 45 (n=100).

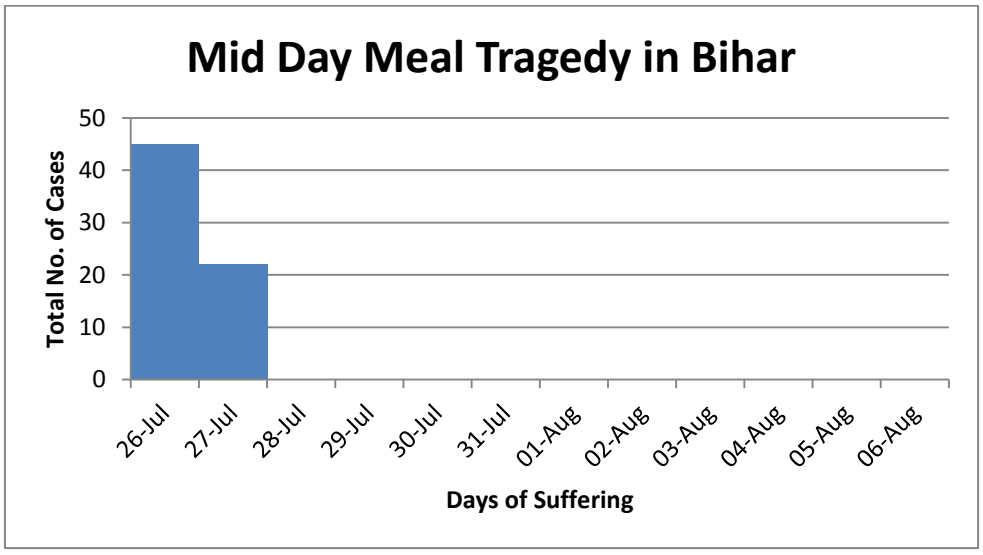

Figure 1: *Mid Day Meal Tragedy in Bihar*

The above figure depicts that the tragedy started on 26th July 2013 after the consumption of contaminated Mid Day Meal and 45 fell ill out of which 23 died and the rest got hospitalized and discharged on 6th of August 2013 as per the reports of various news dailies [5]. The report shows that

the cause of the food contamination is pesticide (may be deliberate or accidental). Here the major concern is the pesticide-laced Mid Day Meal which could have been prevented with proper precautionary measures. What is lacking here is proper food surveillance measure.

Food surveillance is essential for the protection and maintenance of community health which implies the monitoring of food safety and food hygiene. The WHO defines food safety and food hygiene as "all conditions and measures that are necessary during the production, processing, storage, distribution and preparation of food to ensure that it is safe, sound, and wholesome and fit for human consumption" [6]. Alma Ata declaration considered food safety as an essential component of food safety. It is clear from the definition that proper food hygiene could have stopped the menace. In the case of Bihar it is controversial that whether contamination happened intentionally or accidentally but from the public health prospective both the conditions are important and the food surveillance is the solution to it. There are several evaluation studies regarding the efficacy of MDM but here the condition is very basic i.e. the safety of the meals served which needs to be addressed first than the former. The Forensic Science Laboratory's (FSL) report in the Bihar's mid-day meal tragedy stated that high quantity of organo phosphorus pesticide was found in the food items that the children consumed. The Forensic Science Laboratory report found Monocrotophos, an organ phosphorous compound in the samples of oil from the container, food remains on the platter and mixture of rice with vegetables on Aluminium tasla (utensil). It is used as a pesticide for agricultural purposes; it is very toxic to human beings and other animals. This has created a huge havoc as the likelihood of the occurrence of such instances could be anywhere. The political pressure is growing in some of the states (Gujarat) to probe the issue and figure out the use such pesticides in the concerned states [7].

Another important public health concern is the provision of basic and primary health services. The primary health centre in Mashrak did not have adequate facilities to quickly reverse the effects of the poisoning; nor was there a fast enough means of transport to the district headquarters in Chhapra [4]. Many times we talk of accessible, acceptable and affordable primary health but what about availability, if it is not at our disposal the former three components hardly matters. As the first pillar of health care delivery primary health care system has a pivotal role in combating situations like this at their local level as per the situation and as per the scopes at their disposal.

The third important concern is the cost of Mid Day Meal. The per unit cost of midday meal fixed at Rs. 3.11 – Rs. 4.65 is unrealistic as even a bottle of drinking water costs Rs. 10. The Committee on Empowerment of Women had recommended in the Lok Sabha that per unit cost of mid-day meal being served in schools should be fixed on a more realistic basis. It also urged the government to regularly review the implementation of the scheme in terms of per unit cost, calorie contents and mode of preparation of midday meals [8]. This is what is called as nutritional surveillance which is defined as "Keeping watch over nutrition, in order to make decision that lead to improvement in nutrition in population" [9]. The basic objective of which is to aid long-term planning in health and development, to provide input for the programme management and evaluation and to give timely warning and intervention to prevent short term food consumption crisis [9, 10].

4. Conclusion

According to the Human Resource Development Ministry, the Mid Day Meal Scheme benefits around 10.44 crore children in about 12.12 lakh schools in the country. This is indeed huge in its form of delivery thereby it becomes imperative for a proper implementation and subsequent monitoring and evaluation of the programme. Again the millennium development goals to eradicate extreme poverty and hunger and to achieve universal primary education have made it an important programme in India.

The major limiting factor in this study is the use of secondary data as the entire article is based on the news paper findings and the author has not collected the primary data from the site itself.

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Prevalence of Undernutrition and Anemia among the Child Beneficiaries of Mid-Day Meal Program

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Abstract Estimates suggest that over one third of the World's population suffer from anemia; primarily due to iron deficiency and India is projected to be one of the countries with very high prevalence. The National Family Health Survey (NFHS-III) reveals that the prevalence of anemia is 70-80% among Indian children. Anemia is an indicator of gross under nutrition and it affects the physical growth, cognitive performance and behavior of children. The objective of Mid-Day Meal program (MDM) was to control under nutrition and the probable anemic state among school children. In this background the present study was undertaken to examine the prevalence of anemia among the child beneficiaries of 8 to 10 years of age. The Male and Female children (no: 776) attending the Urban municipal upper primary schools in Tirupati (AP) composed the subjects of the study. A general information survey revealed that all the children belonged to low socio economic status. The data on anthropometry indicate that 37.5 and 36.1% children belong to the stunted and stunted & wasted type of malnutrition. Only 26.4% children were in the normal category. Prevalence of anemia was assessed through Cyanmethaemoglobin method. The results were interpreted using WHO cut-off values for classification of anemia into different degrees. The data revealed that of the 776 children studied, 19.6, 40.6 and 0.2% were in mild, moderate and severe anemic states, respectively. About 40% children were in the normal category having Hb values $\geq$ 11.5g/dl. While MDM program is satisfactory in terms its nutrition contribution, the low food intake of children during other meals appear to bring down the days nutrient intake resulting in under nutrition and anemia. The results thus focus on the need for routine iron supplementation for the school going children to decrease the very high prevalence of iron deficiency anemia, till the time the child's daily nutritional needs are taken care-of.
Keywords *Mid-day Meal; School Children; Anemia; Malnutrition*

1. Introduction

Anemia occurs when the tissue stores of iron are depleted, leading to a lowered level of serum iron, a decrease in transferrin saturation and an increase in erythrocyte protoporphyrin. When tissue stores are seriously depleted, hemoglobin levels decline. Thus, low levels of hemoglobin may be taken to indicate IDA (Seshadri and Gopaldas, 1989).

Iron is an essential trace element, involved in three major functions. The delivery of oxygen for the sustenance of life is accomplished by Hb and myoglobin which contain iron as an intrinsic component. As a constituent of cytochrome, iron is also needed for cellular respiration. It is involved in the detoxification of lethal peroxide species formed in the tissues. Iron is a cofactor (the metal component) of many enzymes like cytochromes, catalase, and peroxides, which carry out several vital functions in the body.

ID has profound negative effects on human health and development. In infants and young children, functional consequences include: impaired immune function; showed psychomotor development, coordination, and scholastic achievement; and decreased physical activity levels (UNICEF/ UNU/ WHO, 2001).

IDA, which affects 1.2 billion persons, is the most prevalent nutritional deficiency worldwide. In 1991, above 290 million school-aged children were anemic, of whom 150 million attended school (Viteri, 1991). ID is the most common nutrient deficiency and anemia, as an indicator of iron deficiency, affects nearly 2 billion people worldwide, or about a third of the world's population. Overall, it is estimated that worldwide 39 percent of preschool children and 52 percent of pregnant women are anemic, the majority living in developing countries. Many school- age children, adults (male and female), and the elderly also suffer from anemia. ID can affect all age groups and presents a major hurdle to national development (UNICEF/ UNU/ WHO, 2001).

Two billion people worldwide are estimated to suffer from anemia; approximately 50 percent of all anemias can be attributed to iron deficiency WHO/ UNICEF, 2004. The WHO estimates that most preschool children and pregnant women in developing countries and at least 30-40 percent in developed countries are iron deficient. The prevalence of anemia in developing countries is three to four times higher than that for developed countries. In developing countries, the most affected population groups are pregnant women (52 percent) - although all women in the age 15-59 yrs are affected (42 percent), school age children (48 percent), and preschool children (39 percent). Moreover 45 percent of the elderly and 30 percent of adult men are anemic, highlighting that the problem extends to other population groups. The problem is more extensive in Southeast Asia and sub-tropical Africa where anemia is linked to poverty (WHO/ UCF/ UNU, 2001). The direct contribution of anemia to global burden of disease is 14 DALYs per 1000 population (Viteri, 1999).

In India an analysis of the work carried out during the last about half a century indicates high prevalence of anemia among infants (80 percent) and in preschool children (74-78 percent). The study conducted by the ICMR in 16 districts of 11 states reported that about 90 percent among adolescent girls had hemoglobin levels indicative of anemia. Moderate and severe anemia ranged from about 22 percent among adolescent girls to about 50 percent in pregnant women. The prevalence was uniformly high in different states. The percent prevalence among preschool children and adolescence in Andhra Pradesh was 70.8 and 72.8 percent respectively (ICMR, 2001).

The prevalence of anemia is particularly high in developing countries, where 39 percent of children under five years old, 48 percent of 5-14 yrs old children, 42 percent of all women, and 52 percent of pregnant women are anemic (WHO/ UCF/ UNU, 2001). It is estimated that about half of the anemia is due to ID (Zimmermann and Hurrell, 2006) and the remainder due to other causes, such as nutritional deficiencies (e.g. deficiencies of vit-A, riboflavin), infectious disorders (particularly malaria, HIV and Tuberculosis), hemoglobinopathies, and ethnic differences in normal Hb distributions (Nestel, 2002; Lynch, 2005). The present investigation provides the findings of a pilot study to identify the prevalence of anemia among school children residing in Tirupati urban.

2. Materials and Methods

The present study was carried out on 776 children (342 males and 434 females) between the age group of 8 to 10 years from Tirupati urban, municipal upper primary schools. Which are the beneficiaries of Mid-Day Meal Program? All the subjects were from low income families. The purpose of the study was explained to the children.

Height was measured using portable anthropometric rod and weight by plot form weighing balance with minimum clothing. The height and weight were measured nearest to 0.1 cm and 0.5 kg respectively (Jelliffe, 1966). The measurements were compared with NCHS standards. Venous blood was drawn from 776 children using disposable syringe. Hemoglobin (Hb) level was assessed by cyanmethaemoglobin method (Dacie and Lewism, 1991). Magnitude of prevalence of anemia was assessed using the (WHO, 2001) cut offs.

3. Results and Discussion

Table 1 show that out of the total of 776 children screened only 26.4 percent were found to be normal and the remaining 74 percent belonged to stunted (31.5 percent) and stunted & wasted (36.1 percent) types of malnutrition. The age related distribution reveals that in both boys and girls a high percent of stunted children (41.7 and 41.8 percent respectively) occurred in the 8 yrs age group. Whereas stunting and wasting was high in the 9 yrs age group of both boys and girls (39.7 and 38.4 percent respectively). The percent of normal children was comparatively higher in boys than girls. At the age of 8 yrs an equal percent of boys and girls were in stunted category. Girls were more stunted than boys at 9 and 10 yrs. The percent of SW children increased in boys with the age.

Malnutrition continues to be a problem of considerable magnitude in most developing countries of the world (Som et al., 2006). In the present study, the overall age and sex combined prevalence of N, W, S and SW were 26.4, 0.0, 37.5 and 36.0 percent respectively. While wasting reflects a failure of attainment of wt-for-age only; Stunting reflects a failure to reach linear growth potential due to sub optimal health and / or nutritional conditions; SW reveals low body mass relative to chronological age which is influenced by both, a child's ht and wt (WHO, 1995).

The percent distribution of children according to Hb status is presented in Table 2. The aggregate data combined for boys and girls on Hb status reveal that 60.4 percent children have Hb levels below the normal values (<11.5 g/dl) indicating anemia.

Table 1: *Prevalence of Undernutrition among School Going Children as Assessed from Water Low's Classification*

Nutritional Grades	Boys				Girls				Grand Total %(n)
	8 yrs %(n)	9 yrs %(n)	10 yrs %(n)	Total %(n)	8 yrs %(n)	9 yrs %(n)	10 yrs %(n)	Total %(n)	
Normal	27.1 26	29.3 34	31.5 41	29.5 101	22.5 29	24.4 40	24.8 35	24.0 104	26.4 205
Wasted	-	0.9 1	-	0.3 1	-	-	-	-	0.04 1
Stunted	41.7 40	30.1 35	29.2 38	33.1 113	41.8 54	37.2 61	44.7 63	41.0 178	37.5 291
Stunted & Wasted	31.2 30	39.7 46	39.2 51	37.1 127	35.7 46	38.4 63	30.5 43	35.0 152	36.0 279

(WHO, 2001); out of which 40.6 percent were moderately anemic and 19.6 percent were mildly anemic and 0.2 percent were severely anemic. The age wise distribution of the data revealed that in

the 8 yrs age group 35.6 percent were found to be normal, 12.9 percent were mildly anemic and 50.6 percent were moderately anemic and only 0.9 percent were severely anemic. In the 9 yrs age group the categories of anemia in the same order were 39.3, 22.1 and 38.6 percent and none of them were severely anemic. In the 10 yrs age group the categories of anemia occurred as 43.2, 22.5, 34.3 percent and none of them were severely anemic. A high percent of moderate anemia was prevalent in all the three age groups.

Table 2: *Percent Prevalence of Anemia among School Going Children*

Status (Hb µg/dl)	Boys				Girls				Grand Total %(n)
	8 yrs %(n)	9 yrs %(n)	10 yrs %(n)	Total %(n)	8 yrs %(n)	9 yrs %(n)	10 yrs %(n)	Total %(n)	
Non anemic (≥ 11.5)	36.5 (35)	38.8 (45)	45.4 (59)	40.6 (139)	34.9 (45)	39.6 (65)	41.1 (58)	38.7 (168)	39.6 (307)
Mild anemia (10 to <11.5)	15.6 (15)	41.4 (48)	35.4 (46)	31.9 (109)	10.8 (14)	8.5 (14)	10.6 (15)	9.9 (43)	19.6 (152)
Moderate anemia (7.5 to <10)	47.9 (46)	19.8 (23)	19.2 (25)	27.5 (94)	52.7 (68)	51.8 (85)	48.2 (68)	50.9 (221)	40.6 (315)
Severe anemia (<7.5)	-	-	-	-	1.6 (2)	-	-	0.5 (2)	0.2 (2)

The gender wise segregation of the data revealed that 40.6 percent of boys were non-anemic and the remaining 59.4 percent belonged to the mild (31.9 percent), moderate (27.5 percent) degrees of anemia. A high percent (45.4) of boys in the 10 yrs age group were normal while high percent (41.4) of 9 yrs olds boys were mildly anemic. Further, a high percent (47.9) of 8 yrs age group boys were suffering from moderate anemia.

With regard to girls the percentage in non-anemic group was 38.7. Of the remaining 61.3 percent of anemic group a highest percent 50.9 were in the moderate degree of anemia followed by a lower percent (9.9), in mild anemia when compared with boys. The percent of girls in the non-anemic group was 34.9' 39.6 and 41.1 in the 8, 9, 10 yrs respectively. With the increasing age the percentage of girls in the non-anemic state also increased as in the case of boys. However, the moderate degree of anemia showed a highest prevalence in all age groups of girls when compared to boys.

Due to the utilization of different thresholds to judge Fe deficiency among children belonging to other communities, a comparison of the prevalence rates recorded in the current study may not project the true trends. It is generally observed that ID is the most common micronutrient deficiency. The frequency of ID among school children other developing countries have been reported to vary from 16 to 21.6 percent (Al-Othaimeen et al., 1999; Hashizume et al., 2003). This is higher than that of the 54 percent observed in the present study which is conducted in the same town, Tirupati. The high prevalence observed in ID may be due to the fact that all children are from the low income families.

Any attempts to examine the role of diet in the aetiology of ID must take into account the factors that inhibit or enhance Fe absorption. The consumption of heme Fe having better bioavailability is observed to be low among these children of low socioeconomic classes. Meat products such as red meat, poultry and fish represent excellent sources of heme iron. However, the cost of these products often restricts access to the poorest in developing countries (Bhargava et al., 2001). The limited economic potentiality of Low SES households could probably explain why boys and girls of poor families were found to have lower consumption of red meat and fish and hence lower intake of highly bioavailable iron.

Polyphenol-containing beverages, such as tea, are known to reduce nonheme iron bioavailability by the formation of insoluble complexes (Reddy et al., 2000). Tea consumption is observed to be frequent among the children.

ID was probably the most common cause of anemia. However, anemia could also be due to other factors such as deficiencies of folate, vit-B_{12} or vit-A, chronic infections and inflammations and hemorrhages (WHO, 2001). Low intake of Fe, poor bioavailability of Fe from the Indian diet and rising trend of consumption of “empty calorie” foods were suggested to be the main causes of anemia in the school going children (Verma et al., 1998).

In the present study the nutritional state viz., the Hb levels were different for the different nutritional grades. The Hb levels of N group were in the normal range and that of the S and SW children recorded values below the cut offs. It is evident that better nutrition status maintains better the micronutrient levels of the children.

In the present context 60 percent of school going children was anemic with Hb levels < 11.5 g/dl. The data thus reveal that a high percent of children in every age group were suffering from moderate degree of anemia followed by mildly anemic state. There is a wide range in the prevalence of anemia reported among children. An in-depth survey of 2,998 children ages 8-9 and 12-13 yrs in Ghana and Tanzania revealed that 77 percent of children in Tanzania and 41 percent of children in Ghana were suffering from IDA (Partnership for child development, 1998). In a study of 1,210 primary school children aged 7-14, in Riyadh, Saudi Arabia, an anemia level of 55.4 percent was found. The highest level (71.4 percent) was found among 14 yrs old girls (Alothainmeen et al., 1999).

In a survey of nearly 14,000 rural school children in Africa and Asia, the prevalence of IDA was more than 40 percent in five African countries (Mali, Tanzania, Mozambique, Ghana and Malawi) amongst children aged 7-11 yrs and in four African countries amongst children aged 12-14 yrs. In the two Asian countries studied, the overall prevalence of IDA was found to be considerably lower than in Africa which was around 12 percent in Vietnam and 28 percent in Indonesia among 7-11 yrs olds. Children aged 7-11 yrs were found to have lower mean hemoglobin concentrations, while IDA was found to be more common in the older age group. Girls also were found to have lower hemoglobin concentrations than boys, although the overall prevalence of IDA was higher in boys, particularly in the 12-13 yrs age group. An association between late enrollment in school, as compared to enrolling closer to the correct age, and a higher prevalence of anemia was also found (Partnership for child development, 2001). These data and findings of the present study suggest that ID and IDA continue to be a major nutritional problem projecting the need for effective control measures.

Furthermore, the higher incidence of ID reported for adolescent school girls, compared to boys shows that female gender exerts an extra effect on the prevalence of this medical condition, probably due to accessional iron losses gender might need closer surveillance (Tatala et al., 1998; Abalkhail and Shawky, 2002; Musaiger, 2002; Hashizume et al., 2004). There is a need to understand the factors associated with the high prevalence of ID among urban school children, which may predispose these children to risk of anemia during adolescence.

Among school going children low intakes of Fe, poor bioavailability of Fe from the Indian diet were suggested to be the main cause of anemia (Verma et al., 1998). Iron deficiency was probably the most common cause of anemia (WHO, 2001). The prevalence of anemia corroborates to an extent with dietary intakes of Fe. The differences in iron intake by region could explain variations in anemia prevalence.

4. Conclusion

The results of the study indicate that anemia is widespread among school going children residing in the Tirupati area. Although severe anemia was found in the 8 year old children, as most of the children studied girls were found to have an inferior nutritional status than boys. Not only a higher percentage of girls were anemic, a higher proportion of girls in comparison to boys suffered from more serious grade (moderate) anemia.

The prevalence of anemia clearly point to that particularly in the developing countries. And anemia even now remains a public health problem as evidenced from the prevalence data of the past few decades and the recent.

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Status of Serum Trace Elements among Preschool Children in Rural Bhubaneswar, Odisha, India

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Abstract One in every three malnourished children in the world is from India leading to high child morbidity and mortality. Out of these, preschool children constitute the most vulnerable segment of any community. The present study aimed at assessing the levels of trace elements in preschool children aged 2-5 years in rural Gram Panchayats (GP) of Bhubaneswar block, Odisha, India. A cross-sectional study was undertaken covering 176 children (boys 100 and girls 76) from 8 GPs selected on random basis. Blood was collected and lyophilized serum subjected to trace elements by proton induced X-ray emission (PIXE) technique. Overall, the mean levels of manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), bromine (Br) and lead (Pb) were 19.2±3.39, 0.86±0.52, 3.2±0.9, 4.04±0.87, 15.26±2.27, 1.03±0.95, 59.95±6.55 and 0.29±0.66 ppm respectively. The concentrations of Mn, Ni, Cu and Br were increased significantly with age, while levels of Co, Zn, Se, and Pb showed random variations. The mean Co, Ni, Cu, Se, Pb and Br levels were found to be higher among boys than girls however, only Cu and Br found to be significantly high. There were significant correlations observed between some of elements (Mn-Br, Co-Ni, Ni-Cu, Ni-Br, Cu-Zn and Cu-Br) and Se levels negatively associated with Pb. The mean concentrations obtained for most trace elements with an exception of Mn were within the ranges reported by earlier studies.
Keywords *Preschool Children; Trace Elements; PIXE; Odisha; India*

1. Introduction

Preschool children constitute the most vulnerable segment of any community. Their nutritional status is a sensitive indicator of community health and nutrition [1]. More than half (54%) of all deaths before age five years in India are related to malnutrition [2]. The overall scenario in the nutritional profile of preschool children in Odisha is much inferior to other states in India [3]. A community based studies show that the magnitude of under nutrition in preschool children is still a leading problem in Odisha

[4]. Over the last three decades, increasing attention has been directed towards specific deficiencies of trace elements. Although these elements are required in small amounts, organisms need these substances for their health and all forms of life [5]. Trace element determination in blood serum has become important to investigate the vital role in human metabolism as well as to obtain information regarding the health status of individuals [6]. Several studies [7-17] were undertaken in measuring trace elements in the serum/plasma using various techniques like instrumental neutron activity analysis (INAA), flame atomic absorption spectrometry (FAAS), Electro-thermal atomic absorption spectrometry (ETAAS) and Proton Induced X-ray Emission (PIXE) in different age groups but no such studies were reported for the most vulnerable preschool children in India. The current study aims at evaluating the levels of serum trace elements among the preschool children in Odisha by PIXE method, which can quantify trace element down to parts per million (ppm) or sub-ppm levels [18]. In this study some essential and toxic (Pb) elements were estimated among preschool children. The levels of the trace elements were presented and the results are compared with the data reported in the literature.

2. Materials and Methods

A cross-sectional study was undertaken in rural block of Bhubaneswar covering 8, out of 19 GP selected on random basis. The study sample included 176 children aged 2-5 years after getting informed written consent from caregivers and mothers. Age of children was recorded in months from the birth records available with mothers or Anganwadi Centers. Venous blood samples were collected from each child and allowed 30-40 minutes for spontaneous clotting and then the serum was separated by centrifugation at 3000 rpm for 10 minutes at room temperature. Sera were stored at -20°C in metal free vials until analysis. The serum samples were lyophilized to make powder form using freeze-dried vacuum concentrator (Labconco make no. 010112731E). Aliquots of 250 mg of dried serum was weighed and mixed with high purity graphite powder in 1:1 ratio by mass. The pellets were made by applying 6 Dalton pressure in a hydraulic press [19].

The trace element composition was determined using the PIXE analytical methodology at the Institute of Physics, Bhubaneswar. PIXE is simultaneous, reliable, rapid, multi-elemental, sensitive and non-destructive in nature to analyse the trace elements [20]. The proton beam with energy of 3 MeV and diameter 3 mm obtained from the 3 MV tandem pelletron accelerators was used to irradiate the samples in vacuum (10^{-6} Torr) inside a PIXE chamber. The targets (pellets) were held at 45° to the beam direction on a target holder that was mounted on an insulated stand surrounded by a cylindrical electron suppressor held at negative potential with respect to the target. The sample targets were bombarded with 3 MeV proton beams and the beam current was in the range of 25–30 nA. A Silicon (lithium) detector (active area 30 mm^2) was used with a resolution of 170 eV at 5.9 keV, with beryllium window placed at 90° to the beam direction to detect the characteristic X-rays emitted from the targets. Spectra were recorded using a multichannel analyser calibrated with ^{241}Am X-ray source [21]. No X-ray absorbers were used between the detector and target during data collection. The PIXE spectral analyses were performed using GUPIX-2004 software (University of Guelph, Guelph, Ontario, Canada). This provides a non-linear least squares fitting of the spectrum, together with subsequent conversion of the fitted X-ray peak intensities into elemental concentrations, using the fundamental parameter method for quantitative analysis. The serum concentrations of the trace elements such as Mn, Co, Ni, Cu, Zn, Se, Br and Pb were measured and expressed as ppm. For the checking of the reliability of the technique, a certified reference material NIST Bovine liver (1577b) was used as an international standard and comparison (Table 1). The measured values and the certified values are in good agreement and thus the experimental procedure adopted is reliable in analyzing the serum samples.

Table 1: *Concentration of Trace Elements (ppm) in Certified Reference Materials (Bovine Liver, NIST-1577b) Measured by Proton Induced X-Ray Emission Method*

Trace Elements	Certified Value	Measured Value	Percentage of Recovery
Manganese	10.5±1.7	10.3±1.2	98.10
Copper	160±8.0	157±10	98.13
Zinc	127±16.0	130±11	102.36
Selenium	0.73±0.06	0.71±0.05	97.26
Bromine	9.7	10.1±1.1	104.12
Lead	0.123±0.004	0.128±0.003	99.22

Statistical analysis was performed using SPSS program 11. The data was presented in mean±standard deviation and t-test was used for comparison between groups. Significance was considered when p value was less than 0.05. Pearson's correlation tests were performed for establishing associations between parameters.

3. Results

The characteristics of the study population are provided in Table 2. The study sample included 100 male and 76 female children aged 2-5 years from different socioeconomic and ethnic groups which comprised 93 general (GEN), 49 scheduled caste (SC) and 34 scheduled tribe (ST) children.

Table 2: *Characteristics of Study Population in Bhubaneswar Rural Block, Orissa, India*

Gram Panchayat	No. of Children	Sex		Community		
		Boys	Girls	SC	ST	GEN
Andharua	20	12	8	5	4	11
Chandaka	24	13	11	7	4	13
Daruthenga	26	14	12	8	3	15
Kantabada	18	11	7	4	10	4
Dadha	20	12	8	4	2	14
Raghunathpur	21	13	8	6	4	11
Kalyanapur	23	12	11	8	2	13
Kalarahang	24	13	11	7	5	12
Total	176	100	76	49	34	93

SC-Schedule Caste, ST- Schedule Tribe, GEN-General Caste

The mean serum concentration of trace elements in children by age and sex are presented in Table 3. The mean Mn, Co, Ni, Cu, Zn, Se, Br and Pb levels were 19.23±3.39, 0.86±0.52, 3.20±0.90, 4.04±0.87, 15.26±2.27, 1.03±0.95, 59.95±6.55 and 0.29±0.66 ppm respectively. The level of Mn was high and the selenium deficiency was marked in the population. Levels of Pb in the serum were at very low concentrations indicating that the study population is free from the influence of Pb contamination in excess of the normal level. Mean concentrations of Mn, Ni, Cu and Br were increased with age, however, elements like Co, Zn, Se and Pb showed random variations. The concentrations of Co, Ni, Cu, Se, Br and Pb were found to be higher for boys as compared to girls, while Mn and Zn level tend to be in reverse trend. Sex variation was significant observed for Cu ($p<0.05$) and Br ($p<0.01$). The mean values of Mn, Se and Br showed increasing trend with respect to age.

Table 3: *Mean (±SD) Concentrations of Serum Trace Elements (ppm) by Age and Sex*

Age years	Sex	N	Manganese	Cobalt	Nickel	Copper	Zinc	Selenium	Bromine	Lead
2	T	26	15.85±3.21	1.01±0.50	2.75±0.55	3.50±0.38	15.37±1.29	0.94±1.28	55.14±5.64	0.09±0.13
	B	14	14.36±3.41	1.26±0.24	2.82±0.35	3.52±0.12	14.87±1.27	1.11±1.56	56.10±6.59	0.10±0.22
	G	12	17.59±1.90**	0.61±0.41**	2.67±0.73	3.47±0.56	15.95±1.10*	0.74±0.87	54.02±4.29	0.08±0.02
3	T	41	19.83±3.05	0.64±0.64	3.23±0.59	4.04±0.82	14.51±2.02	1.02±0.89	59.13±5.44	0.42±0.74
	B	20	19.82±3.20	0.73±0.64	3.14±0.63	4.15±0.85	13.95±2.17	0.88±0.81	60.09±5.99	0.44±0.71
	G	21	19.85±2.97	0.56±0.63	3.31±0.55	3.94±0.80	15.04±1.74	1.16±0.97	58.22±4.83	0.40±0.79
4	T	57	19.40±3.54	0.84±0.45	3.20±1.04	3.99±0.96	15.43±2.79	1.01±0.85	61.10±5.25	0.30±0.68
	B	34	19.58±4.12	0.76±0.44	3.30±1.28	4.15±0.97	16.07±2.21	0.98±0.74	63.25±5.00	0.25±0.56
	G	23	19.14±2.51	0.97±0.47	3.05±0.48	3.74±0.90	14.93±2.02	1.04±1.01	57.92±3.88**	0.39±0.84
5	T	52	20.25±2.47	0.96±0.45	3.41±1.03	4.36±0.87	15.43±2.79	1.09±0.91	61.72±7.83	0.27±0.72
	B	32	20.04±2.61	1.02±0.49	3.52±1.24	4.44±0.96	15.35±3.13	1.25±1.06	62.41±8.73	0.35±0.87
	G	20	20.58±2.24	0.85±0.38	3.23±0.54	4.24±0.71	15.56±2.20	0.83±0.53	60.63±6.18	0.14±0.36
Pooled	T	176	19.23±3.39	0.86±0.52	3.20±0.90	4.04±0.87	15.26±2.27	1.03±0.95	59.95±6.55	0.29±0.66
	B	100	19.05±3.86	0.92±0.52	3.27±1.08	4.15±0.91	15.25±2.53	1.07±1.00	61.35±7.11	0.30±0.68
	G	76	19.47±2.64	0.77±0.52	3.11±0.59	3.88±0.81*	15.29±1.89	0.97±0.87	58.10±5.23**	0.27±0.65

B: Boys, G: Girls, T: Total, * $p < 0.05$, ** $p < 0.01$

From the correlation matrix (Table 4), it was observed that the concentrations of elements Mn, Ni, Cu and Br increased with the age. Among the inter elemental relationships, significant correlations observed between elements Mn-Br, Co-Ni, Ni-Cu, Ni-Br, Cu-Zn and Cu-Br. There was a significant negative correlation observed between levels of Pb-Se.

Table 4: *Correlation Matrix for the Trace Element Contents in Children*

Indicator	Age	Manganese	Cobalt	Nickel	Copper	Zinc	Selenium	Bromine	Lead
Age	1.000								
Manganese	0.347**	1.000							
Cobalt	0.061	-0.128	1.000						
Nickel	0.203**	-0.141	0.241**	1.000					
Copper	0.280**	0.042	0.138	0.439**	1.000				
Zinc	0.083	0.093	-0.019	-0.111	0.159*	1.000			
Selenium	0.047	0.146	-0.072	0.000	-0.049	-0.020	1.000		
Bromine	0.316**	0.224**	-0.085	0.316**	0.156*	0.057	0.107	1.000	
Lead	0.037	0.108	-0.040	-0.051	-0.001	0.066	-0.202**	-0.016	1.000

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

Table 5 presents the values (mean±SD) of trace elements in this study population as against earlier reports. In the present study it was found that the levels of Mn and Co were relatively high, and levels of Ni, Cu, Zn, Se and Pb were closer to earlier studies.

Table 5: *Concentrations of Serum Trace Elements (ppm) in Preschool Children, Orissa, India*

Trace Elements	Mean ± SD (95% CI)	Earlier Studies on Preschool Children
Manganese	19.23±3.39 (5.82-32.98)	0.08±0.147, 0.32± 0.18 [18, 24]
Cobalt	0.86±0.52 (0.01-1.87)	0.05±0.08, 0.24±0.25, 0.61±0.33 [24, 14, 18]
Nickel	3.20±0.90 (1.80-9.75)	0.06±0.06, 2.6-7.5, 11.7±6.5 [18, 25, 14]
Copper	4.04±0.87 (2.28-6.53)	1.63±0.37, 3.61±0.10 [27, 31]
Zinc	15.26±2.27 (7.30-20.70)	7.4 ±0.25, 15.15±0.19, 16.8±0.08 [14, 31, 8]
Selenium	1.02±0.95 (0.0-4.7)	0.45-1.21, 0.90 [28, 29]
Bromine	59.95±6.55 (42.60-79.20)	59.1-63.0, 100-187 [7, 31]
Lead	0.29±0.66 (0.0-3.10)	0.08±0.01, 0.21 ± 0.105, 0.20±0.10 [13, 15, 17]

4. Discussion

Trace elements are involved in many biological processes supporting life. The concentrations of trace elements in the body are influenced by a number of factors such as gender, age and dietary intakes. Deficiencies in trace elements are usually diagnosed with common symptoms such as malaise, loss of appetite, anemia, infection, skin lesions, and low-grade neuropathy [5]. Intoxication by trace elements is common in situation like flue, fever, nausea, vomiting, diarrhea, anemia, and affect central nervous system causing neuropathy [22]. On the other hand, excess intake of these trace elements leads to diseases and toxicity, for which a fine balance is essential for maintaining health.

In our study Mn concentration was found to be 19.23±3.39 ppm and the levels increased with age. A significantly high content of Mn detected among children in the present study, which is not the case in earlier reports [18]. In a study conducted on neonates, Mn levels in blood at full-term and 6 months were found to be 0.32 and 0.21 ppm [14]. As compare to earlier studies and reference values, Mn levels in this population seem very high. Food is the main source of Mn for general population, with air and water contributing about 1% of the daily intake. Children often eat soil that contains Mn, however, there is little information on how well Mn in soil can be taken up from the stomach into the body if children eat it. Most soils contain Mn values range from 40–900 ppm. Further, Mn can be absorbed in higher-than-usual amounts if the diet is low in iron. Anaemia is the most common among children in Odisha [3]. It is an established fact that low ferritin levels are associated with increase in Mn absorption [23].

The mean Co level in the study was 0.86±0.52 ppm. Sex wise the level of Co is higher in case of boys than girls. Marriott et al., [14] reported serum cobalt levels among Turkish children as 0.24±0.15 µg/l (ppm). In this study, Co levels were relatively higher as compared with earlier studies [18, 24].

The concentration of Ni among the children was 3.20±0.90 ppm, whereas Iyengar and Woittez [25] reported a concentration ranged 2.6-7.5 ppm. Another study [14] reported the Ni level of 11.7±6.5 ppm among children. The level of Ni in case of boys is more than that of girls.

In case of Cu, the mean serum concentration was 4.04±0.87 ppm and more in case of boys than the girls. The level of Cu increased with increase of age. The serum Cu level in preschool children was found to be 3.61±0.01 [26]. Serum Cu levels in malnourished and well children in Tanzania reported to be 16.5 and 21.2 mmol/l [7]. Co levels reported to be significantly higher in children with iron deficiency anaemic (189±49 µg/dl) than those of controls (163±37 µg/dl) [27]. In the rural community of Canagua, Merida State, Venezuela estimated the Cu among pre-school children aged 2-6 years to be 1.18±0.30mg/l, and the level decreased with age [10]. But in the present study, it is not same and the level of Cu is slightly higher in case of boys than girls.

The concentration of Zn in this study population was 15.26±2.27 ppm. Similar concentrations of Zn (15.15±0.19 ppm) documented for preschool children [26] from Nigeria and for malnourished children it was 12.5 ppm in Tanzania [7]. Serum Zn levels are inversely associated with severity of protein energy malnutrition in children using weight-for-age of Harvard Standards. Zn levels fallen consistently with malnutrition grades -I, -II, -III respectively with 8.27, 6.77, 5.3 ppm as compared to 10.9 ppm in the well-nourished group [16]. Levels of serum Zn found to be low in children with iron deficiency anemia (IDA) group as compared to control group [15, 27]. Zn levels of neonates at full-term and 6 months reported to be at 12.0±2.6 ppm and 13.8±2.5 ppm respectively [14]. The mean serum concentrations of zinc among children aged 2-6 years found to be 7.4±2.5 and the levels increase with age [10].

In the present study, mean Se concentration was 1.03±0.99 ppm ranging from 0.03 to 4.7 ppm. A study by Arnaud et al., [28] on serum Se concentrations among newborns found to be varying 0.45-

1.21 ppm. Muntau et al., [29] reported a statistically significant age dependency: increase in Se levels from 1 to 5 years (0.64-0.90 ppm). Se concentrations found in children is within the normal ranges. The concentration of Se in case of boys is little higher than girls. It is observed that, Se could not be detected (negligible amount) in 31.68% of samples, which indicating the deficiency of Se in the population. It may be due to low soil Se levels and dietary intakes.

The concentration of Br among child population was 59.95±6.55 ppm, with significantly higher levels in boys than in girls. The levels of Br increased with age. Serum Br level among malnourished children (59.1 µmol/l) was found to be lower than the healthy control groups (63.0 µmol/l) in Tanzania [7]. In a study conducted on Taiwan preschool children from revealed significant differences between Atayal and Bunun aboriginal groups [30].

Of the total samples, 20.6% (21.3 % boys and 18.9 % girls) children had detected lead in their serum at very low concentrations indicating that the study population is free from the influence of lead contamination in excess of the normal level. The Pb level in this study was 0.29±0.66 ppm. Pb levels are shown to influence negatively with iron status of population groups. Anemic children from Lucknow, India showed a significant association of elevated Pb levels to an extent of more than 1 µg/dl [13] and those children having iron deficiency anemia shown to be have lower Pb levels than that of their normal (0.13±0.04 vs 0.08±0.01 ppm) [15]. Moreover, a negative linear correlation of Pb with Zn and iron ($P < 0.01$), hence deficiency of Zn and iron reflecting Pb toxicity levels in children [17]. Similar findings were observed in our study showing inverse association between Pb and Se.

5. Conclusion

The concentrations of Mn and Co were relatively high, and levels of Ni, Cu, Zn, Se and Pb were closer to earlier studies which may be due to living environment, preference of dietary intakes of the given population, quality of diet and soil contents of these elements. The results provide baseline information of trace elements for the vulnerable section of child population, which needs further study in assessing its deficiency and intoxicant effects independently and in combinations.

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Botanical Antioxidants for Skin Health in the World of Cosmeceuticals

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Abstract The desire to maintain a youthful appearance in an aging population has accelerated several advancements in the cosmeceuticals market. The term cosmeceutical defines products containing bioactive substances that cannot be considered cosmetics or drugs. A variety of ingredients have been used in cosmeceuticals to improve the health and appearance of aged skin, and during the past decade, the utility of botanical natural products have gained much attention in the West. Throughout this review, the skin aging, and photoaging are discussed, mechanisms which underlie these processes are explored, and treatment options using natural plant extracts are examined.

Keywords *Skin Aging; Dermatology; Antioxidants; UV Rays; Cosmetics*

1. Introduction

Improved life conditions as well as medical and technological developments have led to an increase in life expectancy, with a higher number of people now living to a comparatively longer life, with the elderly population becoming a significantly higher proportion of the population in many countries (Giacomoni, 2005). Biologists and the pharmaceutical industry are seeking ways to achieve more success in improving the quality of life in the elderly. Some believe that it is possible to extend the lifespan of humans and therefore seek ways to increase longevity; while others have more reserved expectations and focus on applications that make it possible to grow older while avoiding aging and its associated pathologies. As a result, aging and longevity are considered as two different fields of research by most scientists (Giacomoni, 2005). A commonly accepted definition of aging is the accumulation of molecular damage with time (Giacomoni, 1992). Research employing this model definition has been able to make several advances in understanding the mechanisms of skin aging, given the easy access to skin tissues. Various research approaches use models such as cell cultures, animal research and human studies, thus facilitating the search for effective rejuvenating treatments (Giacomoni, 2005). Together with the aging of muscular and skeletal systems, skin aging is a process with very direct effects. The skin is a major sensory organ, as it is the body's first line of defence

against infectious organisms and physical harm, and it plays a critical role in controlling body temperature. While slowing the aging processes of the skin will not only help maintain a youthful appearance, but it will also have beneficial effects for the whole body. The skin is an ideal model for studying the onset of aging because it is the easiest organ to observe, and aging of the skin is not a life-threatening process (Giacomoni, 2005).

2. Inflammatory Models for Skin Aging

Collecting knowledge about the skin aging process has led to the micro-inflammatory model, which describes how both internal and external factors can contribute to the process (Giacomoni, 1996a, 2001, 2005). This model focuses on observations indicating that the majority of factors identified to speed up skin aging share common features, such as the ability to initiate the synthesis of intercellular adhesion molecule-1 (ICAM-1) in endothelial cells. Factors known to speed up the aging process include ultraviolet (UV) radiation, trauma, and hormonal imbalance, among several others (Giacomoni, 2005). After synthesis, ICAM-1 is transported to the surface of the endothelial cells in the capillary vessels of the dermis, where it signals monocytes and macrophages to attach to the surface of capillary vessels and migrate into the dermis.

These steps are controlled by the release of pro-oxidants and by hydrolytics enzyme activation which damage the extracellular matrix and surrounding cells (Giacomoni, 1996b). The cell damage triggers an arachidonic -acid dependent inflammatory response to release prostaglandins and leukotrienes which signal the mastocytes to release histamine and tumour necrosis factor 1 alpha (TNF-1α). Histamine and TNF-1α can further induce more endothelial cells of the skin capillaries to synthesize ICAM-1 and to bind circulating monocytes and macrophages. Numerous experiments provide research that this self-maintaining and self-amplifying micro-inflammatory is believed to be responsible for skin damage via production of highly reactive oxygen species (ROS), ultimately resulting in skin aging.

This model focuses on the importance of identifying major environmental and lifestyle factors which cause skin aging and avoid them. The sun is still the greatest environmental factor that accelerates skin aging. Many defences are available against solar radiation, including a number of natural and synthetic sunscreens with varying SPF. Sunscreens are photostable, non-irritant and non-phototoxic substances which are able to absorb UV radiation before it can cause cellular damage (Giacomoni, 2005).

3. Skin Aging Process: Intrinsic Aging and Extrinsic Aging

Several changes commonly linked with skin aging are a direct result of sun exposure. During the 19th century, researchers observed differences in the facial skin of outdoor workers when compared to that of indoor workers (Nghiem et al., 2001). The skin of these outdoor workers had several changes associated with various skin cancers, such as thickening and brownish discoloration on light-exposed skin (Nghiem et al., 2001). The skin aging process falls into two categories: natural skin aging (intrinsic aging) and photoaging (extrinsic aging). Intrinsic aging is induced by internal physiological factors, while extrinsic aging results from exposure to various external factors (Thring et al., 2009).

3.1. Intrinsic Skin Aging

Intrinsic aging is characterized by reduced collagen synthesis, degeneration of elastic fiber networks, and loss of hydration, resulting in laxity, fine wrinkling and the development of benign growths (Criscan et al., 2012). This process occurs as slow but progressive and irreversible tissue degeneration. Telomere shortening and metabolic oxidative damage from ROS play a key role in the process of aging (Kosmadaki et al., 2004). In aged skin, there is elevation of transcription factor

activator protein 1 (AP-1) (Chung et al., 2000), which is involved in cellular processes such as differentiation, proliferation, and apoptosis (Ameyar et al., 2003). AP-1 is also involved in promoting collagen breakdown by upregulating matrix metalloproteinases (MMPs) (Helfrich et al., 2008).

MMPs contribute to remodeling of tissue associated with several physiological and pathological processes including morphogenesis, tissue repair and cirrhosis (Helfrich et al., 2008). The activity of MMPs is elevated in aged skin, and is associated with increased levels of degraded collagen (4-fold higher in aged vs. young individuals) (Fisher et al., 2002). Furthermore, synthesis of types I and III procollagen is reduced in aged skin the combination of increased collagen breakdown and decreased synthesis of new collagen results in an overall decrease in collagen levels (Kim et al., 2004; Varani et al., 2000).

3.2. Extrinsic Skin Aging

Extrinsic photoaging is characterized by wrinkling and furrowing with a thickening of the skin, along with a variety of benign, premalignant, and malignant neoplasms (Gilchrest, 1989). UV irradiation from the sun leads to the generation of ROS which results in the upregulation of AP-1 and downregulation of transforming growth factor β (TGF-β). An increase in AP-1 activity leads to the augmentation of MMPs which would subsequently trigger the breakdown of collagen.

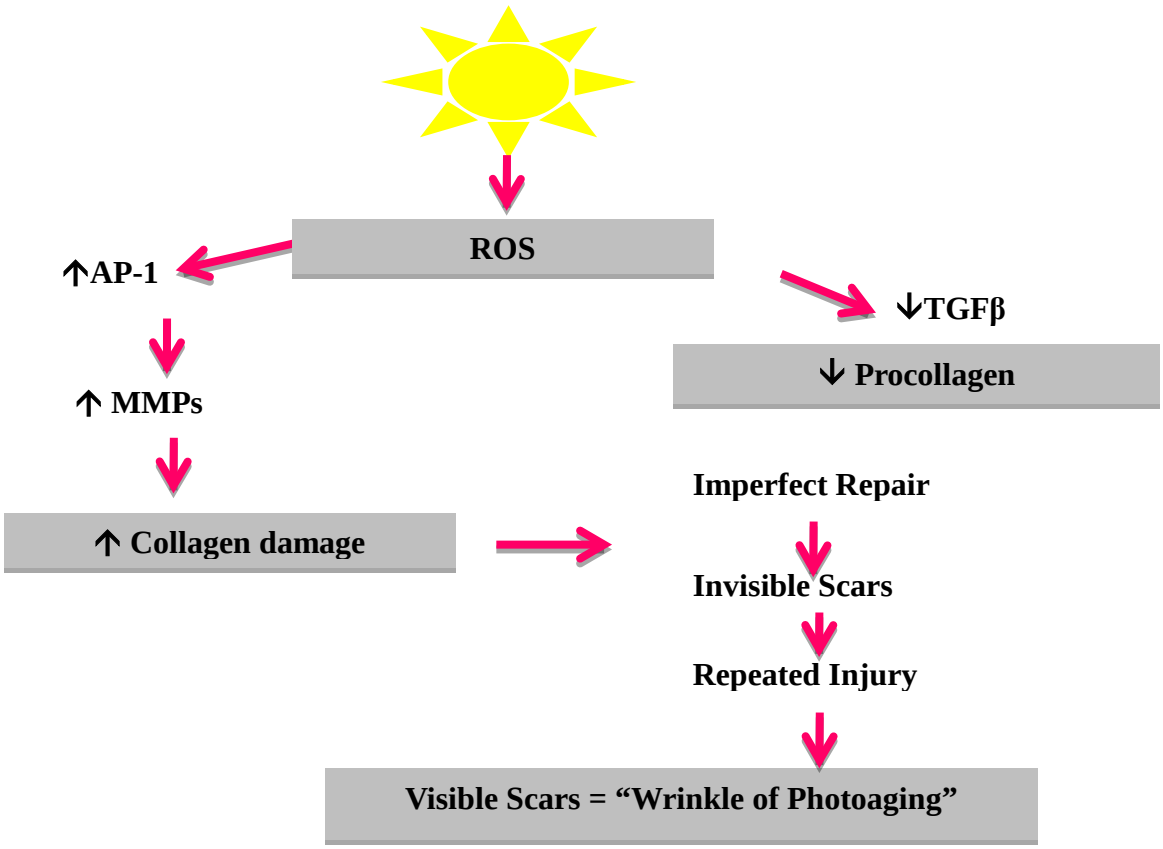

Figure 1: *The Effects of UV Rays from the Sun in the Process of Photoaging Indicating where Botanicals can Mediate Positive Changes, to Prevent and/or Decrease Signs on Photoaging*

As a down regulation in TGF-β activity is associated with a decrease in procollagen production, together an increase in collagen breakdown combined with a decrease in procollagen production results in repeated exposure to UV irradiation. This repeated exposure leads to the accumulation of damage, and eventually results in visible solar exposure scars or wrinkles associated with Photoaging

(see Figure 1) (adopted from Helfrich et al., 2008). Histologically, when compared to sun-protected skin, a 20% reduction in total collagen and a decrease in cellular content were observed in photo-damaged skin (Schwartz et al., 1993). The molecular changes of photoaging are considered to amplify natural skin aging (Fisher et al., 2002). As a result, UV radiation suppresses cell-mediated immunity, and predisposes individuals to skin cancer, immune system failure, and infection. Furthermore, UV radiation induces suppression of the local effector mechanisms involved in immune responses to recall antigens and inhibits the contact hypersensitivity response (Cooper et al., 1992; 1995; Damian et al., 1997; Morison et al., 1985; Yoshikawa et al., 1990). Such exposure causes alterations in the connective tissue through the formation of lipid peroxides, cell contents and enzymes, as well as ROS (Thring et al., 2009). ROS are free radicals, defined as atoms or molecules with an unpaired electron; and it is this electron that causes much of the damage (Giacomoni et al., 1992). Lipid peroxides can be broken down to secondary products that damage the extracellular matrix, while ROS are associated with the loss of skin elasticity (Benaiges et al., 1995; Kaur et al., 2006). Biological systems require ROS for various metabolic pathways, thus the body is able to generate reactive oxygen species such as superoxide and nitric oxide through defined pathways (Han et al., 2001; Lupo et al., 2007). However, overproduction ROS can cause severe oxidative stress and thus damage tissues, through inactivation and degradation of protein, lipid, carbohydrate and cellular DNA components (Brooker, 2011). In addition to nuclear DNA, mitochondrial DNA can also be transformed by oxidative stress. As DNA repair is less efficient in mitochondria, mutations rapidly accumulate. A common deletion in the DNA has been identified and shown to be very common in photoaged cells; this deletion can be generated by UVA and such mutations may alter the ability of cells to carry out oxidative phosphorylation, ultimately generating additional oxidative stress (Pinnell et al., 2003).

4. UV Rays and Skin Cancers

The foundation of photoaging therapy consists of a broad spectrum ultraviolet-A radiation (UVA) and ultraviolet-B radiation (UVB) sunscreens. UVR causes several acute effects in the skin, including immediate pigment darkening, delayed tanning, sunburn, epidermal thickening, as well as several immune responses. Most importantly, UVA and UVB radiation have been observed to contribute to the disruption of extracellular matrix, which is a characteristic symptom of photoaging (Sorg et al., 2005; Talwar et al., 1995). It has long been thought that UVB causes most damage, but it is becoming increasingly evident that the biological effects of UVA is significantly more important; UVA also penetrates the skin more deeply than does UVB (Dekker et al., 2005). The mechanism of UV radiation associated dermal damage includes, decreased collagen I and III synthesis, increased collagen degradation by TGF-β and activator protein A, infiltration of inflammatory cells predominantly by neutrophils into the dermis releasing ROS (Saha, 2012; Sorg et al., 2005; Talwar et al., 1995). Biomolecules weakly absorb UVA, but it can generate ROS, which oxidize proteins, DNA, and lipids (Cooke et al., 2000; Hattori et al., 1996; Struthers et al., 1998). Cells have developed defense systems to protect themselves from ROS, including endogenous, exogenous and enzymatic antioxidants (Dekker et al., 2005). Heme oxygenase-1 (HO-1) has a cytoprotective function and is strongly inducible in several mammalian cell types by chemical and physical stresses (Elbirt et al., 1999; Noel et al., 1997). This has been shown that HO-1 is highly inducible in skin fibroblasts by UVA; however it does not induce HO-1 expression in human but can induce HO-1 expression in epidermis of hairless mice (Allanson et al., 2004; Applegate et al., 1995; Dekker et al., 2005).

UVB irradiation is a carcinogen and can induce squamous cell carcinomas (Pinnell et al., 2003). As DNA absorbs UVB radiation, DNA mutations can arise. The UV action spectrum for generation of squamous cell carcinoma occurs mostly in the UVB region, though there is some activity in the UVA (Pinnell et al., 2003). While UVB contributes to tumor initiation, UVA primarily causes tumor promotion and generates more oxidative stress due to higher lipid peroxidation efficiency. Additionally, UVA extensively damages DNA by causing strand breaks and oxidation of nucleic acids (Pinnell et al.,

2003). In addition, UVA can induce MMP synthesis that can enhance the aggressiveness of skin cancer. Sunlight can suppress the immune function of skin and promote skin cancer formation (Pinnell et al., 2003). Approximately 40% of human beings are susceptible to UV immunosuppression. Although most studies of UV immunosuppression have been conducted using UVB, recent studies demonstrated the role of UVA in immunosuppression, and the capacity of antioxidants to prevent such immunosuppression (Duthie et al., 1999; Nghiem et al., 2001; Pinnell et al., 2003). Moreover, in addition to more efficiently generating ROS in skin, UVA causes additional biological effects different from UVB. Sunlight contains significantly higher amounts of UVA in comparison to UVB, and the UVB is almost entirely absorbed in the epidermis, while UVA is capable of reaching deeper dermal layers and even disrupting circulating blood cells (Pinnell et al., 2003).

5. The Skin Care Industry

Skin care is the largest of the cosmetic products worldwide, valued at approximately 96 billion in 2011 (Tyrimou, 2012), with the Asia-Pacific accounting for 43% of the skin care market in 2011. (Tyrimou, 2012). What's more, the sales for anti-aging products in North America rose by nearly 14% in 2011 and are estimated to continue to increase around the world (Tyrimou, 2012). The realm of cosmeceuticals is rapidly expanding in numerous countries. This expansion is a result of the availability of new ingredients, the financial rewards for developing successful products, consumer demand, and a better understanding of skin physiology (Tyrimou, 2012). The cosmeceutical industry combines the many skills of cosmetic creators, along with the creativity of marketing experts, the requests of an aging population and the understanding of dermatologists into several products (Giacomoni et al., 1996a).

The search for effective and safe sun protection has propelled cooperation and mutual exchange between scientists within the industry and academia, collaboration most successful within the science field of photobiology (Giacomoni, 2005). Sunscreens are the "gold standard" for photodamage protection (Choudhary et al., 2010). However, it has been shown that sunscreens provide much less protection than expected, but providing a false sense of security. Sun protection factor (SPF) of individual products is measured by testing the efficacy of the component to filter UV at an application rate of 2 mg/cm² of skin (Pinnell et al., 2003). In fact, controlled studies of actual sunscreen usage demonstrated that sunscreens are applied to skin at only 0.5 mg/cm² or less, and given that SPF concentration is not linearly proportional, 0.5 mg/cm² application of high SPF sunscreen to skin only provides less than SPF 3 protection (Autier et al., 2001; Pinnell et al., 2003) (not clear). Moreover, synthetic sunscreens can potentially cause harm as free radicals may be produced by ingredients in the products themselves when activated by UV radiation (Cross et al., 2001; Pinnell et al., 2003). Therefore, such problems enhance the introduction of natural products for sun protection, and are considered safer. As a result, this allows for innovation in the cosmeceutical market to develop safer, naturally derived products. Some of these naturally derived products have proven to be helpful, whereas more evidence is needed for others (Amer et al., 2009).

6. Natural Skin Care Therapy

Due to extensive research on different plant species and associated therapeutic principles, traditional medicine is being re-examined (Moulisha et al., 2010). It has been demonstrated that plants synthesize chemicals with powerful antioxidant activity to control the oxidative stress caused by sunlight and oxygen (Moulisha et al., 2010). Anti-collagenase and anti-elastase activities have been found in secondary metabolites and plant extracts (Thring et al., 2009). Collagenase and elastase are enzymes that contribute to the degradation of collagen within the skin. Several Plant polyphenols such as flavonoids, phenolic acids and tannins have been found to be collagenase inhibitory compounds which may serve as a platform for synthesis of other inhibitory molecules (Kim et al., 2004). Polyphenols, such as epigallocatechin gallate (EGCG), extracted from green tea (*Camellia*

sinensis) have been extensively explored and found to be effective inhibitors with particular good anti-elastase activity at concentrations of 250 µM (Kim et al., 2004; Thring et al., 2009).

Triterpenoids known as boswellic acids isolated from frankincense (*Boswellia spp*.) resin have also indicated anti-elastase activity (Mereish et al., 1991). In a study analysing 150 plants extracts for their ability to inhibit elastase, six showed activity over 65%. These included cinnamon (*Cinnamonum cassia*), turmeric *(Curcuma longa*) and nutmeg (*Myristica fragrans*). Polyphenols isolated from persimmon (*Diospyros kaki*) leaf showed anti-collagenase and anti-elastase activity (Lee et al., 1999; Thring et al., 2009). This activity was thought to be a result of flavonoids present in the polyphenol extract. Plant extracts and natural products which have shown anti-enzyme activity represent a wide variety of the types of phenolic compounds found in plants (Lee et al., 1999; Thring et al., 2009). White tea and cleavers extracts also demonstrated high anti-elastase activity, suppressing over 89% and 57.9% of enzyme activity respectively. Similar anti-elastase effects were observed in burdock root (50.9%), bladderwrack (50.2%), anise (31.9%) and angelica (31.6%) (Thring et al., 2009).

Sunscreens are useful but not ideal due to incomplete spectral protection and risk of toxicity (Pinnell et al., 2003). Antioxidants, common ingredients in cosmeceuticals, have been used by the industry for many years as a result of several benefits such as anti-aging and anti-inflammatory properties. In addition to blocking UV-induced inflammatory pathways, antioxidants provide protection by quenching free radicals (Reszko et al., 2009). While skin uses antioxidants for protection against sun damage (Pinnell et al., 2003), the system can be overwhelmed by excess exposure to various sources of pro-oxidants, which induce oxidative stress (Rabe et al., 2006). UV radiation absorbed by various chromophores in skin result in photochemical reactions (Amer et al., 2009). These reactions result in DNA alterations such as oxidation of nucleic acids and gene mutations and can change proteins and lipids, causing changes in cell function and leading to tissue aging (Amer et al., 2009).

Two mechanisms are involved in free radical natural skin defense: 1) enzymatic defense by glutathione peroxidise and extracellular superoxide dismutase; and 2) non-enzymatic processes through components such as vitamin C, tocopherols and other food derived antioxidants (Pinnell et al., 2003). Antioxidants pair up with free radicals, ultimately minimizing cross linkage and DNA damage (Amer et al., 2009). Topical antioxidants provide a great treatment option due to the close proximity of the molecules to the skin where it can block the solar radiation and as oral antioxidants. In some cases the orally administered components may not reach the skin in sufficient amounts to be effective (Amer et al., 2009; Zhang et al., 1999). However, several recent studies have shown that orally administered components (omega -3-fatty acids, lycopene) are more effective when consumed through diet (beauty from within). Several obstacles exist within the industry regarding effectiveness of topical application of antioxidants: 1) instability, such compounds can be reduced or oxidized easily; 2) color, difficulty to produce an acceptable aesthetic product; 3) lack of adequate skin penetration; 4) photo-protection of the antioxidant etc., (Amer et al., 2009). Recent categories of antioxidants include a wide variety of natural plant components such as polyphenols (Amer et al., 2009).

7. Polyphenols

Polyphenols are a large class of chemical compounds synthesized by plants and are rich in fruits, vegetables, tea, cocoa and other plant products, and have been related to health benefits shown by these products. Polyphenols have antioxidant, anti-inflammatory, anti-carcinogenic and other biological properties which may protect from oxidative stress and several diseases (Kanti et al., 2009). Polyphenols are abundant in nature and extremely diverse with over 8,000 different polyphenolic compounds currently identified (Kanti et al., 2009). Although all polyphenols have similar chemical structures, subtle differences exist which allow for subdivision into main subclasses: phenolic acids, stilbenes, tannins, diferuloylmethanes and flavonoids (Kondratyuk et al., 2004; Kanti et al., 2009; Spencer et al., 2008). Flavonoids and phenolic acids are capable of scavenging free radicals and

chelating metal ions such as iron and copper known to participate in the initiation of free radical reactions (Utara et al., 2009). Flavonoids act as scavengers of free radicals and terminate the process of ROS production as well as inhibit the activities of several redox enzymes, and act in redox-sensitive signalling cascades to inhibit cell damage caused by free radicals (Cao et al., 1996; Parmar et al., 2010; Patel et al., 2005; Robak et al., 1988; Svobodova et al., 2003; Torel et al., 1986).

8. Botanicals for Skin Health

8.1. Soybeans

Soybeans and related food products are a rich source of a subclass of flavonoids called isoflavones (Pinnell et al., 2003). Isoflavones have gained increased popularity because epidemiologic studies suggest that they may be responsible for the lower risk of cardiovascular disease and breast cancer in populations that consume large amounts of soy (Glazier et al., 2001).

The most abundant isoflavones in soy are genistein and daidzein, which are present as glycosides that are converted to the free isoflavones forms (Brandenberger et al., 1997). The glycosides are not estrogenically active, and may be used for topical applications (Miksicek, 1995). Isoflavones are weak estrogens; however their affinity to the estrogen receptor is 4-5 folds lower than the hormonal estrogens. Estrogens function by coupling with estrogen receptors in the nucleus, turning linked genes on or off, which leads to proliferative or differentiation responses. Two types of estrogen receptors (ER) have been identified and are both present in the skin: ER alpha (ER-α) and ER beta (ER-β) (Brandenberger et al., 1997). Genistein has a 30-fold higher affinity for ER-β than ER-α; however, greater ER-α agonist activity has been shown (Barkhem et al., 1998; Katiyar et al., 1999). Bioavailability of isoflavones, just as any other polyphenols, is considerably low. Still, the circulating levels of phytoestrogens are capable of inducing a biological effect (Pinnell et al., 2003). Isoflavones may block the estrogen receptor leading to anti-estrogenic effects (Pinnell et al., 2003). Skin properties change dramatically during and after menopause (Affinito et al., 1999; Brincat et al., 1987; Castelo-Branco et al., 1992). The thickness of the skin reduces along with the collagen content. Oral or topical administration of estrogen has shown to increase thickness and collagen content of skin (Brincat et al., 1987; Castelo-Branco et al., 1992; Maheux et al., 1994; Varila et al., 1995). Genistein might also exhibit collagen-stimulating effects. Throughout studies using skin fibroblasts, genistein increased collagen, type I, alpha 2 (COL1A2) gene expressions (Greenwel et al., 1995) which may be an alternative process independent of the estrogen receptor action (Greenwel et al., 1995).

Genistein is an effective antioxidant, as it scavanges peroxyl radicals and protects against lipid peroxidation in vitro and in vivo (Hwang et al., 2000; Wiseman et al., 2000). This isoflavone has also been shown to inhibit in vitro UV-induced DNA oxidation and reduced hydrogen peroxide–generated DNA damage in human lymphocytes (Giles et al., 1997; Widyarini et al., 2001). This antioxidant has also demonstrated anti-inflammatory properties by suppressing UVB-induced expression of cyclooxygenase-2 in keratinocytes and inhibiting UVB-stimulated prostaglandin E2 synthesis in human epidermal cell cultures (Isoherranen et al., 1999; Miller et al., 1994). Finally, genistein has immune-modulating effects as it has proven to inhibit UV-induced immunosuppression in mice (Widyarini et al., 2001).

8.2. Tea

Tea (*Camellia sinensis*) is a potent source of polyphenols, containing approximately 30% to 35% of the dry weight of the leaf. Tea polyphenols are widely studied for their anticarcinogenic activity mostly in animal models of various cancers including that of skin. Tea polyphenols have shown strong skin cancer inhibition in mouse 2-stage carcinogenesis models (Alexis et al., 1999; Bickers et al., 2000; Bode et al., 2000; Katiyar et al., 1996; Yang et al., 2002). Both oral and topical green tea polyphenols

lowered chemically induced and UV-induced skin tumors (Huang et al., 1992; Wang et al., 1991). Green tea also inhibited growth of established skin tumors, as it prevented conversion of benign skin tumors to squamous cell carcinoma (Miller et al., 1994; Wang et al., 1992). Green tea and black tea were equivalent in effect and decaffeinated tea was shown to be less effective (Wang et al., 1994).

While the nature of anticarcinogenic effect is unknown, tea polyphenols are powerful antioxidants as they quench singlet oxygen, superoxide radical, hydroxyl radical, hydrogen peroxide, and peroxyl radical (Grinberg et al., 1997; Guo et al., 2003; Jovanovic et al., 2000; Reszko et al., 2009; Shi et al., 2000; Unno et al., 2002). It has been shown that tea polyphenols reduced UV-induced lipid peroxidation in skin (Kim et al., 2001) and oxidation of proteins in a free radical–generating system in vitro (Nakagawa et al., 2002). Tea polyphenols also regulate cellular redox signal transduction. In human keratinocytes, (-) epigallocatechin- 3-gallate inhibited factors involved in the photoaging process such as UVB-induced AP-1 activity and mitogen-activated protein kinase cell signaling pathways (Barthelman et al., 1998; Katiyar et al., 2001).

Studies indicate that tea polyphenols are anti-mutagenic. Tea polyphenols protected DNA from oxidation by hydrogen peroxide and UVB in vitro (Wei et al., 1999). In human skin fibroblasts, tea polyphenols protected against radiation-induced DNA damage (Parshad et al., 1998). In Jurkat lymphocytes, epigallocatechin gallate decreased DNA damage caused by free-radical generators and hydrogen peroxide (Johnson et al., 2000). Topical application of green tea polyphenols reduced UVB-induced pyrimidine dimers in epidermis and dermis (Katiyar et al., 2000).

Tea polyphenols have anti-inflammatory effects. Topically applied green tea polyphenols reduced UV-induced erythema and sunburn in human skin (Elmets et al., 2001). Topical (-) epigallocatechin-3-gallate decreased UVB-induced inflammatory responses and infiltration of leukocytes in human skin (Katiyar et al., 1999). Green tea polyphenols also have immune-modulating effects. Green tea polyphenols protected human skin from UV-induced Langerhans cell depletion skin (Elmets et al., 2001). Topical epigallocatechin-3-gallate protected against UVB-induced immunosuppression and tolerance in mice, while topical application of EGCG inhibited carcinogenesis and selectively increased apoptosis in UVB-induced skin tumors in mice (Katiyar et al., 1999; Lu et al., 2002).

In a human study, topical green tea extract inhibited UV-induced erythema and reduced DNA damage (Elmets et al., 2001). In another study, the combined use of oral and topical green tea on the clinical and histologic characteristics of photoaging was evaluated in 40 women with moderate photoaged skin (Chiu et al., 2005). The subjects received a green tea 10% cream plus an oral 300 mg green tea supplementation twice daily or a placebo treatment for 8 weeks. Through patient self-assessments, it was revealed that the green tea group had significant improvements in overall appearance (Chiu et al., 2005). Those receiving the green tea regimen also had a significant improvement in elastic tissue (Chiu et al., 2005).

8.3. Coffee Plant

The whole fruit of the coffee plant has been shown to contain a wide range of polyphenol compounds, including proanthrocyanidins, quinic acid, caffeic acid, caffeine and chlorogenic acid (Lupo et al., 2007). In the past, coffee growers discarded the fruit of the coffee plant, harvesting the coffee bean alone, however in vitro and ex vivo studies revealed that the outer fruit of the coffee plant has effective hydroxyl radical scavenging activity (Baumann, 2007a). Another study also found a positive relation between the level of chlorogenic acid in the extract and antioxidant activity (Baumann, 2007a). Additionally it was shown that caffeic acid inhibits UVB induced expression of COX-2 which can eventually lead to skin cancer (Kang et al., 2009). Moreover, positive effects on UVB induced carcinogenesis, as well as patches induced by UVB were ameliorated with caffeine administration and

topical application respectively in mice (Conney et al., 2008). In addition, co-incubation with quinic acid prevented skin death that was caused by UV damage in skin cultures (Mammone et al., 2006).

8.4. Fern

The extract of the *Polypodium leucotomos* (PL), a fern plant grown in Central America, was found to contain active components which include a variety of phenolic compounds such as p-coumaric, ferulic, caffeic, vanillic, and chlorogenic acids (Gombau et al., 2006). These compounds have shown to retain antioxidant, photoprotective, and chemopreventive properties, inhibiting lipid peroxidases and scavanging free radicals (Gombau et al., 2006; Middelkamp-Hup et al., 2004). PL appeared to be an effective photoprotective agent post oral administrations. It has been shown that there was a significant decrease in erythema in treated skin in healthy participants exposed to varying doses of UV radiation; with or without an oral administration of the extract (Middelkamp-Hup et al., 2004). Histologically, treated skin was characterized by less epidermal damage, fewer cells with sunburn, and fewer cyclobutane pyrimidine dimers (mutagenic and carcinogenic compounds), less epidermal proliferation, and less dermal mast cell infiltration (Middelkamp-Hup et al., 2004). Moreover, PL was also able to suppress the production of ROS that was induced by UV, therefore acting as antioxidant agent (Gonzalez et al., 2011). In addition, orally administered PL to mice resulted in inhibition of UVB radiation that triggered skin cancer (Siscovick et al., 2008).

8.5. Pine Bark

Pycnogenol™ is an extract from French maritime pine bark which contains several phenolic and polyphenolic flavonoids (Berson, 2008; Yoshikawa et al., 1990). Antioxidant properties of such compounds include prevention of lipid peroxidation and reductions in oxidative stress via an increase in glutathione (GSH) and the GSH antioxidant defense enzymes (Sime et al., 2004; Yoshikawa et al., 1990). In a mouse model, when a pycnogenol extract was applied after daily irradiation, UV radiation-induced inflammation, immunosuppression and carcinogenesis were reduced (Kanti et al., 2009; Yoshikawa et al., 1990). The wound-healing properties of this extract were further confirmed in an experimental rat model, where application of a Pycnogenol™ 1% to 5% gel significantly shortened wound healing time in a dose-dependent manner (Brincat et al., 1965). Moreover, Pycnogenol™ supplementation resulted in beneficial effects on skin hydration as well as skin elasticity which are mediated by hyaluronic acid and collagen, therefore suggesting this compound role in skin aging (Marini et al., 2012).

8.6. Mushroom

Various types of mushrooms such as shiitake and reishi have been consumed by people in many Asian countries for centuries (Berson et al., 2008). Mushroom extracts have been shown to have potent antioxidant and anti-inflammatory properties, including inhibition of lipid peroxidation, activities of superoxide dismutase and metalloproteinases, and levels of proinflammatory cytokines (Berson et al., 2008; Mau et al., 2002). In addition to these effects, the shiitake complex has been shown to inhibit the enzymes elastase, involved in elastin breakdown, and AP-1, involved in collagen break down (Berson et al., 2008). Moreover, a study on 45 healthy adults demonstrated that mushroom extracts stimulated growth of epidermal skin cells (Berson et al., 2008). Mushroom complex was applied as a serum twice daily or as a cream once daily to randomized sites of treatment (Nebus et al., 2007). Sites treated with all formulations of the mushroom extract were associated with significantly faster cell turnover rates compared with untreated sites of control subjects (Nebus et al., 2007). Similar effects were observed in a study involving 31 women subjects with moderate facial photodamage. Assessments revealed significant improvements in skin texture, clarity, a reduction in overall photoaging, fine lines, and pigmentation within only 8 weeks of treatment (Nebus et al., 2007).

8.7. Milk Thistle

Silymarin, a flavonoid isolated from milk thistle plant, is composed of different flavonolignans including silybin, silidianin, silychristin and isosylibin (Mereish et al., 1991; Wagner et al., 1974). Silybin shows more antioxidant and anti-inflammatory properties than other compounds in milk thistle, and is known to be an antioxidant compound with skin cancer chemopreventive properties (Comoglio et al., 1990; Wagner et al., 1974). Several experiments show that topical application of silymarin significantly inhibited UVB-induced skin edema, formation of sunburn and apoptotic cells (Katiyar et al., 1997). This evidence suggested that silymarin might provide protection against different stages of UVB-induced carcinogenesis (Afaq et al., 2002). It was shown that topical application of silymarin protects against UVB radiation-induced non-melanoma skin cancer in mice (Afaq et al., 2002). Female SKH-1 hairless mice were subjected to UVB-induced tumor initiation, phorbol ester-mediated tumor promotion; as well as DMBA-induced tumor initiation, UVB-mediated tumor promotion, and UVB-induced complete carcinogenesis (Katiyar et al., 1997). In all three procedures, topical application of silymarin prior to UVB irradiation/DMBA exposure significantly lowered tumor incidence, tumor multiplicity per mouse, and average tumor volume (Afaq et al., 2002).

8.8. Grape Seed

Grape seed extracted from various plants such as grapes are rich in pro-anthocyanidins, part of the flavonoid family (Vinson et al., 1995). Pro-anthocyanidins are powerful antioxidants with strong free radical scavenging activities (Guo et al., 1996). A potential antioxidant mechanism of photo protection by grape seed proanthocyanidins (GSP) has been suggested, as GSP inhibited the depletion of antioxidant defense components caused by UVB and appears to enhance SPF in humans (Afaq et al. 2003; Mantena et al., 2006; Mittal et al., 2003). Additionally, grape seed extract demonstrated photochemopreventive effects on skin cancer induced by UVB (Perde-Schrepler et al., 2012). Moreover, oral administration of this compound was also beneficial in reducing hyperpigmentaion (Baumann, 2007b). Additionally, grape seed extract supplemented mice noticed chemopreventive effects on skin cancer induced by UV (Filip et al., 2011a; 2011b). Furthermore, oxidative stress and apoptosis induced by UVB in skin was reduced when mice consumed grape seed extract (Filip et al., 2013).

8.9. Sea Buckthorn (SBT)

SBT is thorny nitrogen fixing deciduous shrub native to Europe and Asia, which is used as a medicinal plant in Tibetan and Mongolian traditional medicines (Lu, 1992; Patel et al., 2012; Rousi et al., 1971). Since the 1950's, many curative preparations of SBT have been clinically used to treat radiation damage, burns, oral inflammation and gastric ulcers in China and the former Soviet Republics (Fu et al., 1993; Geetha et al., 2002; Isoherranen et al., 1999; Mereish et al., 1991).

Leaf and fruit extracts of SBT at a concentration of 500 µg/ml were found to inhibit chromium-induced free radical production, apoptosis, and DNA fragmentation, and restored the antioxidant status. This data suggests that these extracts have cytoprotective properties, which may contribute to the anti-oxidant activity (Geetha et al., 2002). More than 200 bioactive components have been found in SBT plant, containing several chemical compounds including carotenoids, tocopherols, sterols, flavonoids, phenolics, lipids, and ascorbic acid. These compounds are of interest due to their biological and therapeutic activities including antioxidant and antiproliferative effects, hepatoprotective effects, antimicrobial effects and immunomodulation effects (Cheng et al., 2003; Christaki et al., 2012; Geetha et al., 2008; Grey et al., 2010; Nemtanu et al., 2009).

Berries of the SBT are an excellent source of phytochemicals such as ascorbic acid, tocopherols, unsaturated FA, phenols, and carotenoids. Berries have been used for the treatment of radiation

damage, burns, oral inflammation, and gastric ulcers (Kumar et al., 2011). Other observed positive health effects include reduction in plasma cholesterol level, inhibition of platelet aggregation, and regulation of immune function (Yang et al., 2002). Study findings reported that phenolics were main contributors to the antioxidant activity of SBT berries, leading to increased focus on using SBT berries for medical and cosmetic purposes as well as functional foods (Beveridge et al., 1999; Greenwel et al., 1995; Yoshikawa et al., 1990; Zhang et al., 1989). Seventeen phenolic acids were tentatively identified in SBT berries. Salicylic acid was the predominant phenolic acid, as it constituted between 55.0% (Otradnaja and Trofimowskaja cultivars) and 74.3% (Nevlejena cultivar) of the total phenolic acids present (Zadernowski et al., 2005). Another study tentatively identified only four phenolic acids, namely p-coumaric, ferulic, p-hydroxybenzoic, and ellagic acids in SBT berries harvested in Finland (Hakkinen et al., 2000).

Phytosterols are main constituents of sea buckthorn oils. β-sitosterol and 5-avenasterol are the major phytosterols found in sea buckthorn oil (Bal et al., 2011). The amount of phytosterol in SBT is significantly high and may exceed soybean oil by 4–20 times. Research indicates that the total phytosterol content, varied between subspecies and collection sites, in the seeds, fresh pulp/peel, and the whole berries were 1200–1800, 240–400, and 340–520 mg/kg, respectively (Yang et al., 2001).

9. Conclusion

Oxidative stress can occur from many internal and external factors including metabolism, pollution, and sunlight radiation. A wide variety of information supports the photocarcinogenic damage to the skin from sunlight and its relationship to oxidative stress. Antioxidants work together in skin, supporting and regenerating each other. Topical antioxidants may provide several advantages for photoprotection not provided by dietary supplements alone. As antioxidants are delivered into skin, they can provide protection by accumulating in pharmacologic concentrations and targeting exposed skin. As oxidative stress depletes natural antioxidant stores, these concentrations offer protection by supplementing reserves.

There are several natural ingredients found in many plants with antioxidant and anti-inflammatory properties that appear to be effective for photoprotection (see Figure 2). More notably, some of these agents such as soy, mushroom extracts, and tea, also have chemopreventive properties that offer potential for the prevention/treatment of skin diseases and cancers. The botanical compounds discussed here show significant anti-inflammatory, antioxidant and cell protective effects. These protective effects may contribute to their anti-photocarcinogenic effects and act to inhibit various biochemical processes induced by solar UV radiation. Based on the epidemiological evidence and laboratory studies conducted using in vitro and in vivo systems, it is suggested that regular consumption and topical treatment of these polyphenols may provide effective protection against the harmful effects of aging and UV radiation.

Consumer-driven demand has led to rapid development of products to counteract signs of aging skin. Botanicals found in cosmeceuticals may offer skin protection from photodamage and repair skin by improving or stimulation of new collagen production.

Combined with sunscreens and other sun protection, cosmeceuticals can help enhance skin appearance and health. More importantly, as many cosmeceuticals claim different effects, future trends should include multifunctional cosmetics which will allow for optimal skin health benefits to be plausible.

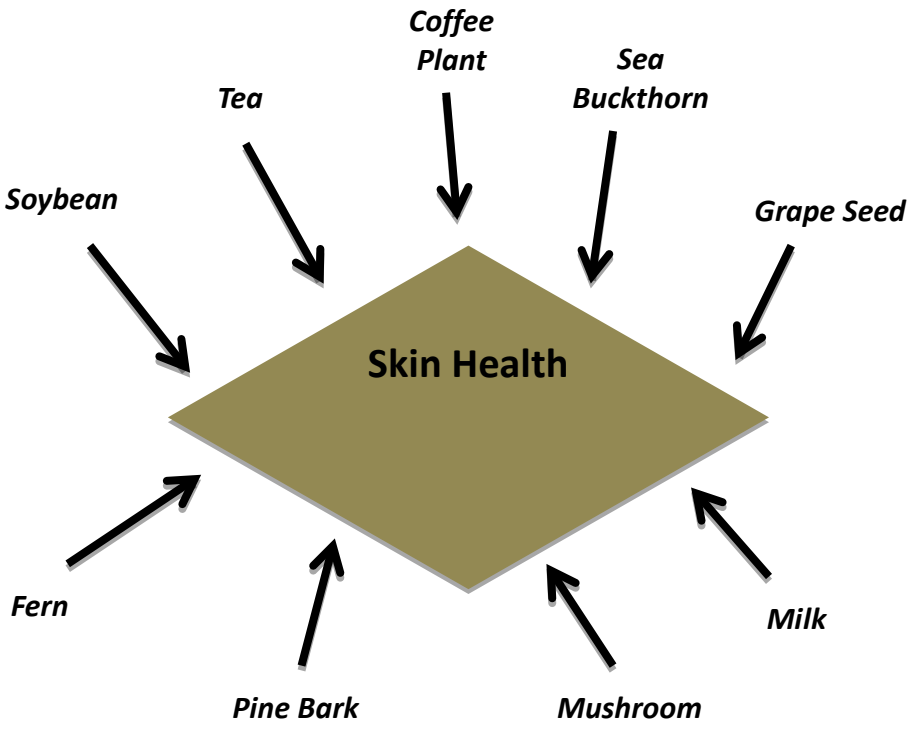

Figure 2: *Botanicals Beneficial for Skin Health*

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Sensory and Objective Evaluation of Pumpkin Bars using Ground Flaxseed or Sweet Potato Baby Food as Egg Replacers

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Abstract The purpose of this study was to produce an acceptable dessert product that met the dietary needs of an individual with an egg allergy. Three variations of pumpkin bars were analyzed to determine sensory and objective differences in taste, mouthfeel, overall acceptability, batter viscosity, and springiness that resulted due to the elimination of eggs in the recipe. The experimental variations consisted of a replacement of eggs with ground flaxseed, a replacement of eggs with sweet potato baby food, and a control recipe utilizing eggs. Samples of each variation's batter were subjected to viscosity testing using a Bostwick Consistometer. The distance that each 30mL batter sample travelled was recorded at two and four minutes. Once the pumpkin bars were baked and cooled, they were cut into 1"x1" squares for sensory analysis. A total of thirty-nine participants completed a randomized taste test of the pumpkin bars and filled out a ballot to rate the samples on a four-point Likert scale for taste, mouthfeel, and overall acceptability. Data collected from the sensory ballots was entered into SPSS, and paired sample *t*-tests were completed to compare each of the three variations. The variation made with sweet potato baby food had the highest average ratings for taste and overall acceptability; however, statistical analysis concluded that there was no significant difference between the control and either variation for taste, mouthfeel, or overall acceptability. The pumpkin bar samples were subjected to a springiness test using a Brookfield Texture Analyzer, with the Control producing the springiest final product. It was concluded that acceptable dessert products, specifically pumpkin bars, can be produced without the use of eggs, and that certain egg replacers may even create products of greater acceptability to consumers.
Keywords *Dessert Product; Egg Allergy; Sensory Analysis*

1. Introduction

Food allergy prevalence is increasing within the population, with eggs being one of the top eight allergens found in foods [1]. This makes it necessary to develop effective ways to replace eggs in food products and discover egg alternatives made with ingredients that consumers readily have access to. A major category of foods that frequently incorporate eggs are baked products. The

diverse function of eggs as leavening agents, binders, thickeners, and emulsifying agents makes it difficult to create a universal substitute [2].

Ground flaxseed when mixed with water will form a gel comparable to the consistency of eggs. The literature revealed limited research studies using flaxseed as a flour replacer [3, 4], but not distinctively as an egg replacer. Borneo, Aguirre, & Leon [5] concluded that a similar gel produced with chia seeds could be utilized as an effective egg or oil replacer, but no research could be found that specifically used flaxseed as an egg replacer in baked products. The effectiveness of this flaxseed gel in replicating the baking properties found in eggs was evaluated.

Previous studies have created appealing dessert products by replacing eggs with pureed fruits or vegetables, however little information could be found on the use of sweet potatoes in this area. One study attempted to utilize sweet potatoes as a fat replacer in brownie products and reported that the variation with 100% replacement of fat strongly correlated with overall acceptability [6]. Sweet potatoes were chosen as the second variation in this study because of the rich orange color that would blend nicely with the canned pumpkin in the Control recipe and the lack of research evaluating the use of sweet potatoes as effective egg substitutes.

The main objective of this study was to create an acceptable dessert product that eliminated the use of eggs for individuals with an egg allergy. The purpose of this specific experiment was to test the overall acceptability of pumpkin bars prepared using ground flaxseed or sweet potato baby food as a replacement for eggs. The following research questions were addressed through the experiment: i) Is there a statistically significant difference in desired sensory properties of taste, mouthfeel, and overall acceptability for pumpkin bars made with a) Ground flaxseed, b) Sweet potato baby food, and c) Control recipe using eggs? ii) Is there a statistically significant difference in desired objective variables of batter viscosity and cake springiness for pumpkin bars made with a) Ground flaxseed, b) Sweet potato baby food, and c) Control recipe using eggs? It was predicted that pumpkin bars made with ground flaxseed would have an equal taste rating, denser mouthfeel, thinner batter viscosity, and decreased springiness. Pumpkin bars made with sweet potato baby food were predicted to have a higher taste rating, denser mouthfeel, thicker batter viscosity, and decreased springiness.

2. Materials and Methods

2.1. Ingredients

Three variations of pumpkin bars were prepared to test quality characteristics of dessert products produced with eggs, flaxseed gel, or sweet potato baby food. Each variation contained the same ingredients except for the substitution of eggs with an egg replacer. The ingredients used in each variation can be found in Table 1. The ingredients utilized in each pumpkin bar recipe were entered into Food Processor (Version 10.12.0), a nutrient analysis software, to determine the total Calories, fat, carbohydrate, fiber, and protein found in one serving size of pumpkin bars. It was determined that one batch of pumpkin bars yielded thirty-five 2.1”x2.2” servings.

Table 1: *Ingredients Incorporated into Each Pumpkin Bar Variation*

Ingredient	Control Recipe – Eggs	Variation #1 – Ground Flaxseed	Variation #2 – Sweet Potatoes
Sugar	392.0g	392.0g	392.0g
Vegetable Oil	118mL	118mL	88.5mL
Canned Pumpkin	425.3g	425.3g	425.3g
Eggs, beaten	200.0g	-	-
Water	-	88.8g	-
Ground Flaxseed	-	16.2g	-

Sweet Potato Baby Food	-	-	168.0g
All Purpose Flour	252.0g	252.0g	252.0g
Baking Powder	7.0g	7.0g	7.0g
Baking Soda	4.7g	4.7g	4.7g
Salt	2.3g	2.3g	2.3g
Cinnamon	4.6g	4.6g	4.6g
Ground Cloves	0.6g	0.6g	0.6g

2.2. Procedure

Three jelly-roll pans with dimensions of 10.5”x15.5” were obtained for the Control and two variation recipes of pumpkin bars. Each pan was greased with one tablespoon of margarine and one tablespoon of flour to prevent the product from sticking to the pan after the bars had cooked and cooled. After the pans were greased, the oven was preheated to 350^{o}F (177^{o}C).

For the Control recipe, all ingredients were obtained and precisely measured. To create consistency in measurements, each individual ingredient was measured by the same researcher throughout the preparation of the variations. The eggs were measured by cracking four eggs into a single bowl and beating them together with a fork. A portion of 200.0g was then measured from the beaten egg mixture.

For Variation 1, using flaxseed and water, the same ingredients and mixing procedure were followed, except for the addition of eggs. Each egg was replaced with one tablespoon (8.1g) of ground flaxseed and three tablespoons (44.4g) of water. The Control recipe required the use of four eggs; however the equivalent of only two eggs were replaced with the ground flaxseed gel. Therefore, a total of 16.2g of ground flaxseed and 88.8g of water were utilized in replacement. The two ingredients were mixed in a small bowl with a metal fork with the flaxseed added first followed by the addition of water. The flaxseed mixture was placed in the refrigerator for twenty-five minutes in order for the mixture to effectively set and thicken. The gel mixture was stirred intermittently throughout the refrigeration period.

Variation 2 also followed the same ingredient and mixing preparations as the Control recipe, except that sweet potato baby food was used in replacement of the eggs, and the amount of oil utilized was decreased to 88.5mL. Each egg was replaced with ¼ cup (56g) of sweet potato baby food; however, the equivalent of only three eggs was replaced with the pureed sweet potatoes. Therefore, a total of 168.0g of sweet potato baby food was utilized in replacement.

After all of the ingredients had been accurately measured, they were combined into a large glass bowl. The ingredients were mixed with an electric hand mixer at a medium speed for three minutes. At this point, volumes of 90mL were removed from each batter preparation to be used for objective testing using a Bostwick Consistometer. The remaining batter was then poured into one of the jelly roll pans and placed in an oven to bake. The bars were allowed to cook for thirty minutes at 350°F (177^{o}C), or until a toothpick inserted in the center came out clean.

The products were prepared in the same room and on the same day to eliminate extraneous environmental variables. Oven temperature remained constant over all variations; however multiple ovens were utilized for preparation. Once removed from the oven, the pumpkin bars were allowed to cool to room temperature while sitting on the counter for thirty minutes. The bars were then covered with plastic wrap and foil and stored on the counter until it was time to cut and serve.

Using a medium sized chef's knife, a 1.25" border was removed from the perimeter of the bars to create uniformity in all samples and to remove the portion of the bars subjected to greater heat at the edge of the pan. The remaining bars were cut into 1"x1" squares for sensory analysis and objective testing using a Brookfield Texture Analyzer.

2.3. Objective Testing

After the ingredients were mixed with an electric hand mixer, volumes of 90mL were removed from each batter preparation. Each batter sample was tested for viscosity using a Bostwick Consistometer. Viscosity specifically measures the resistance to flow of matter [7]. Individual samples of 30mL were poured into the closed gate of the leveled device, and the chamber was opened when the timed trial began. The force applied was the gravitational pull on the batter sample itself. The distance the batter traveled over a two minute and four minute period was recorded. Three trials of each variation were completed, and all results were averaged for comparison.

Three 1"x1" pumpkin bar samples of each variation were reserved for objective analysis. These samples were tested for springiness using a Brookfield Texture Analyzer. This machine applies force to samples using different probes to simulate a variety of stresses. The number two attachment was utilized to complete a springiness test. Springiness refers to how well a product physically springs back after it has been deformed from compression [7]. The spring back is measured at the down stroke of the second compression from the device. Three trials of each variation were subjected to testing, and their results were averaged.

2.4. Sensory Testing

The three samples provided for each sensory analysis were taken from the same location in each pan using a template. Samples were placed on white paper plates that were equally divided into thirds. A random three digit number was assigned to each sample and these codes were transcribed onto the plates prior to starting the experiment. After cutting the bars, samples were placed into their respective portions on the plate.

The sensory analysis was conducted at a large Midwestern public university. The panelists participating in the study were a convenience sample and were not trained for this sensory test analysis. Individuals who had any form of food allergy were not permitted to participate in the study.

The test began by reading an individualized experiment synopsis and handing out a consent form for each panelist to sign. After having all participants sign and date the waiver, sensory ballots and samples were distributed. Sample plates were slightly rotated when presented to panelists to help prevent positional bias. The panelists were encouraged not to speak during the analysis of the pumpkin bars to eliminate suggestive error. The ballot asked the test subjects to rate the three pumpkin bars on their taste, mouthfeel, and overall acceptability on a four-point Likert scale (Table 2). The four choices on the scale ranged from one being very dissatisfied to four being very satisfied. The goal was to test the acceptability of the products and to determine if there was a statistically significant difference among the three variations.

Table 2: *Pumpkin Bar Sensory Evaluation Ballot*

Sample Characteristics	Very Satisfied	Satisfied	Dissatisfied	Very Dissatisfied
Taste- Should experience a rich pumpkin flavor accompanied by a nice balance of spices	4	3	2	1
Mouthfeel- Should feel moist and fluffy, not dry or dense	4	3	2	1
Overall Acceptability- How well would you rate this pumpkin bar when considering both taste and mouthfeel?	4	3	2	1

2.5. Statistical Analysis

The sensory data recorded from panelist ballots was compiled and entered into an Excel spreadsheet. The data was then downloaded into SPSS (Version 19) for statistical analysis to calculate the overall flavor, mouthfeel, and overall acceptability of each pumpkin bar variation. Descriptive and inferential statistics were used to calculate the ratings recorded by the panelists. A paired-sample *t*-test was performed for the variables of flavor, mouthfeel, and overall acceptability for each variation. Descriptive statistics were used to analyze objective data from the batter viscosity and springiness tests.

3. Results and Discussion

3.1. Results

3.1.1. *Nutritional Analysis*

The nutritional analysis values were generated using Food Processor and represent a single pumpkin bar serving. The Control contained the greatest amount of Calories, while Variation 2 contained the least amount of Calories per serving. Variation 2 also contained the least amount of fat at 2.52g. The Control contained the greatest amount of protein and was the only recipe to contribute any cholesterol. The sweet potato variation contained the greatest amount of carbohydrates per serving, while the flaxseed variation contained the greatest amount of fiber. Complete nutritional analysis of the pumpkin bars is found in Table 3.

Table 3: *Nutritional Analysis of Pumpkin Bar Variations*
Totals are Representative of One Serving Size of Pumpkin Bars—2.1"x2.2"

	Control Using Eggs	Ground Flaxseed	Sweet Potatoes
Total Calories	109.87 kcal	103.84 kcal	98.25 kcal
Fat	3.86g	3.48g	2.52g
Cholesterol	21.26mg	0.00mg	0.00mg
Carbohydrates	17.85g	17.95g	18.56g
Fiber	0.77g	0.91g	0.84g
Protein	1.67g	1.06g	1.02g

3.1.2. *Sensory Testing*

Table 4 presents the results from the sensory analysis testing. A total of thirty-nine subjects participated in the study and completed a sensory ballot. For all variables tested, there was no significant difference ($p<0.05$) between any of the three variations. All samples were rated as acceptable on the four-point Likert scale. The lowest average score was given to the taste of the

Control at 3.15, while the highest scores were given to the average mouthfeel of the Control and Variation 2—both at 3.54. Variation 2 scored the highest in acceptability, while Variation 1 scored the lowest.

Table 4: *Paired-Sample t-Test for Taste, Mouthfeel, and Acceptability of Pumpkin Bar Variations*

Variable	Mean n=39	SD
Taste		
Control	3.15	0.670
Variation 1[a]	3.28	0.647
Variation 2[b]	3.33	0.092
Mouthfeel		
Control	3.54	0.505
Variation 1[a]	3.51	0.644
Variation 2[b]	3.54	0.682
Acceptability		
Control	3.31	0.614
Variation 1[a]	3.23	0.627
Variation 2[b]	3.41	0.595

[a]Variation 1 – Ground Flaxseed
[b]Variation 2 – Sweet Potato Baby Food

3.1.3. *Objective Testing*

Table 5 shows the results of the objective testing for all pumpkin bar variations. Three trials were completed for each variation and results were averaged. For the springiness test, the Control had the greatest springiness at 3.57mm while Variation 1 had the least springiness at 3.06mm. For the batter viscosity testing using the Bostwick Consistometer, the Control travelled the furthest throughout the duration of the test. Variation 2 was the most viscous and travelled the shortest distance in the device.

Table 5: *Mean and Standard Deviation for Objective Testing of Pumpkin Bars*

Variable	Mean N=3	SD
Springiness (mm)		
Control	3.57	0.025
Variation 1[a]	3.06	0.012
Variation 2[b]	3.23	0.215
Distance Spread at 2 min (cm)		
Control	0.53	0.076
Variation 1[a]	0.12	0.029
Variation 2[b]	0.02	0.029
Distance Spread at 4 min (cm)		
Control	0.63	0.116
Variation 1[a]	0.22	0.029
Variation 2[b]	0.17	0.289

[a]Variation 1 – Ground Flaxseed
[b]Variation 2 – Sweet Potato Baby Food

3.2. Discussion

3.2.1. *Nutritional Analysis*

The results from the nutritional analysis show that the sweet potato variation would be the best option for individuals concerned with total Caloric intake. Although the difference in total Calories is relatively small, this difference is most likely the result of decreased fat content in Variation 2. The observation

of overly moist pumpkin bar products was observed in pilot trials utilizing both sweet potatoes and ground flaxseed. This is why equivalents of eggs were not replaced in a one-to-one ratio in the final recipes. In attempt to lower the proportion of wet ingredients in Variation 2, the equivalent of three eggs were replaced with sweet potato baby food and the total amount of vegetable oil was reduced by 29.5mL. Findings of overly moist pumpkin bars were unexpected in Variation 1 since findings by Koca and Anil [4] showed bread products made with ground flaxseed to have increased water absorption due to the high fiber content. The discrepancy of egg equivalents was a variable that was not effectively controlled in the methodology of the experiment and can optimistically be perfected in future studies.

Both Variation 1 and Variation 2 contained zero grams of cholesterol as a result of the elimination of the eggs. This would make these alternatives better options for individuals concerned with their total cholesterol levels and risk for cardiovascular disease and additionally allows vegans, who eliminate all animal products from their diet, to consume this specific baked product. It was also noted that the flaxseed and sweet potato variations had over a half a gram less of protein per serving. With protein as such a focus in food products as of late, this may be seen as a negative factor; however, other benefits such as decreased fat, decreased cholesterol and increased fiber may be of greater concern to consumers.

3.2.2. *Sensory Analysis*

Between the Control, Variation 1, and Variation 2 there was no statistically significant difference over all variables tested. The data from the sensory analysis responses supports the main study objective that acceptable variations of pumpkin bars can be made without the use of eggs. The data suggests that the variations prepared without the use of eggs are just as acceptable as the Control. This contradicts previous findings stating that eggs are essential for cake products to meet quality standards [8].

Even though there was no statistically significant difference between the variations, Variation 2 was rated higher in taste and overall acceptability than the Control. This finding suggests that the addition of the substitute ingredients in replacement of eggs added unique flavor profiles that improved the taste of the final product. The results matched the findings of Forrester et al., [6] who concluded that acceptable baked products can be produced by replacing ingredients with sweet potatoes.

The high proportion of canned pumpkin in this recipe may have compensated for the elimination of egg products due to its moisture and binding qualities. Thus, the addition of ground flaxseed or sweet potato baby food may have had little to no effect on the final product. One avenue for future research would be to produce a variation eliminating the use of eggs with no additional ingredients to compensate for the exclusion. This would test how large of an impact the canned pumpkin played in reproducing the quality characteristics of the eggs.

It was expected that the samples containing flaxseed would have a lower mouthfeel rating due to the grainy texture that the seeds added to the batter. This variation was also perceptibly different because the seeds were visible in the final baked product. These factors may have resulted in Variation 1 having the lowest overall acceptability rating. The findings do support that the gel produced when combing water with ground flaxseed does create pumpkin bar products of high acceptability. Further research should be conducted to determine if this substitution could be generalized to all baked products using eggs.

3.2.3. *Objective Analysis*

Since only three samples of each variation were subjected to each objective test, indication of significance cannot be assumed due to small sample size. Future studies with the resources to conduct objective tests in greater depth are needed to strengthen the findings in this study.

Variation 1 was the least springy of all the samples, which was supported by previous research that found decreased springiness in muffins using ground flaxseed [3]. Previous research conducted using chia seeds as egg replacers in cakes also found similar results. The gel formed from chia seeds mixed with water was comparable to how the flaxseed gel was prepared in this study. Cakes prepared with chia seeds were denser than the Control and had a decreased volume [5]. This recurring finding suggests that foods made with flaxseed gel will have denser qualities that decrease the overall height and airiness of the final product.

The decreased quantity of protein found in Variations 1 and 2 may have hindered the overall structure of the pumpkin bars while baking. While eggs would denature and coagulate in response to the prolonged heat in the oven, neither the flaxseeds nor the sweet potatoes would be able to reproduce these stability and structural qualities. This may have contributed to the pumpkin bar products being more compact and less springy upon testing.

Measurements of batter viscosities illustrated apparent differences between the variations. The Control contained the greatest amount of liquid ingredients, which most likely contributed to its decreased viscosity and increased spread. Modifications in the amount of liquid ingredients in the variations likely caused the variance when testing this variable.

4. Conclusion

The purpose of this experiment was to create an acceptable dessert product that eliminated the use of eggs for individuals with an egg allergy. The production of acceptable pumpkin bars can be achieved without the use of eggs, as supported by the experimental data. Ground flaxseed and sweet potato baby food appear to be quality egg substitutes in the production of pumpkin bars; however further research should be conducted to see if these specific egg substitutions can be applied to other baked products.

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Vitamin B_{12} Intake Correlated to Physical and Mental Improvements in Multiple Sclerosis Specific Quality of Life

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Abstract Current literature fails to provide individuals with multiple sclerosis (MS) specific dietary recommendations to advance quality of life (QOL). Due to the important structural and functional roles of vitamin B_{12} in the nervous system, the purpose of this research was to determine possible correlations between dietary intake of vitamin B_{12} and self-reported quality of life (QOL) among individuals with MS. The National MS Society and MS Foundation were used to recruit volunteers age 18 and older with a clinical diagnosis of MS. After the initial response (n = 89), 46 participants completed an online demographic survey/questionnaire and the MS Quality of Life-54 (MSQOL-54). Additionally, participants (n=23), completed a 3-day food record utilizing *MyPyramid Tracker*. Increased consumption of vitamin B_{12} (M=4.63 ± 3.44 µg) was positively correlated to the MSQOL-54 Subscales, *Emotional Well-Being*, *Health Perceptions*, *Health Distress*, and *Overall QOL*, as well as, to the QOL Composite Summary score for Mental Health. Individuals who consumed 5.0 µg or more of vitamin B_{12} exhibited significantly higher QOL scores for eight of the twelve Subscales, including *Pain* and *Overall QOL* (p<0.01). Additionally, both of the QOL Composite Summary scores (Physical and Mental) were significantly higher (p<0.01) than individuals who consumed less than 5.0 µg of vitamin B_{12}. Dietary intake studies like this one can assist in producing dietary guidelines for individuals with MS, which are currently absent from the *Nutrition Care Manual*. Since MS currently has no known cure, efforts of healthcare professionals should focus on influencing QOL through specific micronutrient intake recommendations, especially vitamin B_{12}. Increased vitamin B_{12} requirements may be needed for individuals with MS to achieve greater QOL.

Keywords *Multiple Sclerosis; Quality of Life; Vitamin B_{12}*

1. Introduction

The Academy of Nutrition and Dietetics has produced a medical nutrition therapy diet guide, the *Nutrition Care Manual*, to aid healthcare professionals in recommending appropriate dietary guidelines to individuals with a wide variety of illnesses and disease states (approximately 50 listings, including three neurological disorders). Dietary guideline recommendations specific to multiple sclerosis (MS) are currently not addressed or specified in this widely used and well respected resource. The purpose of this research was to determine possible correlations between dietary intake, specifically vitamin B_{12} and self-reported quality of life (QOL) among individuals with MS.

The National Multiple Sclerosis Society (NMSS) (2012) stated that about 400,000 individuals in the United States and over 2.5 million people worldwide have been diagnosed with MS, a chronic, immune-mediated inflammatory and neurodegenerative disease of the central nervous system (CNS), with an etiology that is not yet fully understood. In MS, the immune system attacks the myelin sheath of nerve cell fibers in the brain and spinal cord [1]. As a result of the autoimmune attack, the myelin sheath becomes damaged, resulting in inflammation and lesions that disrupt messages to and from the CNS [2; 3]. Individuals with MS have demonstrated poorer QOL in the areas of physical symptoms, mobility, emotional life and social interaction compared with persons having other chronic diseases. Many researchers have attributed poorer QOL to the unpredictable course of the disease and the variable symptoms: fatigue, pains, bowel and bladder dysfunction and impairment of mobility and visual acuity [4]. Many other symptoms associated with MS can greatly affect an individual's QOL. Symptoms can be described further as multidimensional experiences along four dimensions, intensity (strength or severity), timing (frequency and duration), level of distress (degree of discomfort), and quality (how the symptom feels) [5].

Various studies have pointed out the possibility of a potential relationship between MS and vitamin B_{12}. The serum B_{12} levels of MS patients are lower than those seen in healthy individuals [6; 7], and multiple researchers have recommended further exploration of this connection. Previous findings have suggested there may be an association between the time of onset for the first neurological symptoms, and a person's vitamin B_{12} status [8]. Vitamin B_{12} and Folate serum levels are typically taken initially during introductory MS diagnosis to rule out nutritional micronutrient deficiencies [9]. This is due to the fact that vitamin B_{12} deficiency and MS share similar manifestations in their physiology [10]. Increased dietary vitamin B_{12} intake may be beneficial in combination with current immunotherapies in patients with MS and has shown promising results when implemented [11]. Results from one study show that malabsorption of vitamin B_{12} may exist in almost 12% of the MS population [12]. Commonality of low vitamin B_{12} levels in association with patients diagnosed with MS poses the question of MS patients' physiological needs for vitamin B_{12}. A 2011 meta-analysis examining the relationship of vitamin B_{12} and other micronutrients in patients diagnosed with MS shows a direct relationship between low vitamin B_{12} levels and patients diagnosed with MS [13].

2. Materials and Methods

2.1. Participants

A sample (n = 49) of individuals living in the United States with a declared medical diagnosis of MS, all of which were at least 18 years old, participated in this study. Out of the 83 individuals who showed interest in the study, 49 of them (59%) completed the Multiple Sclerosis Online Survey, and of those participants, 23 of them (47%) went on to additionally complete a self-administered 3-day food record. The majority (92%) of the participants were Caucasian (n = 45), and all of the participants who completed both parts of this study were female. Of the 49 total participants, 36 of

them (74%) had relapsing-remitting MS, four (8%) had progressing-relapsing MS, five (10%) had secondary progressive MS, three (6%) had primary progressive MS and one participant had benign MS (2%). Of the 23 participants who completed both parts of this study, 87% of them had relapsing-remitting MS.

2.2. Recruitment

After approval from a large, Midwestern university's Institutional Review Board (IRB), the recruitment process began and was conducted according to IRB specifications. Permission was requested and granted by the NMSS to publicize information regarding this study on their website page "Surveys and other Research Studies." Permission was also granted by the NMSS to personally email each state or regional chapter's representative. Every chapter was asked to announce this study on their website, provide information about the study in their monthly newsletters, and included in their chapter's listserv e-mailings. The Multiple Sclerosis Foundation (MSF) was similarly contacted requesting permission to assist in the recruitment process and support groups across the United States were contacted inviting members to participate in this study. Lastly, the researcher's university listserv was employed to gather participants who might meet the criteria (medical diagnosis of MS and over the age of 18 years).

2.3. Instruments and Data Collection

Each participant was first instructed to complete the Multiple Sclerosis Online Survey using the hyperlink provided to them in an email. Upon review of the informed consent, participants indicated their willingness to participate by checking the appropriate box, allowing them to begin the two-part online survey. The first 13 questions contained the Personal Background Questionnaire, which, with permission, was modified from the Personal and Family Questionnaire [14]. This tool gathered demographic information regarding household income, ethnic background, marital status, time since diagnosis, type of MS, current period of relapse or remission, employment status, and current medication regiment. Lastly, the participants were asked to type in their individual MyPyramid Tracker User ID provided by the researcher in the email. This aided the principal investigator in identifying and linking the participants' online surveys to their specific self-administered 3-day food record. The individual participants were not identifiable beyond their assigned User ID and were not required at any time to include other personal identifiers.

The second part of the online survey, the MSQOL-54 (Multiple Sclerosis Quality of Life-54), began immediately after the Personal and Family Questionnaire. The MSQOL-54 is a multidimensional health-related quality of life measure that combines both generic and MS-specific items into a single instrument [15]. This 54-item instrument generated 12 Subscales (*Physical Health, Role Limitations due to Physical problems, Role Limitations due to Emotional issue problems, Pain, Emotional Well-Being, Energy, Health Perceptions, Social Function, Cognitive Function, Health Distress, Sexual Function*, and *Overall QOL*) along with two additional questions, one regarding *Change in health* and the other pertaining to *Satisfaction with sexual function*. These two additional questions are referred to by the researcher as the two Independent Categories. Two composite summary scores were calculated as products of the various Subscales, the *Physical Health Composite Summary* score and the *Mental Health Composite Summary* score. Past researchers have concluded that in spite of the lengthy time of administration, the MSQOL-54 is still favorable and reliable for detecting the QOL in any period of MS, including remission [16]. The results also indicated that MS-specific measures of QOL might be used for detecting the effectiveness of treatment during the relapse period in patients with MS. Due to the nature of MS, QOL may vary greatly over the course of a week or a month depending on the occurrence and/or the severity of an exacerbation or relapse. The MSQOL-54's ability to show effectiveness during any period of the disease has been noted as an important characteristic of this instrument [4].

Following the completion of the Multiple Sclerosis Online Survey, a hyperlink directed the participants to MyPyramid Tracker. This free, online-based dietary self-assessment tool was part of ChooseMyPlate.gov, and translated the principles of the 2005 Dietary Guidelines for Americans (DGA) and other nutrition standards developed by the USDA and Department of Health and Human Services [17]. This tool was used by the participants to perform a self-administered 3-day food record, including everything eaten or drank over the course of three consecutive days, preferably including at least one weekend day, and complete with quantities and specific preparation methods for each item. This type of dietary assessment is preferred since it does not rely heavily on subject's memory and is not as invasive or time-consuming compared to other methods [18-22].

Educational materials attached to the email, provided information on common serving sizes and detailed step-by-step instructions on how to complete a self-administered 3-day food record using MyPyramid Tracker. The educational materials used by participants in this study were adapted from the public domain tool, MyPyramid Tracker Tutorial. Detailed instructions and educational materials can reduce human error and has been shown to be effective in populations with a severe illness [18; 23].

Once the participants accessed MyPyramid Tracker, they were asked five additional demographic questions pertaining to age, gender, entry date, height, and weight. Participants then proceeded to record all foods and beverages consumed over the course of their first day by searching the USDA food database, which is linked to MyPyramid Tracker. Many of the food choices included specified preparation or cooking methods, and required the participants to select serving sizes and number of servings they consumed for each item. The educational materials provided by the researcher included serving size information by comparing different serving volumes to common objects (a baseball = 1 cup), further enhancing the participant's chance of recording an accurate food record [18; 23]. This process was repeated for all foods and drinks consumed by each participant over the course of three days.

2.4. Analysis of the Data

Participants' results from the MSQOL-54 questions were scored using the MSQOL-54 scoring form [15]. Each available answer per question corresponded to a pre-set numerical value. Every question, besides the two questions that inquired about *Change in health* and *Satisfaction with sexual function*, was part of one of the 12 diverse Subscales. Scores for each question relevant to a specific Subscale were added together and then divided by the total number of questions that corresponded to that Subscale. Subscale scores were based on a 0-100 scale, zero being the worst QOL. To determine the Composite Summary scores multiple Subscales were added together based on the nature of their content (mental or physical) and the sum was divided by the number of Subscales included in the Composite Summary. Eight of the Subscales (*Physical Function, Health Perceptions, Energy, Role Limitations – Physical, Pain, Sexual Function, Social Function* and *Health Distress*) were used to calculate the Physical Health Composite Summary score and five of the Subscales (*Health Distress, Overall QOL, Emotional Well-Being, Role Limitation – Emotional* and *Cognitive Function*) were used to calculate the Mental Health Composite Summary score. *Health Distress* was the only Subscale designed to be calculated in both Composite Summary scores [15].

Participants' MyPyramid Tracker accounts were accessed weekly to collect data from individuals who had recorded three days of food entry. The daily consumption over the course of the 3-day food record was used to calculate the mean consumption per individual participant. MyPyramid Tracker provided information regarding 30 different dietary components, including vitamin B_{12}. The

participants' mean vitamin B_{12} consumption over the course of the three days was used for statistical analysis.

2.5. Statistical Analysis

Once all the data were entered and calculated from the MSQOL-54, including results from the Personal Background Questionnaire, data were analyzed using SPSS v.19 for statistical analysis. MyPyramid Tracker's results were collected in a similar fashion using an Excel spreadsheet to record data over time. When the data collection period ended, only the means of the 3-day food records were entered into SPSS v.19 and used in data analysis. Correlation analysis was utilized to determine if a relationship existed between vitamin B_{12} intake and any of the 12 Subscales, two Independent Categories or two Composite Summary scores. Additionally, multiple independent *t*-tests were used to determine if QOL differences existed based on specific vitamin B_{12} intake levels.

3. Results

Possible relationships between vitamin B_{12} intake and Subscale, Independent Categories and Composite Summary scores of the MSQOL-54 were examined. The mean scores for Subscales, Independent Categories and Composite Summary scores of the MSQOL-54 are detailed in (Table 1). The mean vitamin B_{12} intake was 4.63 ± 3.44 µg, with consumption levels ranging from 1.00 µg to 13.60 µg.

Table 1: *Overall Results of the MSQOL-54*

MSQOL-54 Subscales	Scores[1]
Physical Health	75.65 ± 32.94
Role Limitation (Physical)	35.87 ± 32.71
Role Limitation (Emotional)	79.71 ± 39.87
Pain	72.32 ± 25.26
Emotional Well-Being	77.74 ± 14.71
Energy	42.0 ± 25.97
Health Perceptions	54.78 ± 27.36
Social Function	68.12 ± 32.63
Cognitive Function	60.43 ± 28.48
Health Distress	66.74 ± 30.36
Sexual Function	65.59 ± 29.01
Overall QOL	72.75 ± 20.66
MSQOL-54 Independent Categories	
Change in Health	50.0 ± 26.11
Satisfaction with Sexual Function	57.61 ± 35.7
MSQOL-54 Composite Summary	
Physical	60.29 ± 24.84
Mental	73.4 ± 19.04

[1]Data are presented as mean ± SD, *n*= 23

There was a positive, moderately strong, and statistically significant relationship between vitamin B_{12} intake and the Subscale *Emotional Well-Being* (*r* (21) =0.52, *P* =0.010). Additionally, three other Subscales displayed a positive, moderate, and statically significant relationship to vitamin B_{12} intake, *Health Perceptions* (*r* (21) =0.44, *P* =0.034), *Health Distress* (*r* (21) =0.45, *P* =0.030) and *Overall QOL* (*r* (21) =0.47, *P* =0.024). Similar significance was found for the *Mental Health Composite Summary* Score. There was a positive, moderately strong, and statistically significant relationship between vitamin B_{12} intake and the *Mental Health Composite Summary Score* (*r* (21) =0.47, *P* =0.025).

Due to the statistically significant correlation between vitamin B_{12} intake and some measures of the MSQOL-54, vitamin B_{12} intake and QOL scores were further assessed through multiple independent *t*-tests. The Recommended Dietary Allowance (RDA) (2.4 µg) was used to assess if consumption at levels recommended influenced QOL. The mean scores for the participants who consumed less than the RDA were not significantly different compared to the mean scores for the participants who consumed at or above the RDA for vitamin B_{12} (Table 2).

Table 2: *MSQOL-54 Scores Based on Vitamin B_{12} Intake compared to the Recommended Dietary Allowance (RDA)*[1]

MSQOL-54 Subscales	Vitamin B_{12} Intake	
	≥2.4µg[2]	<2.4µg[3]
Physical Health	76.18 ± 33.71	74.17 ± 33.68
Role Limitation (Physical)	36.76 ± 32.01	33.33 ± 37.64
Role Limitation (Emotional)	84.31 ± 35.59	66.67 ± 51.64
Pain	76.57 ± 19.7	60.28 ± 36.48
Emotional Well-Being	81.18 ± 11.02	68.0 ± 20.24
Energy	44.35 ± 26.49	35.33 ± 25.6
Health Perceptions	57.65 ± 28.51	68.33 ± 24.22
Social Function	73.04 ± 30.83	54.17 ± 36.42
Cognitive Function	57.65 ± 31.48	68.33 ± 27.79
Health Distress	72.65 ± 33.62	50.0 ± 26.18
Sexual Function	69.61 ± 26.52	54.17 ± 27.39
Overall	76.66 ± 21.95	61.68 ± 11.7
MSQOL-54 Independent Categories		
Change in Health	50.0 ± 26.52	50.0 ± 27.39
Satisfaction with Sexual Function	61.77 ± 32.01	45.83 ± 45.87
MSQOL-54 Composite Summary		
Physical	63.3 ± 23.81	51.75 ± 27.94
Mental	76.69 ± 18.81	64.07 ± 17.94

[1]Sample consisted of all non-pregnant females between 24-64 y.
[2]Data are presented as mean ± SD, *n*= 17.
[3]Data are presented as mean ± SD, *n*= 6.

Another independent *t*-test was implemented to determine if individuals who consumed at least 5.0 µg had QOL scores different from individuals who consumed less than 5.0 µg. Participants who consumed 5.0 µg or more of vitamin B_{12} had MSQOL Subscale scores for *Physical Health* (*M*=92.50 ± 9.35) and *Health Perceptions* (*M*=76.67 ± 16.02) greater than participants who consumed less than 5.0 µg (*M*= 69.70 ± 36.33) and (*M*=47.06 ± 26.58). The difference between means for *Physical Health* and *Health Perceptions* were statistically significant at the *P*<0.05 level (*t* (20.28) = 2.37, *P*= 0.028) and (*t* (15.01) = 3.22, *P*= 0.019) respectively.

Additionally, participants who consumed 5.0 µg or more of vitamin B_{12} had QOL Subscale scores for *Role Limitation-Emotional* (*M*=100.00 ± 0.00), *Health Distress* (*M*=85.00 ± 13.04), and *Emotional Well-Being* (*M*=88.67 ± 6.89) greater than participants who consumed less than 5.0 µg (*M*= 72.55 ± 44.47), (*M*=60.29 ± 32.33) and (*M*=73.88 ± 14.91). The difference between means for *Role Limitation-Emotional*, *Health Distress,* and *Emotional Well-Being* were statistically significant at the *P*<0.05 level (*t* (16) = 2.55, *P*= 0.022), (*t* (20.33) = 2.61, *P*= 0.017) and (*t* (18.98) = 3.23, *P*= 0.031).

The last Subscale score that showed a significant difference between groups was the Subscale *Pain.* Participants who consumed 5.0 µg or more of vitamin B_{12} had a mean score for *Pain* (*M*=88.89 ± 7.65) that was greater than participants who consumed less than 5.0 µg (*M*= 66.47 ±

26.83). The difference between means for *Pain* was statistically significant at the *P*<0.01 level (*t* (20.71) = 3.11, *P*= 0.005).

The Independent Category *Social Function* exhibited a significant difference between means scores. Participants who consumed 5.0 µg or more of vitamin B_{12} had a *Social Function* mean score (*M*=86.11 ± 15.52) that was greater than participants who consumed less than 5.0 µg (*M*= 61.77 ± 34.99). The difference between means for *Social Function* was statistically significant at the *P*<0.05 level (*t* (19.46) = 2.30, *P*= 0.033).

Participants who consumed 5.0 µg or more of vitamin B_{12} had MSQOL mean scores for *Physical Health Composite Summary* (*M*=76.09 ± 8.38) and *Mental Health Composite Summary* (*M*=86.79 ± 7.66), greater than participants who consumed less than 5.0 µg (*M*= 54.71 ± 26.45) and (*M*= 68.67 ± 19.72). The difference between means for *Physical Health Composite Summary* and *Mental Health Composite Summary* was statistically significant at the *P*<0.01 level (*t* (20.97) = 2.94, *P*= 0.008) and (*t* (20.58) = 3.17, *P*= 0.005).

Also, participants who consumed 5.0 µg or more of vitamin B_{12} had a mean score for *Overall QOL* (*M*=88.05 ± 4.64), greater than participants who consumed less than 5.0 µg (*M*= 67.36 ± 21.48). The difference between means for *Overall QOL* was statistically significant at the *P*=0.001 level (*t* (19.43) = 3.73, *P*= 0.001).

4. Discussion

In this present study, Subscales that were found to have three of the lowest mean scores were *Role limitation- Physical, Energy, and Health Perceptions* (Table 1). These three Subscales may be decreased due to their relationship with depression and fatigue, two of the most common and disabling symptoms, as well as, important predictors of QOL [24; 25]. Increased vitamin B_{12} intake led to statistically significant improvement in, *Health Perceptions,* and others QOL measures that can be assimilated with either depression or fatigue, *Emotional Well-Being*, *Health Distress, Overall QOL* and the summative *Mental Health Composite Summary Score*. These QOL indicators allude to the fact that individuals may have felt less depressed compared to individuals consuming less vitamin B_{12} based on their reported QOL.

While QOL benefits were observed with increased vitamin B_{12} intake, further analysis regarding specific intake levels may help to identify an appropriate dietary recommendation for individuals with MS, which is missing from the literature. Much of the current dietary advice is centered on a general healthy diet with micronutrient recommendations consistent with Dietary Reference Intakes, which for vitamin B_{12} equates to 2.4 mcg per day. Participants who consumed 5.0 mcg or more, as seen in this study, showed improvements directly related to many common symptoms of MS, including a great overlap with the two most prevalent symptoms, depression and fatigue.

Vitamin B_{12} intake has recently been shown to be correlated with reduction in pain levels in a number of medical conditions, including neuropathy [26]. While neuropathy is not caused by MS, manifestations of MS in the CNS are similar to the damage to the peripheral nervous system seen in neuropathy. Other studies have found that decreased plasma vitamin B_{12} can impair physical performance, but correctable through dietary consumption [27]. Vitamin B_{12} is also known to potentially have a role in the maintenance of neurophysiological health, cognitive health, and function [28-31]. Existing literature is clear; vitamin B_{12} can positively affect mental health and physical functioning, which is concurrent with the results presented here, and further supports the importance of vitamin B_{12} for normal physiological functioning and QOL [32-35]. Current dietary recommendations for individuals with MS may be too simplistic or general for this complex

disease. Findings here, while just preliminary, suggest that QOL may be improved significantly with intake levels of 5.0 mcg of vitamin B_{12}, double the current RDA for this population.

Suspected reasons as to why individuals with MS may need to consume greater dietary vitamin B_{12} may be due to the nature of MS and the possible connection to pernicious anemia (PA) noted by earlier researchers [36; 37]. It has been observed in multiple studies that the occurrence of PA is significantly increased in MS samples [37; 38]. The manifestation of mild macrocytosis has also been shown to be statistically significant among individuals with MS with low levels of vitamin B_{12} [38]. Irregularity in vitamin B_{12} metabolism, may explain the enlargement of red blood cells without the clinical diagnosis of PA [10; 36].

Treatment options for MS have been previously described in great detail [39] and thus will not be discussed in depth here. Reductions in serum vitamin B_{12} levels in individuals with MS treated with Copaxone and Interferon-h have been noted and attributed partially to the drug's role in enhancing the need for vitamin B_{12} for myelin repair [11]. Another common drug therapy prescribed to individuals with MS that may influence vitamin B_{12} status are corticosteroids and are often used for individuals experiencing an acute relapse [40]. This approach has been observed, in high doses, to lead to a decrease in both cerebral spinal fluid (CSF) and serum levels of vitamin B_{12} [41]. Often used in conjunction with corticosteroid treatment are medications such as histamine receptor-2 (H2R) antagonist, proton pump inhibitor (PPI) or antacid to combat the prevalent dyspeptic pain associated with corticosteroids [40]. Despite which drug therapy is used to treat this side effect, the goal is to raise the gastric pH either directly or indirectly. When administered over long periods of time, a vitamin B_{12} deficiency may develop due to the increased gastric pH contents as a result of the decreased acid production. This has been observed in previous literature, particularly in older individuals and those experiencing atrophic gastritis [42-44].

Future research in the area of dietary intake and MS is necessary. Current treatments cannot cure the disease or reverse its progression, so alternative treatments are needed. Most importantly, patients need treatments that can preserve abilities as long as possible, optimize function, and promote QOL at all stages of the disease. With the potential role that vitamin B_{12} has shown in QOL amongst the physical and mental dimensions and possible drug-nutrient interactions, an increase in dietary vitamin B_{12} intake above current the recommendation is justified.

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