Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 2, pp. 219-225, Article ID Med-255
ISSN 2348-5140

Research Article | Open Access

Factors Influencing Parents' Eating Behaviors: Insights for Childhood Nutritional Intake

Valerie Jarvis McMillan[1], Patricia A. Lynch[2], Havalah J. Moore[2] and Vinaya A. Kelkar[3]

[1]Child Development and Family Studies, Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United States
[2]Food and Nutritional Sciences, Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United States
[3]Statistician/Research, Department of Biology, North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United States

Publication Date: 9 July 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-255

Abstract It is well recognized that parents can directly affect the consumption patterns and eating behaviors of children. The purpose of this study was to examine parents' responses to external factors that influence their eating behaviors. Parents completed a questionnaire that assessed family eating practices and determined factors influencing eating behaviors. Additionally, parents participated in a focus group session designed to dialogue about real-life scenarios related to factors influencing eating practices. Significant findings were revealed through the themes of healthy eating, cooking practices, and eating away from home. Findings also showed the major factors that influence the eating behaviors of parents were time, convenience and conscious choice.
Keywords *Nutrition; Child Nutrition; Family Nutrition; Food Choices; Nutrition Patterns*

1. Introduction

The parent can be instrumental in controlling the nutritional intake of young children. Many studies have looked at peer environmental and institutional influences. However, the basis for the child nutritional intake is influenced mostly by the parent. It is well recognized that parents can directly affect the consumption patterns and eating behaviors of children through their feeding styles (Patrick, Nicklas, Hughes, Morales, 2005) and ultimately prevent the consequences of childhood obesity. The role the family plays in determining children's dietary intake and eating habits is an important one (Cullen, Klesges, Sherwood, Baranowski, Beech, Pratt, et al., 2004). Children learn about eating from observing the eating behaviors of parents. Therefore, the factors that influence the eating behaviors of parents may prove to be pivotal in changing disordered eating behaviors of young children. The purpose of this study was to examine factors that parents say directly influence their eating behaviors.

2. Literature Review

The problem of childhood obesity has reached epidemic proportions. One in four children under the age of 18 is at risk for overweight and 15% are overweight. Preschool children who are overweight or obese will more than likely be overweight or obese as adults (Patrick, Nicklas, Hughes & Morales, 2005). Childhood obesity has both short-term and long-term health consequences including asthma, high blood pressure, psychosocial disorders, osteoarthritis, and adversely effects both glucose and lipid metabolism. This is a serious public health problem. Thus, the discerning question to ask is the following: Where to start to address this raising health problem in children? It is generally recognized that an individual's food consumption patterns begin at an early age. What an individual chooses to eat, portion sizes, and eating frequencies are often established by primary school age. This suggests that a critical area for the development of better nutrition practices is the evaluation of children's food consumption. If we can determine the processes by which children's eating habits are established, we are likely to be able to develop means to modify children towards healthier food choices and eating behaviors. To this, it is essential that we gain a better understanding of the dynamics of the family (i.e., parents or parental figures) and it influences children's food preferences, selections and eating practices.

It is well recognized that parents can directly affect the consumption patterns of children through their feeding styles (Patrick, Nicklas, Hughes, Morales, 2005) and ultimately prevent the consequences of childhood obesity. The role the family plays in determining children's dietary intake and eating habits is important (Cullen, Klesges, Sherwood, Baranowski, Beech, Pratt, et al., 2004). Children learn about eating from observing parents' food choices. Research has demonstrated that two-year old children's food preferences were associated with their mothers' food preferences (Skinner, Carruth, Bounds, & Zeigler, 2002).

The initial setting for a child's exposure to food choices, eating habits, and involvement in physical activity is at home with the family. Research has also shown that two year olds' food preferences are associated with mothers' preferences and parents' beliefs about healthy foods (Patrick et al., 2005). Feeding styles also determine the food choices of young children. Feeding styles represent how the caregiver maintains or modifies children's eating behaviors and have been associated with dietary intake and weight status (Patrick et al., 2005).

The literature indicates that the family plays an important role in affecting children's dietary intake and eating habits (e.g., Cullen, Baranowski, Rittenberry, Cosart, Hebert, & de Moore, 2001; Cullen, Klesges, Sherwood, Baranowski, Beech, Pratt, et al., 2004). The family provides the primary social learning environment for children and the primary setting for exposure to food choices, eating habits, and involvement opportunities for play and other physical activity (Golan & Crow, 2004). Parental influence is a critical determinant of children's food preferences (Benton, 2004) and food intake. Family environmental factors such as parental feeding practices have been related to overweight in children (Stang, Rehorst, & Golicic, 2004).

There are three types of parental feeding behaviors: authoritarian, permissive, and authoritative. Authoritarian feeding includes behaviors like restriction of certain foods and forcing consumption of other foods. In other words, attempting control of the child's eating patterns with little regard for their choices. Permissive feeding allows consumption of favorite foods in quantities determined by the child with little regard to nutritional value. Authoritative feeding involves communicating to children the importance of eating healthy foods but giving some choices about eating options (Patrick et al., 2005). In general, parental control of feeding practices, especially those that are restrictive tend to be associated with decreasing a child's ability to self-regulate food consumption and can cause increased reliance on external cues to dictate an amount to eat (Rhee, 2008).

Because of traditional values, social networks, patterns of inter- and intra-familial support, food preferences and ethnic and socio-cultural perspectives must be considered. Food habits are deeply rooted in a family's culture representing both their ethnic and community identity (McArthur, Anguiabo & Nocetti, 2001). Families must content with outside influences affecting the availability, accessibility, and selections of preferred foods along with the introduction of new foods and different ways of food preparation.

Parent behaviors can be influential in shaping children's food preferences by frequently exposing them to healthy foods at home and making them accessible (Reinaerts et al., 2007). Parents can also model good eating behaviors and can positively influence their child's eating habits. Some ways to accomplish this are: children and parents eating together and parents consuming healthy foods. Eating meals as a family is positively associated with consumption of fruit, vegetables, grains and calcium-rich foods, vitamins and minerals. McCafferey et al. also report that frequency of family meals may have healthier eating habits. Some habits that may be learned are: learning to eat at a slower pace, learning to follow internal satiety cues, reduced consumption of energy-dense fast foods, and higher consumption of fruits and vegetables compared to those who do not eat regularly as a family.

Understanding children's eating attitudes, behaviors and preferences is important in promoting good health in children (Brown & Ogden, 2004). Likewise, understanding parents' attitudes, behaviors and preferences about eating is equally important in promoting children's good nutrition and health. Although widespread attention is given to the childhood obesity problem in the literature, limited information is known about what primarily influences family's (parents or parental guardians) food preferences and selections and their perspectives on eating behaviors. This gap in knowledge surfaces a particular need to study the factors that influence family's (parents or parental guardians) eating behaviors.

The purpose of this study was to examine parents' responses to external factors that influence their eating behaviors. The study addressed the following question: What are the primary factors influencing parents' decisions related to eating behaviors?

3. Methodology

3.1. Participants

The participants were a convenience sample of parents who voluntarily enrolled their young children aged 2 ½ to 5 at a child development laboratory on a university campus in a south eastern community. The total number of parent participants in the overall research study was 39. For the purposes of the present research study, 19 out of 39 parents participated. All of the parents were female (100 %). From parents' self-reports, 95% were Black/African American, 3% were African, 1% was White/Caucasian, and 1% was Latino/Hispanic. More than 86% of the parent participants reported a household income in the range of $60,000 - $80,000.

3.2. Measures

The *Family Eating Practices Questionnaire (FEP-Q)* was developed by the researchers of study in the year 2010. It was based on a comprehensive review of the literature, informal clinical parent interviews, and adaptations from the works of Schulundt's (1994) *Situational Obstacles to Dietary Adherence Questionnaire* and Williams and Christensen's (2004) *Diabetes Stepping Up to the Plate: Eating Behaviors Patterns Questionnaire.* The FEP-Q is a 44-item scale using a 5-point Likert scale (1 = strongly disagree; 2 = disagree; 3 = neutral; 4 = agree; 5 = strongly agree). The FEP-Q is designed to assess family eating practices and determine factors influencing eating behaviors. Sample items are: I buy and eat low-fat food products (item 5); On Sunday, I eat a large dinner with my family (item

29); I would rather buy takeout food and bring it home than cook for my family (item 36); When choosing fast food, I pick a place that offers healthy foods for my family to eat (item 39). Validation of the FEP-Q was assessed with a convenience sample of parents (N = 16) for the purposes of determining whether the items are relevant to the intended content. The intended content of the FEP-Q is about eating practices and/or behaviors of parents/families. Parents participating in the validation of the FEP-Q rated each item once by marking "Y" for yes and "N" for no as it pertained to their beliefs of importance relative to family eating practices. The FEP-Q was validated.

3.3. Procedures

Parents of young children enrolled at a child development laboratory on a university campus in a south eastern community were recruited for participation in the overall research study. Letters detailing the purpose and procedures of the study were distributed and explained to parents at an evening Parent Teacher Organization (PTO) meeting. Parents were asked to indicate their willingness to participate in the overall research study by completing and returning a consent form. Later, a letter along with the FEP-Q were mailed to parents (N = 39) requesting voluntary participation in the present research study by completing and returning the questionnaire to one of two focus group sessions. The letter explained that parents would receive a $100.00 gift card at the completion of the 1 ½ hour focus group session and submission of the completed FEP-Q. Out of 39 parents, 19 parents voluntarily attended one of two focus group session and submitted a completed FEP-Q. The focus group session was designed for parents to engage in dialogue about eating practices and their family's decision-making processes of food selections and preferences, and factors that influence food consumption. A case study method approach (i.e., case scenarios) using six (6) real-life case scenarios via a PowerPoint presentation was displayed and read to the parents. Following each narrated case scenario, focused questions (i.e., what are the successes in this situation? What are the concerns in this situation? What are some influencing factors in this situation?) Were posed to generate discussion?

4. Results

Data for 19 parents were collected from the 44-question FEP-Q survey. Responses to 16/44 questions shown to be significant ($p < 0.025$) are given below (See Table 1).

Table 1: *Summary of Significant Responses from Family Eating Practices Questionnaire (FEP-Q)*

Theme Topic	Question	Frequency Agree	%	Frequency Disagree	%	N	p
Healthy Eating	I choose healthy foods for my children and me to prevent disease.	16	84.2	1	5.3	19	.024
	My emotions affect what and how much I eat.	11	31.6	6	57.9	19	.024
	I allow my child to eat cookies or ice cream in place of dinner.	0	--	19	100	19	.004
	I eat 3-4 servings of vegetables per day.	10	52.6	6	31.5	19	NS
	My child eats 3-4 servings of vegetables per day.	12	66.7	5	27.8	18	.017
	My child rarely eats breakfast.	0	--	17	94.4	18	.016
	If I am busy, I will eat a snack instead of a meal.	14	73.7	3	15.8	19	.000
	Sometimes I snack when I am not hungry.	8	42.1	10	52.6	19	.019
	My child snacks when not	8	42.1	11	57.9	19	.005

	hungry.						
	I carefully watch my child's food portions.	16	84.2	1	5.3	19	.000
	I watch my food portions.	13	68.4	3	15.8	19	NS
Cooking Practices	My family and I eat vegetables seasoned with fatty meat.	1	1.7	17	89.5	19	.008
	I would rather buy takeout food and bring it home than cook for my family.	1	5.3	18	94.7	19	.029
Eating away from home	I stop for fast food breakfast for my child on my way to school.	1	5.3	18	94.7	19	.018
	I eat at a fast food restaurant at least 3x/week.	1	5.3	18	89.5	19	.008
	When choosing a fast food restaurant, I choose	0	--	16	84.2	19	.005

4.1. Healthy Eating

Parents (84.2%) tend to choose healthy foods for their children and themselves to prevent diseases (16/19). One-third reported that their emotions affect what and how much is eaten. All of the parents reported that they did not allow their children to eat cookies, chips, ice cream instead of dinner. When asked if they eat at least 3-4 servings of vegetables/day, only 52.6% of the parents reported eating the minimal servings of vegetables, while more parents reported (66.7% - 12/18) that their children ate the recommended amount of servings. When presented with the statement, "my child rarely eats breakfast," 94.4% (17/18) disagreed. In a focus group discussion, parents said "sometimes children don't eat breakfast because of time" and the "children prefer to eat breakfast at home." Parents reported eating snacks instead of a meal if they are busy (14/19) and 52.6% sometime snacked even when they were not hungry (10/19), while 57.9% (11/19) reported their child also snacked when they were not hungry. When asked if they carefully watch their children's portion sizes, 84.2% (16/19) agreed that they do; however, they are less likely to watch their own portions.

4.2. Cooking Practices

Eighty-nine percent (17/19) of the parents did not use fatty meats to season their vegetables. Nearly all (94.7) of the parents would rather cook than bring takeout food home to their families.

4.3. Eating Away from Home

Parents disagreed with a statement indicating they would stop for fast food breakfast for their child on the way to school, 94.7% (18/19) of the parents disagreed. Only 1.7% (1/19) of the parents report eating at a fast food restaurant at least three times a week.

However, when choosing a fast food restaurant, parents did not make the choice. Only 5.2% (3/19) of the parents reported choosing the restaurant.

5. Discussion

Although the need for parental guidance is ever present, particularly in a changing environment, where so many factors known and unknown affect eating behaviors, we found that this population group was knowledgeable and attentive to their children's eating behaviors. However, we also found some areas that might help in programming planning and intervention to reduce childhood obesity when focusing on parental biases. For example, parents carefully watched the portion size of their

children's intake more often than their own; they were more likely to ensure the children were getting the recommended servings of vegetables more often than they were. However the children are modeling their parents' behaviors. Contrary to many studies, this population did not report frequent visits to fast food restaurants or seasoning their vegetables with fatty meat products. They preferred cooking and eating at home. In order to alter parent's behavior, there must be a change in their belief system as it relates to their children and/or family. After reviewing the parent participant responses to the FEP-Q it was evident that many of the eating behaviors could be attributed to time/convenience and conscious choice. Responses to the FEP-Q were also compared to the qualitative data gathered from parent participants at focus group sessions at the conclusion of the study. The participants of the focus group sessions discussed factors that influenced their decisions regarding eating behaviors which affected their families' eating behaviors. Participants commented that convenience/time and conscious choices influenced eating behaviors. A main theme that was prevalent throughout the sessions was that it was more convenient to eat out because of the lack of time to prepare meals at home with the many extracurricular activities in which families were involved. A concern that was also expressed was that even though eating out was more convenient it was also difficult to find restaurants that were inexpensive. The results also show that many of the families often ate at large social gatherings where one's eating behaviors may be influenced socially and culturally. It seems evident that with all the questions asked of the parents, conscious choice was the most influential factor.

6. Conclusion

In conclusion, there are many factors that influence the eating behaviors of parents. Although time and convenience and conscious choice are major, our data has also shown significant differences in factors related to eating healthy, cooking practices, and eating away from home. Further research needs to be conducted to determine the association between influential factors and eating behaviors of adults and how those behaviors relate to those of the family.

References

Benton, D. *Role of Parents in the Determination of the Food Preferences of Children and the Development of Obesity.* International Journal of Obesity. 2004. 23 (27) 858-869.

Cullen, K.W., Baranowski, T., Rittenberry, L., Cosart, C., Hebert, D. and de Moor, C. *Child-Reported Family and Peer Influence of Fruit, Juice and Vegetable Consumption: Reliability and Validity of Measures.* Health Education Research. 2001. 16 (2) 187-220.

Cullen, K.W., Klesges, L.M., Sherwood, N.E., Baramowski, T., Beech, B. and Pratt, C. *Measurement Characteristics of Diet-Related Psychosocial Questionnaires among African-American Parents and their 8 to 10 Year Old Daughters: Results from the Girls' Health Enrichment Multi-Site Studies.* Preventive Medicine. 2004. 38 (Suppl. 1) s34-s42.

Cullen, K., Klesges, L., Sherwood, N., Baranowski, T., Beech, B. and Pratt, C. *Measurement of Outcomes, Mediators, and Moderators in Behavioral Obesity.* Prevention. 2004. 38; 1-13.

Dovey et al., Dovey, T.M., Staples, P.A., Gibson, E.L. and Halford, J.C.G. *Food Neophobia and Picky/Fussy Eating in Children: A Review.* Appetite. 2008. 50; 181-193.

Golan, M. and Crow, S. *Targeting Parents Exclusively in the Treatment of Childhood Obesity: Long-Term Results.* Obesity Research. 2004. 12 (2) 357-361.

McCaffrey, T., Rennie, K., Wallace, J. and Livingstone, B. *Dietary Determinants of Childhood Obesity: The Role of the Family.* Current Medical Literature, Clinical Nutrition. 2006. 15; 51.

Nicklaus. *Development of Food Variety in Children.* Appetite. 2008. 52; 253-255.

Patrick, H. and Nicklas, T. *A Review of Family and Social Determinants of Children's Eating Patterns and Diet Quality.* Journal of the American College of Nutrition. 2005. 24; 83-92.

Patrick, H., Nicklas, T., Hughes, S. and Morales, M. *The Benefits of Authoritative Feeding Style: Caregiver Feeding Styles and Children's Food Consumption Patterns.* Appetite. 2005. 44; 243-249.

Reinaerts, E., de Nooijer, J. and de Vries, N. *Parental versus Child Reporting of Fruit and Vegetable Consumption.* International Journal of Behavioral Nutrition and Physical Activity. 2007. 4; 1-8.

Rhee, K. *Childhood Overweight and the Relationship between Parent Behaviors, Parenting Style, and Family Functioning.* The Annals of the American Academy of Political and Social Science. 2008. 615; 12-37.

Stang, J., Rehorst, J. and Golicic, M. *Parental Feeding Practices and Risk of Childhood Overweight in Girls: Implications for Dietetics Practice.* Journal of the American Dietetic Association. 2004. 104 (7) 1076-1079.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 1, pp. 174-187, Article ID Med-276
ISSN 2348-5140

Cloud Publications

Review Article | Open Access

Fundamentals of Randomization in Clinical Trial

Fatemeh Baghbaninaghadehi, Susan Armijo-Olivo, and Linda Woodhouse

Department of Physical Therapy, Faculty of Rehabilitation Medicine, Edmonton, Alberta

Publication Date: 22 February 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-276

Abstract The randomized control trial (RCTs) is widely accepted to be the best design for evaluating the efficacy of new therapies, and thus it is accepted as the gold standard to evaluate treatment effects. Random assignment of patients to the treatment ensures the internal validity of the comparison of new treatment with a control group. Unfortunately, the randomization process in most research studies is not implemented properly. The purpose of this review is to provide researchers and scholarly clinicians with a better understanding of different options to achieve proper randomization. The information presented in this article will also help to better design and interpret the results of clinical trials. Therefore, a brief definition of randomization plus its concise benefits in clinical trials, and the processes of an accurate randomization procedure, generation of unpredictable random allocation sequence and allocation concealment are considered. Recommendations are made to select the suitable techniques of generation of random allocation and allocation concealment. Finally, the authors describe how the appropriate implementation of these two procedures reduces the potential for biases throughout the study and improves the power of the study.
Keywords *Allocation Concealment; Study Power; Randomized Control Trial; Random Allocation*

1. Introduction

Randomized controlled trial (RCT) is defined as a clinical trial in which the participants are assigned randomly to different treatments groups. Thus, each participant has a known and equal chance of being assigned to a given group, and the group assignment cannot be predicted. Randomized experimental design is the preferred method of research for evaluating treatment effects in health research since it provides the highest degree of control over a research study and allows the researcher to draw causal inferences with the highest degree of confidence [1]. They use a systematic methodology that allows controlling for confounder variables [2]. In real life, clinical experiments never control for all confounders; however, RCT design offers the most convincing evidence of the effect of one variable has on another [3].

Most health research journals have been increasingly interested in publishing the results from RCTs [4]. The increasing recognition toward using randomized control trials (RCT) in health care started in the 20th century. In the 1920s, randomized control trial was developed by R.A Fisher to be a fundamental of experimental design [5] in agricultural research [6]. Later, in the 1940s, owing to the

advocacy of Sir Austin Bradford Hill, the RCT design was promoted in health care. His efforts resulted in using the random allocation of an experimental trial and after publishing his results, randomization became known as a secure experimental design to avoid bias in between group comparisons [7].

In RCTs, randomization refers to the use of the probability theory used to assign subjects to different treatment groups. It is a method, based on chance alone, by which the study participants are assigned to the various treatment groups being studied. Randomization minimizes the differences among groups by equally distributing participants with particular characteristics among all the trial arms. It is a curial procedure in RCTs without which the treatment effect could be overestimated by up to 41% [8]. Results from randomised and non-randomised studies could generate completely opposing results [8]. Unpredictable random allocation sequence is a crucial factor that requires attention when implementing any randomized control trial. The process of concealing the randomization until at least all the participants are assigned to their groups is the most important component of randomization without which the randomization fails in a trial [9]. Contrasting and combining results from different studies have demonstrated that the treatment effect is exaggerated with inadequate or unclear random allocation and allocation concealment [10]. We are unaware of a suitable article that comprehensively discusses the benefits and procedures of these two crucial elements of randomization (random allocation and allocation concealment) together, which is necessary to give a comprehensive understanding appreciation for the randomization process. In addition, this article outlines the benefits of using randomization, describes its characteristics, and provides examples of how to perform proper sequence generation and allocation concealment. Finally, discussions in which how proper randomization and allocation procedures reduce biases and increase the study's power are performed. Thus, the content of this paper will help researchers in the area of health sciences to understand how randomization and allocation concealment operate and decrease several flaws regarding knowledge when analyzing, criticizing, and designing future trials.

2. Benefits of Randomization

According to Altman et al. [11], randomization has three major advantages. First, it eliminates bias in the assignment of treatments (selection bias). Second, random allocation facilitates masking the identity of treatments to investigators, participants, and evaluators. Third, randomization allows the use of probability theory and increases the likelihood that changes in the dependent variable are attributable to the independent variables rather than extraneous factors or confounding variables; thus, it decreases the possibility of confounding bias. Randomization tends to distribute individual differences equally across the groups, so that the groups differ systematically in only one way: the intervention being examined in the study. Randomization reduces the chance of any systematic error; for example, let's suppose that the study of a specific rehabilitation technique after osteoarthritis-surgery is intended. A subject could be assigned to two different groups: to a new rehabilitation technique and to a control group. When subjects with good health condition are intentionally or unintentionally (non-randomly) channelled to treatment groups, biases infiltrate to the study. Randomization uses probability theory to ensure that a specific patient will not be consciously or unconsciously assigned to the desirable group to receive a specific intervention. In this case, avoiding the introduction of bias in group assignment helps to ensure that difference in outcome between treatment groups is merely due to chance [7]. Above all else, randomization helps to equally distribute participants with particular characteristics (covariates) among all the trial treatment arms; while non-randomized trials may lead to covariate imbalances in clinical trials. Covariate imbalances can be adjusted in the data analysis stage by statistical methods such as the analysis of covariance (ANCOVA). Although ANCOVA uses the average across the slopes of subgroups to adjust the covariate effect on outcome, different subgroups of the covariate may have different slopes which could be problematic if there is imbalance in the distribution between subgroups. Proper randomization should prevent this problem by equally distributing participants with various covariates among the treatment groups [12].

The success of randomization depends on two interrelated aspects in clinical practice: adequate generation of an unpredictable allocation sequence and concealment of that sequence until assignment occurs. Thus, the person enrolling participants does not know in advance which treatment the next person will get. This is called “allocation concealment”.

3. Generation of Allocation Sequence’s

To minimize bias in a study, researchers should randomly assign each participant to the various treatment groups. There are different types of random allocation techniques used to achieve the desire of an unbiased study. The three most predominant techniques that deliver true randomized allocation are: simple, block, and stratified randomization. These will be the focus of the next section. Then, the less accurate but more pragmatic method, covariate adaptive randomization, along with the other randomization techniques will be discussed.

3.1. Simple Randomization (Un-Restricted Randomization)

Simple randomization is a kind of randomization procedure that is based on a single sequence of random assignments [13]. Simple randomization preserves complete unpredictability for each intervention assignment, and no other allocation method surpasses the bias prevention and un-predictability of this method. Simple randomization involves assembling a sample in such a way that each independent and same-size subset within a population is given an equal chance of being allocated to the various groups [14].

Simple randomization could be generated by coin-tossing, dice throwing, and dealing with previously shuffled cards which represent reasonable approaches for the generation of a simple complete randomization sequence. These are the manual methods of drawing lots. Owing to the threat to randomness, difficulties in implementation, and lack of an audit trail, the use of these methods is not recommended, and investigators are advised to avoid the use of manual procedures [7]. Other methods such as computer random generators or table of random numbers may be used. These options represent reliable, easy, unpredictable, and reproducible approaches that provide an audit trail [7]. Unless the researchers report clearly the randomization method that was used, study results should be treated with caution. When researchers report the use of either a computer random number generator or a table of random numbers, the reader can be more confident that the randomization process was adequate with the sequence generation approach [7].

Simple randomization is problematic when using small sample sizes ($n < 100$) since the sequence can be predicted, and disproportionate sample sizes per group can be obtained [15]. For example, randomizing a total sample size of 10 to two groups using a coin-toss, simple randomization procedure might yield to unequal groups (ratio imbalance) of 7 participants to the control and 3 to the treatment group (Figure 1) [4, 7]. For these reasons, simple randomization is not recommended for trials of less than 200 subjects [14].

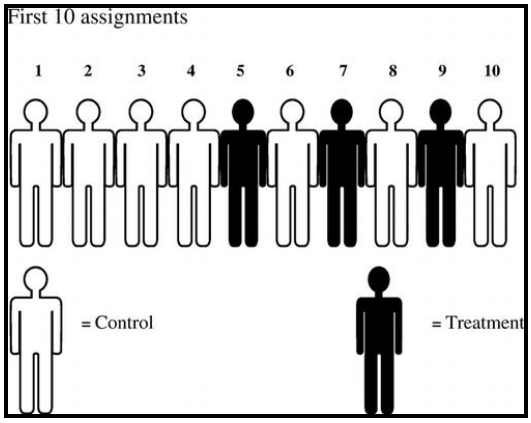
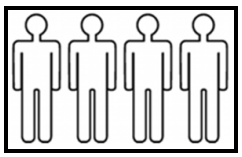


Figure 1: *Imbalance Sample Size due to Simple Randomization in Small Groups (N=10)*

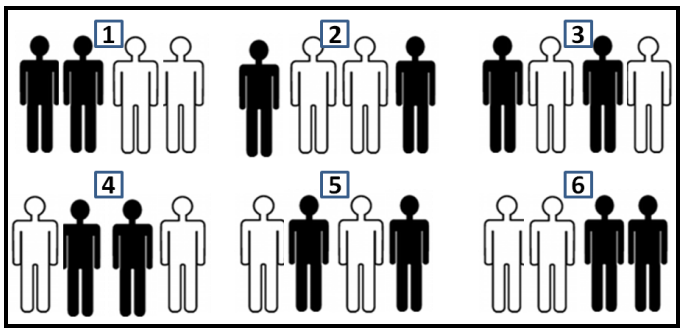
3.2. Permuted-Block Randomization or Blocked Randomization (Restricted Randomization)

The restricted randomization procedure is a useful method to control to the likelihood of obtaining an allocation sequence with an undesirable sample size [16]. In other words, restricted randomization is recommended to use in small randomized control trials when the researchers want treatment groups of equal sizes. It attempts to obtain an unbiased study and yield comparison groups with equal sample size throughout the trial [15]. Permuted blocks or blocking is the most commonly used method to attain balanced randomization. A “blocked size” or “the allocation ratio” is specified and the subjects are allocated within each block [7]. The block size is determined by the researcher a priori and should be the multiple of the number of treatment groups. For example, with two treatment groups, block sizes of 4, 6, and 8 would be appropriate. The “allocation ratio” is the ratio of the number of subjects in one group versus the other group. After specifying the block size, all the potential contribution of the assignments within each group must be calculated, and then the blocks are randomly chosen to determine the assignment of all participants. For example, with a total of 20 participants randomized to two treatment groups, a randomized block method would be: (A) block size is four, (B) possible balance combinations with two T1 (Treatment one) and two T2 (Treatment two) in each block are calculated as 6 and (C) where each block is randomly chosen to determine the assignment of all 20 participants (Figure 2).

(A) Block Size



(B) Possible Balance Combination (e.g. 2 to Treatment 1, and 2 to Treatment II)



(C) Random Selection of Blocks (E.G. 2, 1, 3, 4, 6) to Assign All the 20 Participants

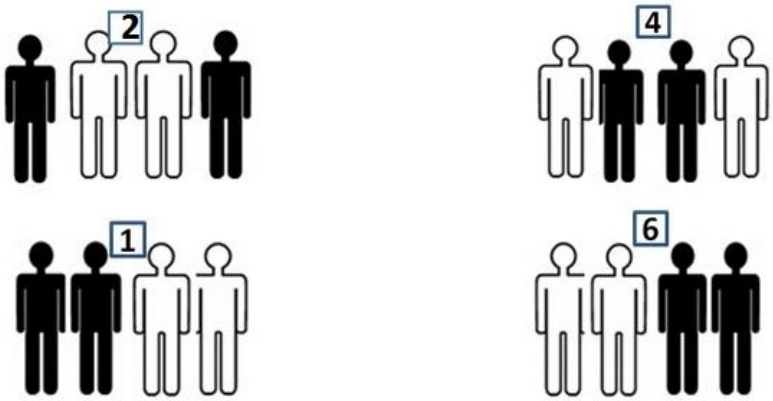


Figure 2: *Balance Sample Size Produced with Block Randomization Even with Small Sample Size (n=20)*

The block size may remain fixed throughout the trial or may change randomly. Whenever blocked randomization is used in a trial that is not double-blinded and the block size is less than 6, it should be varied to reduce the chance of deciphering the assignment scheme by those who are responsible for recruiting the participants. This avoids the risk that sequence is recognized when the treatment allocation becomes known after the assignment based on recognition of past assignments, resulting on inadvertent introduction of selection bias. This is of particular concern when small block sizes are used. To preserve unpredictability of the allocation sequence, the use of a large block size (e.g. 10 or 20 instead of a small block size) and the random variation of block size are recommended [17]. When blocking is used, details regarding the block size (or sizes if varied), allocation ratio, and the random method of the selection at the final stage (e.g. computer random number generator or random number table) must be clearly reported. This allows the reader to be certain about the unpredictability of the random sequence [7].

3.3. Stratified Randomization

There is also a possibility that certain prognostic factors influence the study results. To control these covariates, investigators need to use the stratified randomization method to generate a random sequence [7]. This method helps to achieve balance among groups in terms of participants' covariates (characteristics). To reap the benefits of randomization, specific covariates must be identified by investigators who are knowledgeable about the potential effect of each covariate on a dependent variable a prior. Then, to do the stratification, investigators should generate separate blocks for the combination of each covariate and assign each participant to appropriate blocks of covariate. Finally, simple randomization should be done within each block to assign each participant to one of the treatment groups [4].

As previously mentioned, results of trials can be endangered by influence of the possible covariates. To adjust for this problem, investigators may use the stratified randomization method [7]. For example, when examining the effect of different rehabilitation techniques after a certain surgery, there are a numbers of covariates that influence the result of the trial. It is believed that the patients' age has an effect on the rate of healing and mortality. Therefore, age might be a confounding variable in this case as it influences the outcome of the study. Using stratified randomization, investigators can balance the control and treatment groups for age, sex, and other similar covariates [4].

Suppose we want to perform a stratified randomization with our sample based on gender (2 levels: female/male) and age (2 levels: under 50/above 50) having 2 groups and total of 30 participants. With the combination of 2 covariates, stratification results in 4 blocks. Then a simple randomization procedure, such as coin-tossing, is needed within each block to assign the participants to one of the treatment groups (Figure 3).

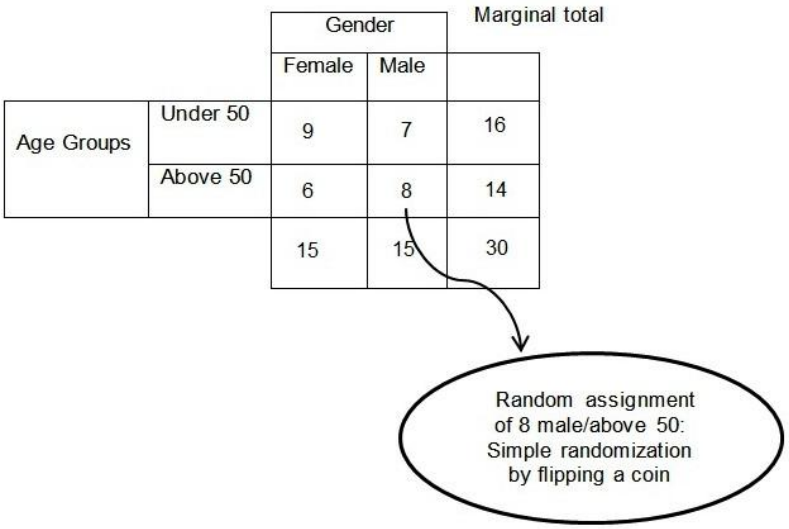


Figure 3: *Scheme of the Stratified Randomization Which Controls the Covariates of Gender and Age between Treatment Groups. It Results 4 Blocks, and the Participants Within Each Block will be Assigned to the Treatment and Control Group by Flipping a Coin to Have an Equal Sample Sizes Terms of Covariates*

As discussed, one of the major benefits of randomization is that it can avoid severe imbalances of the most important prognostic factors across groups and make the groups comparable in terms of covariates [17]. It may also present an ample quantity of balance (on the stratified factors) and may yield slightly more statistical power and precision [17]. Besides the aforementioned advantages, using stratified randomization in small clinical trials is relatively simple. However, in large trials with more stratified covariates to control, the complexity of the trial makes stratified randomization less useful [7]. Using too many block combinations may lead to a large number of blocks and small participant numbers within each block, potentially resulting in large imbalances in the overall treatment allocation. As a general guide, it is believed that if the number of blocks moves towards one half of the sample size, balance in covariates tends to fail [18]. Regarding the number of participants for a trial, although there is no absolute cut-off, trials with more than 200 subjects probably do not benefit from stratification [19]. For these purposes, in small studies in which the number of stratification is more than 1or 2 covariates, the number of blocks can rapidly move toward the number of participants and reduce the usefulness of the procedure to balance the participants [13]. Finally, using stratified randomization is very difficult, because it requires baseline characteristics for all participants, and all participants must be identified before group assignment, which is difficult, as in clinical research trials participants are often enrolled one at a time on continuous basis [14].

3.4. Covariate Adaptive Randomization

Covariate adaptive randomization has supporters [20] and detractors [14]. Although many research studies have suggested using covariate adaptive randomization in clinical trials as means of achieving a valid randomization [4, 21], it is not as usable as the simple, block, or stratified randomization methods [7, 22]. Using this method, only the first subject's assignment is, in fact, selected at random [23]. For remainder newly enrolled subjects, the probability of being assigned to a particular group

varies. The newly recruited subjects are sequentially assigned to a particular group by considering the specific covariate and the previous assigned group [12, 24]. This happens in order to make small groups closely similar with respect to several characteristics [7] and reduce the covariate imbalances [14].

The covariate adaptive approach was first described by Taves [25]. To use Taves' covariate adaptive randomization method, researchers should look back to the examination of previous participant group assignments and then assign each individual, who is newly enrolled in the study, by making a case-by-case decision on group assignment [25].

Consider again the example of 2 groups involving 30 participants, with gender (2 level: male/ female) and age (2 level: under 50/ above 50) as covariates, when enrolment in the trial is being done in a continuous manner. The five participants are already assigned from which the first participant is assigned by flipping the coin, then a newly enrolled participant number 6 who is under 50 and female needs to be assigned to one of the treatment groups, either 1 or 2. Taking into account the characteristics of the 6th participant and using Taves' method, the marginal totals of the corresponding covariate categories for each group must add together. Then, the participant will be assigned to a group with the lowest covariate to decrease imbalance. Since in this example the total number of participants randomized to Treatment 2 is lower than Treatment 1 (2 < 3), the 6th participant is assigned to Treatment 2 (Figure 4).

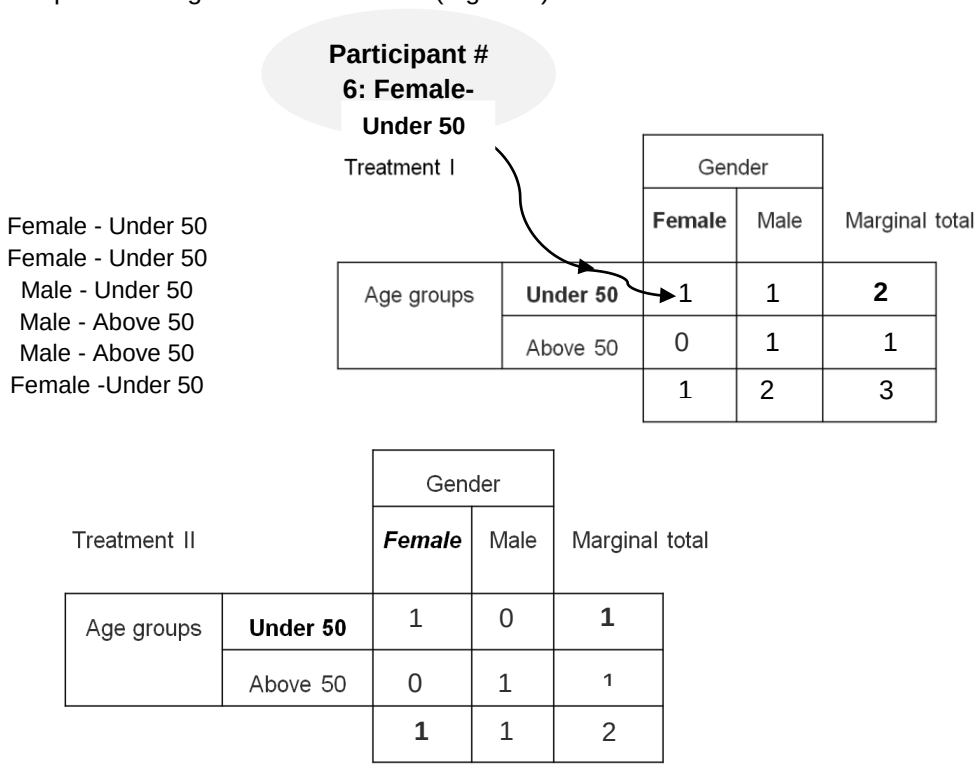


Figure 4: *Schematic of the First 5 Participants' Group Assignments by Two Covariates (Gender and Age) and Assigning of the Newly Enrolled (6^{th}) Participant Who will be assigned to the Treatment II into the Block of Female/Under 50*

4. Summary of Random Allocation Sequence

In summary, to select a proper randomization method, researchers must consider several factors including sample size, balance in sample size, covariates, and participant enrolment. For large sample sizes (n > 200), simple randomization is a good choice. Block randomization, however, is desirable when the balance in sample size is required. To reach the balance in baseline

characteristics, stratified randomization is suggested. Covariate adaptive randomization can achieve better balance than other methods when participants are continuously enrolled into a study [4]. Figure 5, which is adapted from the study by Kang et al., (2008) [4] describes the best method selection process clearly.

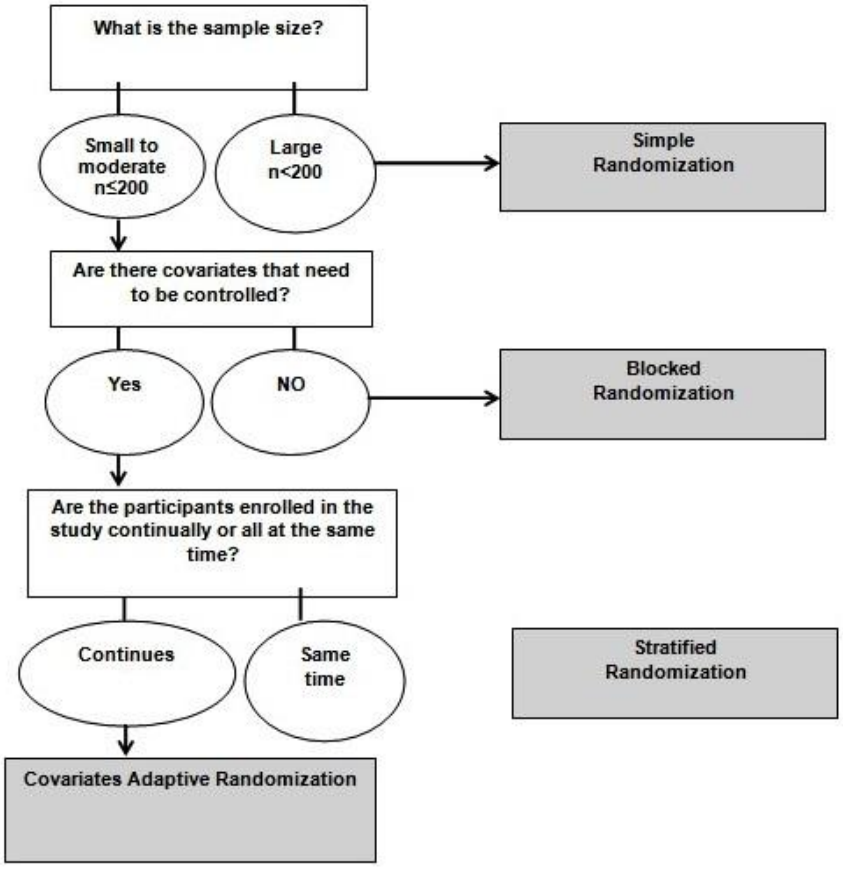


Figure 5: *Flowchart for Selecting a Proper Randomization Technique. Appropriate Techniques are indicated in Gray Boxes. Adapted from Kang et al., 2008*

5. Allocation Concealment

Randomization is a chain of procedures that consists of the generation of a random sequence and allocation concealment. Although the process begins with sequence generation, it does not finish until all the subjects are actually assigned to the groups. Therefore, the process of allocation concealment and the proper implementation of randomization must follow the generation sequence [26]. Allocation concealment keeps clinicians, participants, investigators, and everybody involved in a trial unaware of upcoming assignments [9]. No matter which randomization technique is used (e.g. simple random, stratification) to randomly allocate each participant to a group, if one of the members involved in the trial is able to identify the upcoming assignment the value of randomization is compromised [27,28]. Allocation concealment is a term used for the implementation of the sequence [27], not for the generation of it. Moreover, most researchers confuse allocation concealment with the blinding of treatment [27, 29]. While allocation concealment is always possible, blinding is not always feasible [30] and is considered after assigning the participants. If the researcher is aware of the next assignment, he/she may intentionally or unintentionally influence the selection of participants. For example, if researchers or health care providers know that the upcoming assignment would be an exercise treatment, he/she may tend to influence who is randomized next by selecting a specific participant who may need more exercise. Besides, when the subject becomes aware of the allocation

scheme, knowledge of allocation to control or placebo group may cause the subject to withdraw from the study or to wait to ensure assignment to the active treatment [26]. Therefore, the prognosis of the upcoming allocation group introduces bias that the designed randomization was supposed to eliminate. Some standard methods of ensuring allocation concealment include: sequentially numbered, opaque, sealed envelopes, pharmacy controlled, numbered or coded containers, and central randomization from which the centralized assignment protocol does not involve any person associated with the research trial [26, 31]. Before using this method, researchers should screen each patient to ensure that they meet the eligibility criteria before they call to the randomization center to receive the treatment assignment. It is clear that by using this method neither the patients nor the researchers are able to predict the next allocation; then one can feel more confident about the results of the study [9].

Pharmacy controlled, numbered or coded containers, and central randomization methods require a broad infrastructure support that may be beyond the resources available to investigators in single-center trials [13, 32]; therefore, sequentially-numbered, opaque, sealed envelopes are considered easy and complete methods to implement, but are susceptible to manipulation. Herbison et al. (2011) [33] found through a meta-epidemiological approach that sealed envelopes with some form of enhancement (opaque, sequentially numbered, and so forth) may give adequate concealment when compared with more sophisticated methods of allocation concealment. Hence, to do a secure opacity researchers are recommended to carefully develop and supervise the allocation process to conserve concealment. Thus, researchers should ensure that all the envelopes are numbered beforehand and opened in sequence, after the participants' name and other information is written on each envelope in detail. Using carbon paper to transfer written information to the allocation paper, inside the envelope, generates a valuable unbiased appraisal trial. It is also recommended to use foil inside envelop and around the carbon copy and allocation paper to make randomization completely safe from being deciphered [7, 13, 32]. Finally, it would be best if different persons perform different parts of the event to decrease the chance of predicting sequences and assignments and introducing bias to the study (30). A proper sequence of procedures using opaque concealed envelopes is shown in Figure 6.

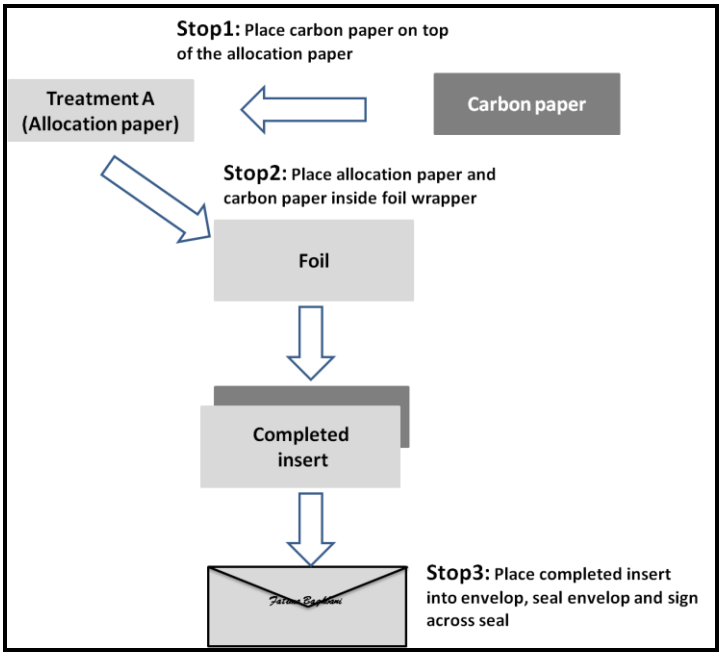


Figure 6: *Preparation of Envelop Insert – Adapted from Doig et al., 2005* [32]

6. The Effect of Randomization on Power of the Study

The power of the study is affected by many factors including the size of actual difference between the mean of treatment groups (effect size), and the amount of the error variance. As the effect size increases, the power increases; while, the greater the error variance, the less the power. Any factors that increase the error variance decrease the researcher ability to detect the true effect size [34]. When the ability of the researcher to measure the true treatment effect interferes with unwanted differences between groups (heterogeneity), due to either random error or systematic error, the internal validity of interventional trials may be threatened. The random differences between groups are unavoidable, but can be made less likely by ensuring that a study has an adequate sample size [35]. Whereas, bias or systematic error can be introduced intentionally or unintentionally and is far more likely to interfere with the study execution and interpretation of the results, and unfortunately it is much more difficult to avoid. Bias can arise at three stages of the research study: during initial enrolment of the participants, implementation of the study, and analysis of the results [35]. The next section is focused on the sources of systematic error or bias during the initial enrolment of the participants, the effect of errors on the power of the study, and how randomization can overcome these biases throughout the study.

Selection and confounding biases are common systematic errors in clinical trials [36]. Selection bias occurs when the sample is not representative of the target population. This may happen if investigators can intentionally or unintentionally preferentially enrol patients between treatment arms; consequently the bias seeps into the study [37]. Selection bias exaggerates the effect size of trials. For example, it has been shown that inadequate allocation concealment can overestimate treatment effects on average by 18% [27, 38]. This has been confirmed by several researchers in different areas of health science [39, 10]. However, other research failed to see the association between allocation concealment and effect size [40, 41]; findings of a met-epidemiological study demonstrated a possible reason for this inconsistency is the type of intervention or outcome assessments. They demonstrated that effect size was exaggerated by inadequate allocation concealment and lack of blinding just in trails with subjective measures and All-cause mortality outcomes [38]. Other factors such as method of randomization have also been shown to influence the results of trials and can potentially inflate type I error to 100% that leads to a “false positive”: the error of rejecting a null hypothesis when it is actually true [42, 43]. Effect size appears to be exaggerated when the random-sequence generation, and also the allocation concealment, is inadequate or unclear [10]. Without allocation concealment, the effects of the intervention tend to be overestimated. In fact, trials with inadequate allocation concealment yield estimates of treatment effect up to 40% larger than trials where adequate allocation concealment was achieved [9, 27]. Therefore, a large treatment effect from a “randomized” trial without adequate allocation concealment might simply reflect biased allocation. The results of a meta-analysis demonstrated that the treatment effect is exaggerated in trails with inadequate or unclear random allocation (ratio of odd ratios = 0.89 for all outcomes), and with inadequate or unclear allocation concealment versus adequate (ratio of odd ratios = 0.93 for all outcome) compared to the trials where adequate concealment was achieved [10]. Randomization can overcome selection bias by increasing block size and varying block size during allocation sequence generation, and allocation concealment [44].

Confounding bias, another common systematic bias (in clinical trial), is defined as a fake association between a factor and outcome, which is not a real factor itself and arises when the factor is related to a range of other characteristics that do increase the outcome risk. It appears when a researcher propose an exposure to an outcome, but in fact measures the effect of a third factor, termed a confounding variable [37]. This may happen if covariates (characteristics) that influence the outcome are not equally distributed between treatment groups [7]. Neglecting randomization will have no influence on the effect size if the patients are actually from a homogenous population, while it has great influence when there is significant heterogeneity in some systematic way among the participants

enrolled to the trial. Therefore, neglecting stratification or other types of restricted randomization (permuted block) may substantially distort the effect size [16]. Randomization can avoid severe imbalances of the most important covariate factors across groups and make the groups comparable in term of the covariates [17]. Therefore, it may slightly improves systematic power to measure differences between two groups, as well as precision if the out-come is correlated with the covariates for participants with significant heterogeneity [17].

The other benefit of randomization is yielding equal sample size. It is well known that, by equally distributing sample size, systematic power to measure differences between two groups improves. Therefore, effects to achieve near or exact equality of sample sizes for treatment and control groups in designs of interventional trials are increasing [45]. Although large imbalances in sample size may appear alarming, the power of a trial is not sensitive to small deviations in equality of the sample sizes [16]. However, in restricted randomization situations (e.g. stratification) group sizes do not have to be exactly equal. Thus, restricted approaches that produce similar sample sizes would yield power much the same as those that generate equal sizes provided the number of participants is slightly more than equal sample sizes [16]. For example, a trial with a number of 60:40 sample size imbalances and with 13% more subjects will have the same power as a balanced trial [46].

Randomization is currently accepted as the most important factor to objectively measure the effect of the treatment, and sets the gold standard for clinical research trials [32]. Unfortunately researchers, for unintentional reasons, fail to implement proper randomization. Common errors include: failing to describe the details regarding the methods of random allocation, concealment (or both) and once it is described, it does not appear as if the subjects were truly randomized. A study of four medical journals dealing with obstetrics and gynecology revealed that approximately 5% of the published RCT reports have assigned participants based on a non-random method [31]. This must represent a gross underestimation as 63 % of the publications failed to indicate a specific method used to generate a random sequence [31].

7. Conclusion

Since randomization is considered the gold standard in most clinical trials, the purpose of this manuscript was to introduce randomization, review several randomization techniques, and to discuss factors related to optimizing randomization procedures. This review paper may be used as a guide for researchers and scholar clinicians to better analyze, criticize, and design randomized clinical trials. Several factors lead a research study to a pure treatment effect size and increased power; randomization is one of the major factors that deserve attention in designing and carrying out clinical trials. It eliminates selection bias, ensures balance of sample size and baseline characteristics, and is an important step in ensuring the validity of statistical tests of significance used to compare treatment groups.

References

[1] Marczyk, G.R., DeMatteo, D., and Festinger, D., 2005: *Essentials of Research Design and Methodology*. Hoboken, NJ: John Wiley & Sons. 306.

[2] Moher, D., Jadad, A.R., Nichol, G., Penman, M., Tugwell, P. and Walsh, S. *Assessing the Quality of Randomized Controlled Trials: An Annotated Bibliography of Scales and Checklists*. Control Clinical Trials. 1995. 16 (1) 62-73.

[3] Portney, L.G. and Watkins, M.P., 2000: *Foundations of Clinical Research: Applications to Practice*. 2nd Ed. Upper Saddle River, NJ: Pearson/Prentice Hall, 722.

[4] Kang, M., Ragan, B.G. and Jae-Hyeon, P. *Issues in Outcomes Research: An Overview of Randomization Techniques for Clinical Trials.* Journal of Athletic Training. 2008. 43 (2) 215-221.

[5] Fisher, R.A., 1977: *The Design of Experiments*. 9th Ed. Edinburgh: Oliver and Boyd. 250.

[6] Armitage, P. *The Role of Randomization in Clinical Trials*. Statistical Medicine. 1982. 12 (1) 345-352.

[7] Schulz, K.F. and Grimes, D.A. *Generation of Allocation Sequences in Randomised Trials: Chance, Not Choice.* The Lancet. 2002. 359; 515-519.

[8] Moore, R.A., 1994: *Evidence-Based Health Care*. Bandolier, 6, Oxford: Anglia and Oxford Regional Health Authority. 7.

[9] Schulz, K.F. and Grimes, D.A. *Allocation Concealment in Randomised Trials: Defending Against Deciphering*. The Lancet. 2002. 359; 614-618.

[10] Savovic, J., Jones, H.E., Altman, D.G., Harris, R.J., Jiini, P., Pildal, J., Als-Nielsen, B., Balk, E.M., Gluud, C., Gluud, L.L., Ioannidis, J.P., Schulz, K.F., Beynon, R., Welton, N.J., Wood, L., Moher, D., Deeks, J.J. and Sterne, J.A. *Influence of Reported Study Design Characteristics on Intervention Effect Estimates from Randomized, Controlled Trials*. Annals of Internal Medicine: Journal. 2012. 157 (6) 429-438.

[11] Altman, D.G., Schulz, K.F., Moher, D., Egger, M., Davidoff, F., Elbourne, D., Cotzsche, P.C. and Lang, T. *The Revised CONSORT Statement for Reporting Randomized Trials: Explanation and Elaboration.* Annals of Internal Medicine: Journal. 2001. 134 (8) 663-94.

[12] Frane, J.W. *A Method of Biased Coin Randomization, Its Implementation, and Its Validation*. Drug Information Journal. 1998. 32 (2) 423-432.

[13] Altman, D.G. and Bland, J.M. *Statistics Notes: How to Randomise*. British Medical Journal. 1999. 319 (7211) 703-704.

[14] Lachin, J.M., Matts, J.P., and Wei, L.J. *Randomization in Clinical Trials: Conclusions and Recommendations*. Control Clinical Trials. 1988. 9 (4) 365-374.

[15] Schulz, K.F. *Randomized Controlled Trials*. Clinical Obstetrics Gynecology. 1998. 41 (2) 245-256.

[16] Lachin, J.M. *Statistical Properties of Randomization in Clinical Trials*. Control Clinical Trials. 1988. 9 (4) 289-311.

[17] Meinert, C.L., 2012: *Clinical Trials: Design, Conduct, and Analysis*. 2nd Ed. New York: Oxford University Press. 469.

[18] Therneau, T.M. *How Many Stratification Factors are "Too Many" to Use in a Randomization Plan?* Control Clinical Trials. 1993. 14 (2) 98-108.

[19] Kenneth, F.S. and David, A.G. *Unequal Group Sizes in Randomised Trials: Guarding Against Guessing.* The Lancet. 2002. 359 (9310) 966-970.

[20] Treasure, T. and MacRae, K.D. *Minimisation: The Platinum Standard for Trials? Randomisation Doesn't Guarantee Similarity of Groups; Minimisation Does.* British Medical Journal. 1998. 317 (7155) 362-363.

[21] Neil, W.S., Gladys, C.M., Craig, R.R. and Marion, K.C. *The Method of Minimization for Allocation to Clinical Trials: A Review.* Control Clinical Trials. 2002. 23 (6) 662-674.

[22] Pandis, N. *Statistics and Research Design: Randomization*. *Part 1: Sequence Generation.* American Journal of Orthodontics. 2011. 140 (5) 747-748.

[23] Moher, D., Hopewell, S., Schulz, K.F., Montori, V., Peter, C.G., Devereaux, P.J. Elbourne, D., Egger, M. and Altman, D.G. *Guideline: CONSORT 2010 Explanation and Elaboration: Updated Guidelines for Reporting Parallel Group Randomised Trials*. British Medical Journal. 2010. 10; 28-55.

[24] Fleiss, J.L., Levin, B.A. and Paik, M.C., 2003: *Statistical Methods for Rates and Proportions*. 3rd edition, New York: John Wiley & Sons, Inc. 768.

[25] Taves, D.R. *Minimization: A New Method of Assigning Patients to Treatment and Control Groups.* Clinical Pharmacology Therapeutics. 1974. 15 (5) 443-453.

[26] Viera, A.J. and Bangdiwala, S.I. *Eliminating Bias in Randomized Controlled Trials: Importance of Allocation Concealment and Masking.* Family Medicine. 2007. 39 (2) 132-137.

[27] Schulz, K.F. Chalmers, I. Hayes, R.J. and Altman, D.G. *Empirical-Evidence of Bias - Dimensions of Methodological Quality Associated with Estimates of Treatment Effects in Controlled Trials.* Journal of the American Medical Association. 1995. 273 (5) 408-412.

[28] Schulz, K.F. *Subverting Randomization in Controlled Trials*. Journal of the American Medical Association. 1995. 274 (18) 1456-1458.

[29] Schulz, K.F., Chalmers, I. and Altman, D.G. *The Landscape and Lexicon of Blinding in Randomized Trials.* Annals of Internal Medicine: Journal. 2002. 136 (3) 254-259.

[30] Pandis, N. *Randomization. Part 3: Allocation Concealment and Randomization Implementation*. American Journal of Orthodontics and Dentofacial Orthopedics. 2012 141 (1) 126-128.

[31] Schulz, K.F., Chalmers, I., Grimes, D.A., and Altman, D.G. *Assessing the Quality of Randomization from Reports of Controlled Trials Published in Obstetrics and Gynecology Journals.* Journal of the American Medical Association. 1994. 272 (2) 125-128.

[32] Doig, G.S. and Simpson, F. *Methodology in Clinical and Health Services Research: Randomization and Allocation Concealment: A Practical Guide for Researchers*. Journal of Critical Care. 2005. 20 187-191.

[33] Herbison, P., Hay-Smith, J., and Gillespie, W.J. *Different Methods of Allocation to Groups in Randomized Trials are Associated with Different Levels of Bias.* A Meta-Epidemiological Study. Journal of Clinical Epidemiology. 2011. 64 (10) 1070-1075.

[34] Gravetter, F.J. and Wallnau, L.B., 2013: Statistics for the Behavioral Sciences. 9th Ed. Wadsworth Cengage Learning. 792.

[35] Lambert, J. *Statistics in Brief: How to Assess Bias in Clinical Studies?* Clinical Orthopaedics Related Research. 2011. 469 (6) 1794-1796.

[36] Chow, S. and Liu, J. 2013: *Design and Analysis of Clinical Trials: Concept and Methodologies*. 3rd Ed. Wiley-Interscience. 892.

[37] David, A.G. and Kenneth, F.S. Bias and Causal Associations in Observational Research. The Lancet. 2002. 359: 248-252.

[38] Wood, L., Egger, M., Juni, P., Gluud, L.L., Schulz, K.F., Altman, D.G., Gluud, C., Martin, R.M., Wood, A.J.G. and Sterne, J.A. *Empirical Evidence of Bias in Treatment Effect Estimates in Controlled Trials With Different Interventions and Outcomes: Meta-Epidemiological Study.* The British Medical Journal. 2008. 336: 601.

[39] Nuesch, E., Reichenbach, S., Trelle, S., Rutjes, A.W., Liewald, K., Serchi, R., Altman, D.G., and Juni, P. *The Importance of Allocation Concealment and Patient Blinding in Osteoarthritis Trials: A Meta-Epidemiologic Study.* Arthritis Rheumatology. 2009. 61 (12) 1633-1641.

[40] Balk, E.M., Bonis, P.A., Moskowitz, H., Schmid, C.H., Ioannidis, J.P., Wang, C., and Lau J. *Correlation of Quality Measures with Estimates of Treatment Effect in Meta-Analyses of Randomized Controlled Trials.* Journal of the American Medical Association. 2002. 287 (22) 2973-2982.

[41] Als-Nielsen, B., Chen, W., Gluud, L.L., Siersma, V., Hilden, J. and Gluud, C., 2006: *Are Trial Size and Reported Methodological Quality Associated with Treatment Effects?* Observational Study of 523 Randomised Trials. 12th International Cochrane Colloquium, Ottawa.

[42] Trowman, R., Dumville, J.C., Torgerson, D.J. and Cranny, G. *The Impact of Trial Baseline Imbalances should be considered in Systematic Reviews: A Methodological Case Study*. Journal Clinical Epidemiology. 2007. 12; 1229-1233.

[43] Berger, V.W. and Weinstein, S. *Discussion: Ensuring the Comparability of Comparison Groups: Is Randomization Enough?* Control Clinical Trials. 2004. 25 (5) 515-524.

[44] Peto, R. *Why do we Need Systematic Overviews of Randomized Trials?* (Transcript of an Oral Presentation, Modified by the Editors). Statistics in Medicine. 1987. 6 (3) 233-240.

[45] Elliott, M.N., McCaffrey, D.F. and Lockwood, J.R. *Brief Article: How Important is Exact Balance in Treatment and Control Sample Sizes to Evaluations?* Journal of Substance Abuse Treat. 2007. 33 (1) 107-110.

[46] Herbert, R.D. *Randomisation in Clinical Trials.* Australian Journal of Physiotherapy. 2005. 51 (1) 58-60.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 1, pp. 155-173, Article ID Med-280
ISSN 2348-5140

Cloud Publications

Research Article **Open Access**

Quality Nutrition Education and Its Impact on Haemoglobin Levels of School Pupils of Muranga County, Kenya

Gladys Njura Gitau, Judith O. Kimiywe and Judith N. Waudo

Department of Foods Nutrition and Dietetics, Kenyatta University, Nairobi, Kenya

Publication Date: 22 February 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-280

Abstract Anaemia can dramatically affect school children with adverse impact on their cognitive development and school performance. Nutrition education has not been given the priority it deserves in primary schools due to the busy school curriculum although it is concerned with changing an individual's behavior. It is in this light that this study was designed. The main objective of this study was to evaluate the effects of three main nutrition education facilitators on nutrition knowledge among primary schools children in Gatanga Sub-County. The facilitators used were researcher, 5 pupil peer educators and an agriculture staff in nutrition education classes using the FAO curriculum chart. A baseline survey was conducted in 12 randomly selected schools for class six pupils' households. Questionnaires for pupils and an interview schedule for caregivers were used to collect data, with pre and post-tests. Demographics and socio-economic factors, food production hygienic practices, dietary intake and biochemical data were obtained. The intervention schools were Mabanda, Kigio and Kirwara (experimental) and Gakurari (control school). Baseline data were analyzed by use of Statistical Package for Social Sciences (SPSS) version 17 and Nutri-Survey software using both descriptive and inferential statistics. The data were coded to search for emerging themes and gaps identified. On average, the mean mark in nutrition knowledge at baseline was 30.05%. In the post-tests all experimental schools significantly improved in nutrition knowledge with the highest school scoring an average of 52% and lowest 40%. A total of 31.4 % pupils at baseline and 21.4% after interventions were found to be anaemic after altitude adjustments at a calculated factor 0.5 for Gatanga altitude (2237m ASL). Improvement in the adoption and use of the projects that enhance nutrition and health significantly occurred in the experimental schools as opposed to the control school. Pupils' haemoglobin status were not significantly different ($p>0.05$) between the experimental and control schools at baseline. However, notable differences in haemoglobin levels occurred in the experimental schools after the interventions. The relationship between nutrition knowledge and nutrient intake was positive and there was a significant relationship between nutrition knowledge and haemoglobin levels at $p<0.05$ ($r=0.253$, $p=0.025$). Anaemia was found to be a significant problem and therefore the need for a comprehensive intervention strategy by all stakeholders. The study findings would contribute towards operationalization of the Kenyan National School Health Policy and Guidelines, the National Food Security and Nutrition Policy in prevention and control of IDA by enhancing nutrition education.

Keywords *Agriculture Extension Staff Anaemia; Haemoglobin Levels; Peer Educator*

1. Introduction

Iron is a micronutrient that is required in the tissues of the body for basic cellular functions and is critically important in the muscle, brain and red blood cells. Iron is a component in many proteins including enzymes and haemoglobin, the latter being important for the transport of oxygen to tissues throughout the body. Although there are many health surveys which have been done on the general population the adverse effects of iron deficiency anemia on school children has been somehow neglected and it becomes difficult to lay strategies of interventions [8]. About 1.8 million of Kenyan children are malnourished and affected by anaemia [15]. Iron deficiency anaemia affects all age groups whereby it affects 21% school girls in Western Kenya [34]. Iron deficiency anemia is characterized by low levels of haemoglobin in combination with abnormal levels of other iron indicators such as transferrin saturation (i.e. iron stores). IDA can lead to weakness, poor physical growth, increased morbidity, impaired cognitive performance and delayed psychomotor development. In particular, iron deficiencies early in life are thought to potentially inhibit the function of neurotransmitters, thus compromising brain function [6]. Impaired gastrointestinal function, altered patterns of hormone and metabolism and reduced DNA replication and repairs have also been noted as other consequences of iron deficiency anaemia [23]. Anaemia is simple to measure and has been used as a marker of iron deficiency severe enough to affect tissue functions. However, iron deficiency is not the sole cause of anaemia in most populations. Even in an individual, anaemia may be caused by multiple factors such as food access and health status [18].

Iron deficiency may, however occur throughout the lifespan where diets mainly consist staple foods and little of animal products. The cause is a one-sided diet based mainly on grains. These contain phytates, substances which bind the nutrient iron from plant sources as insoluble salts [4]. In Kenyan households, studies have been undertaken and diets are mainly cereal-based with tubers and a variety of vegetables and fruits when available. White maize, sorghum and millet are high in phytate and fiber, which inhibit the absorption of micronutrients such as zinc and iron. Communities growing cash crops such as coffee have little land for food crops. Although households may own cattle, goats and poultry, these are not commonly consumed but sold in order to earn income. In addition, products of these animals are sold to earn income [7]. There are multiple sources of dietary iron including heme and non-heme iron, contamination iron and fortification iron. Heme iron is usually of animal origin and of high bio-availability with sources including meat, fish and blood products. Dietary intake of heme iron is negligible in developing countries while iron status and health status (infection, mal-absorption) are the host factors influencing iron absorption [39]. Iron deficiency could also be due to inadequate folic acid, riboflavin, copper, vitamin A, & B_{12} and zinc intake [54]. Micronutrients such as vitamin A, zinc and iron interact with each other to promote appetite which leads to increased food intake and intake of other macro-nutrients and micro-nutrients [38].

According to [18] a study in Egypt, it was revealed that teachers perceived that the unhealthy feeding habits of the school children especially lack of breakfast, affected the interaction between the school children and the teachers. Food security coupled with nutrition education is an important factor that should be considered and addressed in tackling malnutrition [30]. In Kenya, school feeding programs have not been implemented in high potential areas with success [28]. The aim of this study was to offer nutrition education to primary school pupils and assess the iron status after consumption of iron rich foods.

2. Nutrition Knowledge, Attitude and Practices of School Children

2.1. Nutrition Knowledge

Improving nutrition brings greatest benefits to the poor and the most vulnerable especially school going children. According to a Food and Agriculture Organization report [10], it is important to incorporate nutrition education into the curriculum of primary education which considers priority

nutrition issues affecting children and their families in the country [13]. Children are eager to learn, are role models for their peers and schools can stimulate and support children to develop skills and knowledge to face daily challenges now and in the future [16].

Knowledge is necessary for practice of good nutrition in the school environment and households. Without adequate nutrition, health and hygienic education, school nutrition programme seem to be less effective [46]. Poor health and stunting continue to occur during the school years which can be reversed by appropriate nutrition interventions. This was revealed by a study undertaken in Zambia on children 6-15 years [14].

2.2. Agronomic and Small Livestock Production Practices

Poorer households have less land, produced less food; have less food storage and little purchasing power [7]. Intervention measures that promote green leafy vegetable production and small livestock production should be embraced to enhance consumption of micronutrient rich foods. School-based gardening programmes can be an excellent means of introducing new ideas about gardening and a useful channel for reaching others in the community and hence promoting consumption of the vegetables and other iron rich foods [13]. Gardening also promotes agriculture as a dignified and important vocation, increases the knowledge and use of best practices in farming and increases the quantity and quality of available food. Unfortunately, young people often have a poor opinion of agricultural work. Moreover, agriculture is not taught as a school subject in primary schools in Kenya and many students do not take it seriously since they consider it a financially and morally impoverishing vocation. Hence the importance of introducing it to the young primary school pupils when their habits are being formed. This study sought to use three nutrition education facilitators to address iron deficiency anaemia among school children in the coffee growing area of Gatanga Sub-County, Muranga County.

A study in Nepal in which pupils aged 10-14 years and their teachers were trained on agriculture, the study showed much appreciation of the great importance and dignity of agricultural activities. A school agricultural programme provided the resources to establish a model farm on each school's grounds; participants carried out the work, from tilling and digging of compost pits to final harvest. Students also passed knowledge along to their farming families [29].

2.3. Iron Status, Nutrition Knowledge, Attitudes and Practices

Some strategies for the prevention and control of micronutrient deficiencies are; food-based strategies and dietary diversification, combined with nutrition education. Nutrition education by all stakeholders can greatly improve the nutritional status of populations [44]. Food-based strategies are an essential component of a long-term global strategy for the control of micronutrient malnutrition. Nutrition education engages in establishing existing levels of nutrition knowledge, attitudes and practices. In a study [3] it was found out that nutrition education can improve knowledge of healthy nutrition and lifestyle choices. Focused nutritional education using available resources and correcting current dietary habits in a vulnerable group of adolescent girls resulted in dietary changes and practises that ultimately improved iron intake. In another study [24] they reported that nutrition education did have a positive effect on the iron status possibly by improving the dietary iron intake. The study also concluded that long-term community-based approaches involving dietary education emphasizing optimum feeding schedules and adequate diets for children may possibly reduce the risk of anaemia and raise iron status. Food beliefs, preferences and habits in Kenyan families have been passed from generation to generation hence has become a custom or tradition which influences food choices. School-based approaches accord the children the excellent chance to practically participate in the Nutrition Education programme, food selection, preparation and consumption as well as understanding why good nutrition is important [19]. Nutrition education and promoting good nutrition practices in schools are known to have a significant effect in fostering healthy eating habits [33].

2.4. Nutrition Education, Iron Rich Food Consumption and Iron Status Outcomes

The pressures from population growth and poverty contribute to severe malnutrition and continue to affect nearly half of the world's population. Also alarming is that an 18 percent rise in the number of malnourished children is projected for Africa by 2020. Over 2 billion people suffer from malnutrition in their diets, including protein-calorie deficiencies and micronutrient malnutrition like iron deficiency anaemia. Such malnutrition prevents much of the world's population from reaching their full potential - mentally, physically or financially [15]. Dietary intervention may be a safer and more feasible solution to address iron deficiency anaemia in the long run compared to other strategies like supplementation. This intervention requires nutrition education to improve knowledge and practices that support healthy outcomes. Integrated rural nutrition projects and nutrition education have a significant impact on knowledge and attitudes leading to long-term beneficial health effects than activities that only aim at increasing food availability. Nutrition education is therefore a viable, sustainable solution in resource limited setting [23]. In their study [24] in a population group in India, where, the iron and nutritional status was highly compromised, nutrition education intervention was effective, as it improved the dietary iron intake and prevented the children from suffering the sharp decline in iron status noted in the control group.

2.5. Food Sources

Children require iron for their expanding red cell mass and growing body tissue. In addition, iron is needed in increased amounts by girls as they begin to menstruate. Children have lower total energy requirements and therefore eat less and thus at a greater risk of developing iron deficiency, especially if their dietary iron is of low bio-availability [53]. The best food sources of easily absorbed iron are animal products which provide heme iron. Vegetables, fruits, grains and supplements provide non-heme iron which is of low bio-availability.

Food sources of high bioavailability iron include liver, lean red meat (especially beef), poultry, fish, iron fortified cereals, dried fruits and dark leafy green vegetables. Reasonable amounts of iron are also found in lamb and pork. Non-heme iron is found in whole grains such as wheat, millet, oats and brown rice; legumes (beans, *dolicos lablab*, soybeans and peas); dried fruits (prunes, raisins, and apricots); vegetables (broccoli, spinach, kale, beetroots, collards) [57]. Meat, fish, poultry, ascorbic acid (vitamin C) and organic acids are all thought to enhance non-heme iron absorption [40]. Tea, coffee, phytates (storage form of phosphate and minerals) and calcium hinder the absorption of non-heme iron and may contribute to the overall lower bioavailability of non-heme iron compared with heme iron [41].

2.6. Food Guide Pyramid for Meal Planning and RDA

The food guide pyramid is an excellent tool to help make healthy food choices. It assists in selection of foods in order to provide the body with nutrients needed and at the same time the right amounts of calories to maintain a healthy weight. The food guide pyramid is used for food servings and meal plans. The food guide will assist one to organize foods according to the energy and nutrients that they supply, so that the prepared meals are balanced and nutritious. The food guide illustrates how foods should be selected and indicates the foods that should be eaten more (at the base of the food guide), moderately and generously (center) and in small amounts (at the top of the food guide). The food guide also recommends consumption of a minimum of 8 glasses of clean safe water per person per day. It serves as a general guide that lets one choose a healthy diet that is right for them with adequate macronutrients and micronutrients [32; 50]. Figure 1 shows a food guide pyramid.

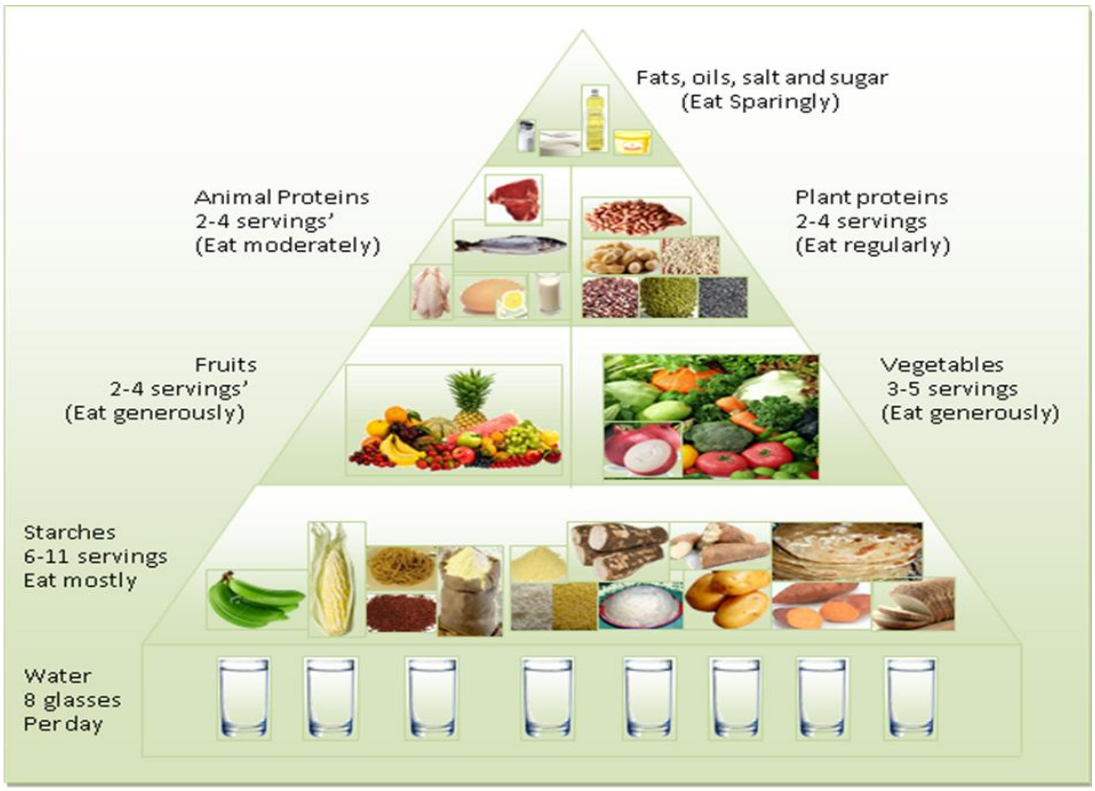


Figure 1: *The Food Guide Pyramid, Source: MOPHS 2012*

The Recommended Dietary Allowance for Iron is, 8-13 mg for 10-18 years old pre-adolescent and adolescent children both girls and boys [48]. An average school lunch must contain at least 3mg of iron in primary schools [56]. A modification of culturally acceptable foods to include iron-rich foods may provide a sustainable approach to controlling and preventing iron deficiency in the population of school aged children [9].

2.7. Effect of Different Nutrition Education Strategies in Addressing Iron Deficiency Anaemia

Schools are the natural development zone for nutrition education. They are one of the main social contexts in which lifestyles are developed. Children of school-going interact with their environment at home, their communities this influences their eating habits [13]. The primary goal of nutrition education should be to help young people adopt eating behaviours that promote health and reduce risk for disease. Behaviourally based education encourages specific healthy eating behaviours for example eating more fruits and vegetables. Learners or target audience from different cultural groups have different health concerns, eating patterns, food preferences and food-related habits and attitudes. These differences need to be considered by teachers and facilitators when designing lesson plans or discussing food choices.

Learners are more likely to adopt healthy eating behaviours when they learn about these behaviours through fun, participatory activities rather than through lectures [35]. Food-based approaches to addressing malnutrition should include educational input. School-garden interventions are most effective when combined with promotional and educational interventions. Strategies combining information, education and communication are needed, and these should be combined with community mobilization and agricultural inputs. School-based nutrition education can improve dietary practices that affect a young person's health, growth and intellectual development [2]. For effective coverage of agricultural extension work in the farms and schools agricultural extension workers fully assist the school pupils in the already formed clubs. The projects they promote include agriculture,

health and nutrition that promote healthy eating, growth and income generation for schools and households. Therefore, embracing any strategy in nutrition education content delivery would go a long way in addressing malnutrition issues

The research was designed to include Peer and Agriculture staff in-order to compare their effectiveness in content delivery to the learners. Peer education is a flexible social strategy within a prevention and early intervention delivery system. It usually focuses on children and youth among others to reach high-risk populations. It is a process in which trained supervisors develop and support a group of suitable people to educate, strengthen and support their peers to contend with the health threats and decisions they face (trainer -of -trainer approach). Peer educators create a safe place for candid and genuine examination of attitudes, choices and situations. Through their role as educators they become informal influences, helpers and advocates for systemic change [20]. Peers can influence each other positively for better nutrition [55]. This can be achieved by having both formal and informal teachings amongst themselves [47]. Where resources are limited and large numbers have to be reached, peer education can have a multiplier effect [55]. Therefore the researcher designed the three strategies of researcher, peer and agriculture staff in-order to compare their effectiveness in nutrition education content delivery to the learners.

3. Methodology

The study targeted pupils in upper primary class 6 and their parents who in our view were appropriate change agents in the community. The study population was chosen because at class six they would be able to grasp nutrition knowledge and practice it at their homes. The pupils at this stage were not under any pressure of external examinations like those in class eight. It was important for them to understand the importance of iron as a micro-nutrient as they enter puberty. This study was designed to assess and address the gap in nutrition knowledge using three facilitators.

3.1. Baseline Study

Baseline study was undertaken in-order to have a general overview of the study area and get the schools that met the required criteria for intervention. The intervention schools acted as a representative sample for the primary schools in the study area. Questionnaires were administered to class six pupils in 12 baseline schools. The structured interview schedule was administered to caregivers of the sampled households (those who prepared meals for the children). The researcher gathered socio-demographic and health data, dietary practices based on a 24-hour dietary recall, seven day food frequency using a questionnaire (FFQ for pupils from sixty seven [67] households in the 12 baseline survey schools from the caregivers. Focus group discussion at baseline was done in two schools with the teaching staff to assess the general need for a nutrition education study in the schools. The researcher convened parent meetings at each intervention school to inform them about the study discuss child health issues and obtain a written informed consent for their children's participation in the study.

3.2. Pretests Done Before Interventions

Pre-tests questionnaires on nutrition knowledge were administered before embarking on the interventions to all the 154 study pupils in the experimental schools and the control school. This was done in March 2012 and interventions started immediately in the same month. For nutrition knowledge, each pupil in class six in the experimental and control schools did a pre- test (pre and post-test questionnaire) which was marked and marks recorded for each pupil to test the effect of the intervention on nutrition knowledge. Other nutrition education topics included food production mainly iron rich foods, selection of a balanced diet from locally available foods and recipe modifications and various projects at household level after the assessment. The assessment of iron status of the study

pupils was done to 89 pupils at baseline and 79 at end-line. The procedures were done by a laboratory technician from Kirwara Sub-District hospital (level 4) on the first week of April 2012.

The purpose of the study and the procedures for blood sample collection for haemoglobin levels were explained to the head teacher, senior teacher, class teacher and the children in class six whose parents had signed the informed consent forms. Random sampling of boys and girls was done to come up with the sample required, from the 154 pupils' enrolled from both experimental schools and control school at baseline for the intervention. Information about name, sex, age and sample numbers were recorded. The sample size for both boys and girls were similar since none of the girls reported to have started menarche. The gender specific sample size was determined based on discussions with the laboratory technician who suggested a greater sample size for females than males if they had menarche since there is an established higher prevalence of anaemia in menstruating females. Pubertal status was assessed by the status quo method: Female pupils were asked whether they had experienced menarche before the blood samples were taken and the males' nocturnal emissions. None of them said they had menarche or emissions in both the pre-tests and post- tests.

Venous blood sample was drawn with a sterile disposable needle and syringe from the sampled pupils asceptically from antecubital veins of the arm and 2ml of blood was alliquotted into BD vacutainer tubes with K_2E anti-coagulant (EDTA-sequestrene) after disinfections with methylated spirit swap and drying of skin. The tubes were then packed in a cooler box at 20^0C for maximum 6 hours before being transported to Kirwara Sub-district hospital (level 4). Full blood count was analyzed using haematology analyser (Celltac) - Model MEK 6410K within 15 hours. A safety box and incineration container for used syringe and needles plus the used swaps was provided to carry the waste. The children were categorized as having iron deficiency anaemia when found with Hb <12g/dl for girls and <13g/dl for boys of the specified ages based on WHO standards.

The researcher had identified what was to be taught (content) in nutrition knowledge. Facilitators for each experimental school were identified and the notes offered by the researcher (same notes were used in all the facilitations). From the notes, the researcher had identified, the topics covered were relevant fitting with psychological development of class six pupils by carefully considering existing dietary needs, local foods, nutritional practices and the children's perceptions. From age 11-13 children learn about food supply and that plants are the basis of food chain (food production). It was therefore important to use different parties or strategies in carrying healthy messages to children and also evaluate their effectiveness in the delivery of the contents. Nutrition education was taught using the three facilitators of the researcher, agricultural officer and peer educators. Among other things the effectiveness of each facilitators was to be evaluated. At each visit, facilitators determined whether each child from the baseline roster was present, absent, had left school or had transferred to another school. Lesson plans showing the lesson organization and presentation were also made with guideline from the classroom curriculum chart and Class 5 and 6 Science books [13; 1; 51]. The pupils' were taught using visual aids where necessary. Real objects like, foodstuffs, vegetable seeds and seedlings, rabbits and fireless cookers were used. The effectiveness of each facilitator in delivery of nutrition education messages was measured during the study. The lessons took 30-35 minutes each for 10 weeks in March to May 2012. This took place in the evenings, weekends and during the holidays to avoid interfering with the school teaching programme. Charts and notes were validated by teachers and pre-tested as needed. Specific micro-nutrient rich foods by the facilitating team were used for demonstrations in the school gardens, including beetroots, Blacknightshade (*Solanum scabrum*) or *Managu*, *Amaranthus bitum* (*terere*) slenderleaf (*Crotalaria ochroleucia*) or *mitoo*, jute mallow (*Corchorus olitorius*) or *mrend*a, capsicum, kales, spinach, onion and cowpeas (-*Vigna unguiculata*) or *kunde* in local names.

The school gardening activities (establishment) included conventional and multistory gardens in the school at the plots allocated by the head-teacher. Cookery of the iron rich locally available foods was

taught in all the experimental schools to class six pupils in about one hour of practical classroom setting. The sustainability projects meant to reduce micronutrient deficiencies, thus multistory gardens, conventional gardens, rabbit and poultry projects were promoted at pupils' households. Improved cookers (*jiko kisasa)* and fireless cookers were also promoted for nutrient and fuel conservation. The lessons that took 30-35 minutes also included the nutritive value of food, the balanced diet, the food guide pyramid, deficiency diseases, health and sanitation, hygiene and food safety, cooking methods and selection of balanced meals from locally available foods and recipe improvement. More emphasis was laid on the importance of iron rich foods and how to grow and eat them. The pupils' responses and teachers' responses on Likert items were analyzed at 3 point and 5 point scales respectively. Two continuous assessment examinations validated by teachers and the post-test were done by the pupils during the study. An end-term survey was undertaken to assess the impact of the intervention to the pupils' households. The dietary intake on 24hr dietary recall (taken twice and averaged), seven day food frequency and technologies adopted were assessed at the household level for the follow-up pupils. A focus group discussion was conducted at the end-term of the study in one school with some teaching staff to evaluate the effectiveness of the study. The post-tests and final home follow-up were done in July-August 2012.

3.3. Data Management

In Phase 1, biochemical results were keyed in the laboratory information system and copies of results kept in manual and an electronic laboratory notebook. These results were to be compared with post-tests of the experimental and control school class six pupils who consented to be included in the biochemical data collection. In phase two, the questionnaires were ordered numerically and edited before data entry. Averaged dietary data from the 24 hour dietary recall were entered into a modified version of Nutri-survey computer nutritional assessment package for quantitative calculations. The resulting data from socio-demographics and economic characteristics, health and sanitation, nutrition knowledge, attitude and practice among others were transferred to Statistical Package for Social Sciences (SPSS version 17) for comparison and contrasting between the independent variables.

3.4. Logistical and Ethical Considerations

A letter to get clearance to carry out research was obtained from the Kenyatta University Board of postgraduate and the Deputy Vice Chancellor (Academics). Research permit was obtained from the National Council for Science and Technology. Administrative permission was also granted at Sub-County and Municipality level and by the schools management committees. Ethical clearance for biochemical data collection was sought from Kenyatta University Ethics Review Committee and also approved by Medical Officer of Health Thika, the Sub-county Public Health Officer and Kirwara Sub-county Hospital (Level 4). Head teachers, pupils and parents were informed about the aim of the study, its procedures and then written consents were obtained from the head teachers and parents.

4. Results

4.1. Nutrition Knowledge across the Experimental Schools after Interventions

The primary outcome was nutrition knowledge while iron status was considered as secondary outcome resulting from the intervention. Post-tests showed a significant difference with experimental schools under three facilitators performing significantly better than the control school (Figure 1). The peer facilitated school performed best with (51.52+24.79) marks, followed by the researcher facilitated school (48.39+22.23) and the agriculture staff (39.29+9.87). The pre-test post- test improvement in the control school (31.21+12.74) was however not significant ($p>0.05$) as compared to the performance of the experimental school. The hypothesis that there is no significant difference between nutritional knowledge in the experimental schools across the three nutrition education

facilitations compared to the control schools in the coffee growing area of Gatanga Sub-County is therefore rejected at p< 0.05 (Figure 1). The hypothesis is rejected because T-test revealed differences in nutrition knowledge before and after the interventions at p<0.05. The results shown in Figure 1 are the performance in nutrition knowledge before and after interventions across all the intervention schools.

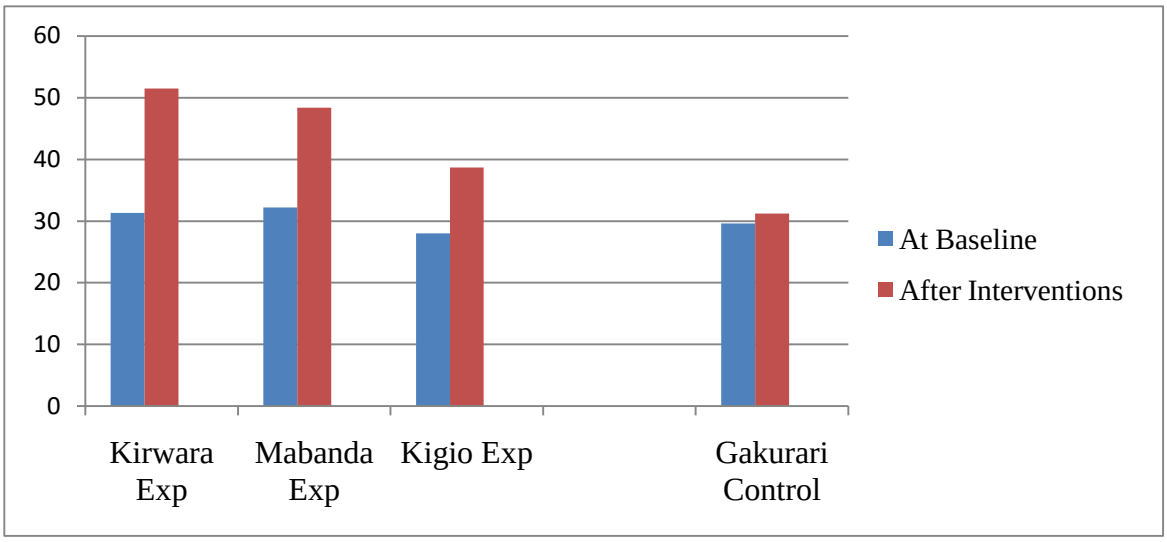


Figure 1: *Nutrition Knowledge Before and After Interventions*

The results show the improvement attained in experimental schools compared to the control school after the interventions. The improvements in the experimental schools were significantly different compared to the control school.

4.2. The 24hr Dietary Intake After interventions

Dietary intakes increased significantly after the interventions in the experimental schools as compared to the control school (Table 1). T –test was computed on food consumption and iron status and food consumption patterns and iron status were not significantly different (p>0.05) between the experimental and control schools at baseline. Notable differences occurred in the experimental schools after the interventions in the intake of some nutrients. Consumption of all nutrients increased and was statistically significant at p<0.05 except for Vitamin E, Calcium (Ca), iron (Fe) and Magnesium (Mg). Based on the results the hypothesis that there is no significant relationship between nutrition knowledge and the consumption of iron rich foods in the experimental schools (Kirwara, Kigio, Mabanda) across the three nutrition education strategies and the control school (Gakurari) of Gatanga Sub-county at p<0.05 is rejected. Table 1 shows the 24hr dietary intakes at post intervention.

Table 1: *The 24 hr Dietary Recall Nutrient Intake after Interventions*

Nutrient	**Control School**	**Experimental Schools (n=43)**			
	Control[0]	Peer facilitated[1]	Researcher facilitated[2]	Agriculture Staff facilitated[3]	p value
Energy (Kcal)	1125+34[a]	1554 +30[b]	1662+28[b]	1627+24[b]	p<0.001
Protein (g)	32.8 +3.0[a]	42.3+9.2[b]	45.9+12.0[b]	46.4+9.8[b]	P<0.05
CHO (g)	315+21[a]	305+23[a]	402+27[a]	465+17[a]	P=0.146

Vit. A (ug)	259+48[a]	488+41[b]	502+45[b]	576+30[b]	P<0.05
Carot. (mg)	554+46[ab]	249+35[a]	244+39[a]	1230+33[b]	P<0.05
Vit. E (mg)	0.72+0.62[a]	1.28+1.07[a]	0.75+0.33[a]	0.71+0.62[a]	P=0.181
Vit. B_1(mg)	0.93+0.19[a]	2.26+3.35[a]	3.5+7.4[a]	1.28+0.28[a]	P=0.416
Fol.acid (ug)	71+19[a]	179+10[b]	141+42[b]	128+44[b]	P<0.05
Vit. C (mg)	44.4+19.4[a]	65.2+17.1[a]	76.0+19.6[b]	81.2+18.4[b]	P<0.05
Ca (mg)	248+22[ab]	228+26[a]	268+21[ab]	306+25[b]	P=0.129
Mg (mg)	398+19[a]	425+11[a]	373+10[a]	358+13[a]	p=0.670
Zinc (mg)	1.3+0.5[a]	3.4+2.4[b]	2.8+1.4[b]	2.6+1.2[b]	P<0.05
Iron (mg)	10.2+1.7[a]	11.0+2.3[a]	11.7+3.6[a]	11.5+2.6[a]	P=0.368

NB: Means in the same row with the same letter are not significantly different.
[0=]**Control school:** Gakurari**, Experimental Schools**: [1=]Kirwara, [2=]Mabanda, [3=] Kigio

4.3. Haemoglobin Levels after the Interventions

After the intervention, the haemoglobin levels were compared at pre and post intervention to establish if there was any change (Table 2). Pupils' haemoglobin status were not significantly different (p>0.05) between the experimental and control schools at baseline but, notable differences occurred in the experimental schools after the interventions. In the peer facilitated school (Kirwara) the mean Hb levels were 12.8g/dl before interventions and after the interventions the mean levels improved to 13.41g/dl. For researcher facilitated school (Mabanda) the mean levels improved from 14.26 g/dl to 14.50g/dl, and the agricultural officer facilitated school (Kigio) the levels improved from 13.50g/dl to 13.83g/dl. For the control school the Hb levels decreased from 13.70g/dl to 13.33g/dl. There was a statistically significant difference between the pre-test and post-test mean Hb values (p= 0.038). Table 2 shows changes in mean haemoglobin levels of pupils at post intervention.

Table 2: *Mean Haemoglobin Levels Before and After Intervention*

	Mean Hb levels g/dl (+) SD		
Schools	Before	After	p- value (t-test)
Control[0]	13.8+0.9	13.3+0.7	0.038
Peer facilitated[1]	12.8+0.9	13.4+1.0	0.002
Researcher facilitated[2]	14.2+1.2	14.5+0.7	0.268
Agriculture staff facilitated[3]	13.6+0.8	13.8+0.8	0.075

[0=]**Control school:** Gakurari**, Experimental Schools**: [1=]Kirwara, [2=]Mabanda, [3=] Kigio

There was a statistically significant difference in the Hb levels before and after the interventions in the peer facilitated school at p= 0.002. Notable improvement occurred in the other two schools but was not significantly different at p=0.268 in the researcher facilitated school and p=0.075 in the agriculture staff facilitated school. The results rejected the hypothesis that there is no significant difference between iron status in the experimental schools across the three nutrition education facilitations compared to the control schools in the coffee growing area of Gatanga Sub-County at p<0.05. The hypothesis is rejected because t-test revealed significant differences in iron status before and after the interventions at p<0.05.

4.4. Effect of Nutrition Education facilitation on Nutrition Knowledge and Practices

The study partly aimed to translate the knowledge transferred through the three facilitators into practices that promote good nutrition and health for pupils and their households. Experimental schools grew vegetables at school in the school gardens. During the study period, the researcher facilitated school did best in vegetable production (12kgs) followed by peer facilitated (10.5kgs) and agriculture staff (8.0kgs).

4.5. Presence of Multi-Storey Gardens in Pupils' Households

Training and use of demonstrations to children on food production showed increase in the crop yields and the variety of foods grown at their households. This improves nutrition and combats chronic hunger. Follow up on the pupils to their households revealed that twelve households in the peer facilitated and researcher facilitated schools had adopted growing of vegetables using the gunny bags since land sizes are very small in this area and such technologies were easily adopted (Plates 1 and 2)

Plate 1: A Sack (Multi-storey) Garden at School growing kales

Plate 2: A Pupil with a Sack Garden at Home growing kales

The researcher and peer facilitated school did significantly better than the agricultural staff facilitated school at $p<0.05$. The control school improved but not statistically significant at $p>0.05$. Peer and Researcher facilitated schools showed improved adoption in food production with peer and researcher facilitated schools showing a statistically significant different improvement at $p<0.05$. Knowledge gained from school gardens was transferred to the community by the experimental pupils. There was an evident improvement in the introduction of new varieties of foods such as cowpea leaves, kales, jute mallow (*mlenda*), beetroot, capsicum, amaranth and black nightshade in the experimental schools. The establishment of gardens with a variety of vegetables was significantly different in the experimental schools households than in the control school. Children from two experimental groups in the peer facilitated and researcher facilitated schools were more likely to have consumed several of the individual micronutrient-rich foods.

Effectiveness of the Different Nutrition Education facilitations Amongst the Study Pupils at Pre and Post-test

The different nutrition education strategies had different impact on the study pupils. Table 3 shows effect of different nutrition education facilitations on the study pupils at pre and post-test.

Table 3: *Performance of Nutrition Education Facilitations Before and After Interventions*

Variable	Strategy used in schools	P value(t-test)
Nutrition knowledge	Control	0.337
	Peer facilitated	0.000
	Researcher facilitated	0.000
	Agriculture staff facilitated	0.000
Haemoglobin levels	Control	0.038
	Peer facilitated	0.002
	Researcher facilitated	0.268
	Agriculture staff facilitated	0.075
Multi-storey gardens	Control	0.324
	Peer facilitated	0.001
	Researcher facilitated	0.044
	Agriculture facilitated	0.323

Performance in all the nutrition education strategies showed improvement in all the selected variables. Most of the selected variables showed a significant difference at p<0.05.

4.6. Relationship of Nutrition Knowledge and Selected Nutrient Intakes after Interventions

Nutrition interventions are known to help in management of all kinds of malnutrition amongst the populations. Table 4.6 shows correlation and p values post interventions.

Table 4: *Correlation and p values post Interventions*

Nutrition knowledge and:	r	p value
Protein intake	0.218	0.080
CHO intake	0.667	0.000
Kcal intake	0.661	0.001
Vitamin A intake	0.217	0.036
Vitamin C intake	0.272	0.068
Zinc intake	0.037	0.261
Folic acid intake	0.230	0.057
Iron intake	0.349	0.007
Haemoglobin levels	0.253	0.025 (p<0.05)

Bivariate correlations (r) explain the relationship between nutrition knowledge and selected nutrients at post-test. The Kcal, Carbohydrate, Vitamin A, folic acid and iron intake relationships were statistically significant except for protein, zinc, folic acid and vitamin C (Table 4). There were significant positive correlations between nutrition knowledge and hemoglobin levels (Table 4). The pupils in the experimental schools improved their nutrition knowledge, iron rich food intake which could have positively influenced their hemoglobin levels (Table 4). The hypothesis that there is no significant relationship between nutrition knowledge and the iron status in the experimental schools compared to the control school (Gakurari) in the coffee growing area of Gatanga Sub-County at (r= 0.253, P=0.025) p<0.05 is rejected. The results show that the interventions in nutrition knowledge had an effect on the levels of haemoglobin levels. The school gardens would be used as a simple and cost effective means to improve nutrition education. Children learn by doing therefore an effort was made to continue training them and involving them in food production, record keeping, sales and utilization by involving the NGO. Linear regression for post-test nutrition knowledge had a positive trend and the haemoglobin levels were increasing. Nutrition knowledge scores increased the

haemoglobin levels. The results show that the interventions in nutrition knowledge had an effect on the levels of haemoglobin levels.

5. Discussion

5.1. Pupils' Nutrition Knowledge at Pre-test

The pre-intervention tests showed that pupils lacked adequate nutrition knowledge (an average of 30.05%) and identified the need to increase effort on nutrition education in Gatanga Sub-County. Interventions on nutrition education to improve knowledge and practice that support healthy eating to address iron deficiency are required in any given population [23]. School gardens and other resources that could have been used as teaching aids for nutrition education were not actively used in the study area. Consistent with the findings, nutrition practices in the area before the intervention were poor since the learners could not practice what they did not know. This is comparable to a study undertaken in Machakos County whereby most pupils scored an average of 35% at pre-test in nutrition knowledge [30].

5.2. Pupils' Haemoglobin Levels at Pre-test

In the current study almost 1/3 of the total pupils were found to be anaemic after altitude adjustments at a calculated factor 0.5 for Gatanga altitude (2237m ASL). The findings compared well with [5] report that in rural public schools in Delhi anaemia was 23% among boys and, 15.3% among girls. This also compares with a study done in Turkey, [26] whereby 12.6% of pupils' in two primary schools were anaemic. In the current study, it was revealed that iron deficiency in a population may be masked by altitude induced polycythaemia as increased prevalence of anaemia after altitude adjustment amongst the population stood at 31.4%. The results are in agreement with [5] in a study undertaken in Chandigarh India where 25.4% rural pupils (age 12-18years) sampled from two rural schools were anaemic. A similar study in Ouagadougou, Burkina Faso revealed that 45.6% of primary school pupils of ages 13-14 years were anaemic. In a report on six African and two Asian countries, 40.2% of children aged 7-11 years and 54.4% of those aged 12-14 years were anaemic [8]. In their study [45] found iron deficiency anaemia prevalence of 31% among Tanzanian school going children.

5.3. Pupils' Dietary Intakes at Pre-test

This study showed a low intake of most nutrients except carbohydrates which were above RDA. The low mean intakes could have been influenced by the poor harvests, large family sizes and the level of inflation that affected food access during the study. In poor countries, diets tend to be deficient in multiple micro-nutrients and not only iron and folate, but deficiency in vitamin B_{12}, vitamin A as well as zinc contribute to iron deficiency anaemia [7]. Many factors influence dietary intake at individual and household levels. It has been documented in some studies that the bigger the land the more diversified crops and livestock breeds are grown and kept respectively meaning diversified diets and more nutrient intake. Research has shown that the higher the income, the better the dietary intakes and the bigger the household the higher the competition for various resources [37]. Low dietary iron intake is an attributed cause of iron deficiency and anaemia in many parts of the developing world. Food-based intervention should be one of the important strategies for reducing the magnitude of the problem of anaemia in school children and their communities [45].

5.4. Pupils' Nutrition Knowledge after Interventions

Post-tests showed a significant difference in nutrition knowledge with the experimental schools performing significantly better than the control school. The performance of the control school also improved in some nutrition knowledge aspects such as the knowledge of enhancers of iron uptake, food guide pyramid and iron rich green vegetables. This could have been contributed by the first

sensitization during the pre-test assessment, which may have provoked the pupils thinking and understanding of the nutrition knowledge. The pre-test post-test improvement in the control schools was however not significant ($p>0.05$) as compared to the performance of the experimental schools. Findings in an integrated rural nutrition project in Kawambwa, Zambia indicated that nutrition education programs have a significant impact on knowledge and attitudes than activities that only aimed at increasing food availability [23]. The pre-intervention test had shown that without adequate training no sex would have an advantage over the other in nutrition knowledge. Significant improvement in nutrition knowledge and practice were observed in all the experimental schools under the different facilitators, while the control school improved but not significantly. In the study, the control school could have improved because the children were exposed to similar questions and discussions may have occurred after the first exposure meaning an improvement in the repetition (post-test).

Peer education can produce leaders in nutrition education who act as positive role models for other pupils. Peer education makes a conscious use of peer influence in a positive way (meaning in a way that contributes to everyone's well-being) [55]. It is well documented that peers have confidence with one another and this could have resulted into the good performance under the peer facilitation strategy. The other reason could probably because the class was fairly small with twenty seven pupils. The primary outcome was improved nutrition knowledge while the iron, worm status and practice were considered as secondary outcomes resulting from the positive effect of the intervention. Changes in practice after intervention demonstrate the effectiveness of the intervention programmes in improving nutrition both among school pupils and household members. Class participation and iron rich food consumption improved greatly. These findings are in agreement with a study done in India whereby there was improved nutrition knowledge after interventions [42]. Pupils in the experimental schools adopted new projects as compared to the control school. Nutrition education leads to general improvement in dietary patterns and other practices like better farming practices.

5.5. Pupils' Nutrition and Health Practices after Intervention

After the interventions, nutritional practices improved greatly at schools and the pupils' households. For example, in this study, consumption of traditional vegetables before the nutrition intervention was poor although almost every family had a small plot to grow the crops. After the intervention, growing of vegetables, consumption of cowpeas, amaranth and black nightshade increased. Increase in the hemoglobin levels was noted in the experimental schools compared to the control school. The results were in agreement with a study in Machakos primary schools whereby the pupils transferred nutrition knowledge to their community/households and improved various practices like consumption of various foods which in turn improved their nutritional status [30].

The findings of the intervention study leads to the conclusion that the food based approach using the three facilitators (Peer facilitated, Researcher facilitated and agricultural extension worker facilitated) could have some influence and hence effective to combat deficiencies and promote good health and well-being of the pupils. Gains made may be attributed to the interventions made, when comparison were made between the experimental schools and the control school. Similar findings are reported in a study done in Chennai district, India where by haemoglobin levels of school children who used micronutrient rich foods improved significantly. Sound nutrition knowledge imparted to the children and their households may also have helped to promote their home food intake [42]. The impact was the improved nutrition and haemoglobin levels. The School Health and Nutrition Policy emphasize the promotion of school gardens to enhance integration of nutrition interventions into routine school activities [34].

5.6. Relationship of Variables

In the current study, haemoglobin levels were low before the interventions but significantly improved in the experimental schools. The peer facilitated school had the best improvement in haemoglobin

levels, followed by the agriculture facilitated and then the researcher facilitated school. It is well documented that nutrition knowledge, good dietary practices can reduce disease prevalence. The results in the current study are in agreement with a study undertaken in Tanzania on school children whereby there was a significant correlation between iron intake and serum ferritin at $p<0.05$. Food-based intervention was an important strategy for reducing the magnitude of the problem of anaemia in the school children [45].

Iron deficiency anemia is a serious health problem that affects school going children reducing pupils' school performance and productivity. Iron deficient children tend to exhibit irritability and a low level of engagement with an interest in their immediate environment. These traits inhibit the development of a child's active learning capacity and impinge upon school achievement. Poor performance in a variety of achievement tests by iron deficient children enrolled in school has been reported by several authors. Iron deficiency anaemia is the end stage of a relatively long process of deterioration in Hb levels. Hb levels are indicators of the final stage of IDA. In the current study it was observed that the Gatanga primary school going children were moderately anaemic at baseline but improvements were noted in the experimental schools after the interventions. The facilitations used to address iron deficiency anaemia through nutrition education and associated measures to increase dietary intake were effective measures which can be up-scaled to other locations with a similar population. Signs and symptoms of anaemia are non-specific and difficult to detect though simple laboratory tests can be used to diagnose and determine its severity. Nutrition knowledge offered to the pupils impacted positively as the results revealed. The experimental schools improved in all aspects of the intervention as compared to the control school. The experimental school pupils households adopted various practices and increased food production in vegetable growing and small livestock keeping.

6. Conclusion

At Baseline

From the pre-test assessment most pupils were not knowledgeable in most practices and confidence in nutrition knowledge was inadequate hence more knowledge was needed as indicated by the pupils performance at baseline. All schools scored very poorly in the pre-tests on nutrition knowledge at baseline study. Many pupils had an interest in learning more in nutrition as a subject. The baseline study revealed that the households had low consumption of animal products and high consumption of plant foods. There was minimum consumption of traditional vegetables which were generally overcooked. Few households consumed organ meat, eggs and red meat. The mean nutrient intakes were all below the RDAs for iron.

Effects of the Intervention

Nutrition knowledge given to the experimental schools improved their dietary intake and agronomic practices. The hemoglobin levels increased after the intervention in the experimental schools while it decreased in the control schools. All nutrition education facilitators showed some impact such that there was improved performance in all aspects with the peer facilitated school doing the best. Relationship between nutrition knowledge and iron rich food intake showed increased nutrient intake and iron status. There was an increased iron rich food intake and hence increased hemoglobin levels. These interventions provided an opportunity to link food security interventions and nutrition outcomes.

References

[1] Alex, B; Njenga, J and Embeywa, H, 2010: *Science in Action, Pupils Book 5.* Oxford New Syllabus, University Press, East Africa Ltd, Nairobi Kenya. Pg 56-63.

[2] Ali-Olubandwa, A.M., Kathuri, N.J., Odero-Wanga, D., Shivoga, W.A. *Challenges Facing Small Scale Maize Farmers in Western Province of Kenya in the Agricultural Reform Era*. American Journal of Experimental Agriculture. 2011. 1 (4) 466-476.

[3] Amani, R. and Soflaei, M., 2006: *Nutrition Education Alone Improves Dietary Practices but Not Hematologic Indices of Adolescent Girls in Iran.* Food Nutr Bull. 2006. 27 (3) 260-4.

[4] Andang'o, P.E.A., Osendarp, S.J.M., Ayah, R., West, C.E., Mwaniki, D.L., De Wolf, C.A., Kraaijenhagen, R., Kok, F.J., and Verhoef, H., 2007: *Efficacy of Iron-Fortified Whole Maize Flour On Iron Status of School Children In Kenya: A Randomized Controlled Trial.* The Lancet. 2007. 369 (9575) 1799-806.

[5] Basu, S., Srikanta, B., Hazarika, R., and Parmar, V. *Prevalence of Anaemia among School Going Adolescents of Chandigarh*. Indian Pediatr. 2005. 42 (6) 593-597.

[6] Bobonis, G.J., Miguel, E., and Sharma, C.P. *Iron Deficiency Anaemia and School Participation.* Poverty Action Lab Paper No. 7. 2004. 1-10.

[7] Bwibo, N.O., and Newmann, C.G. *The Need for Animal Source Foods by Kenyan Children.* Journal of Nutrition. 2003. 133 (11) 3936S-3940S.

[8] Dabone, C., Delisle, H.F., and Receveur, O., 2011: *Poor Nutritional Status Of School Children in Urban and Peri-Urban Areas of Ouagadougou (Burkina Faso).* Nutrition Journal. 2011. 10; 34.

[9] Duncan, B.S. and Mcdade, T., 2006: *The Cultural Ecology of Iron Deficiency among Northern Kenya School Children*. Journal of Human Ecology. Human Ecology Chapter 15, Special Issue (14) 107-116.

[10] Food and Agriculture Organization, 2013a: *Guidelines for Measuring Household and Individual Dietary Diversity*. Prepared by FAO Nutrition and Consumer Protection Division Rome, Italy. 1-49.

[11] Food and Agriculture Organization, 2013b*: Nutrition Education in Primary Schools in Argentina*. UN: Ministry of Education, Science and Technology (MECyT); Secretary of Agriculture, Livestock, Fisheries and Foods, (SAGPyA) of the Ministry of Economy and Production. FAO Web Site Retrieved 25th March 2013.

[12] Food and Agriculture Organization, 2005a: *Classroom Curriculum Chart.* Learning Objectives for Nutrition Education in Primary Schools in Developing Countries. Promoting Lifelong Health.

[13] Food and Agriculture Organization, 2005: *Nutrition Education in Primary Schools. A Planning Guide for Curriculum Development Vol 1 and 11.* FAO viale delle terme di caracalla, 00100 Rome Italy. 1-8.

[14] Freund, P., Graybill, E. and Keith, N., 2005: *Health and Education Working Together.* A Case Study of a Successful School Health and Nutrition Model. 1-10.

[15] Government of Kenya, 2012: *National Food and Nutrition Security Policy*. Ministry of Agriculture. Agricultural Sector Coordination Unit (ASCU). 1-20.

[16] Guiney, A.M., 2010: *Call to Action for WASH in Schools*. Drinking Water, Sanitation and Hygiene-WASH in schools. http://www.unicef.org.

[17] Guiney, A.M., 2010: *Call to Action for WASH in Schools.* http://www.unicef.org. 3-10.

[18] Gunde, R., 2004: *School Children in Developing World: Health, Nutrition and School Performance.* UCLA International Institute, Los Angeles, CA90095-1487.

[19] Hongo, T., 2004: *School Nutrition Programs in Kenya*: *Successes and Problem Areas*. A Paper Presented in Global Child Nutrition Forum and AFSA Annual National Conference, Indianapolis, Indiana USA. Retrieved 15th August, 2013.

[20] HPES, 2009: *Peer Education Systems – Overview*. Harvard School of Public Health- Harvard Peer Education Systems, Education, Prevention, Support.

[21] IFAD, 2013: *Calculating Sample Size.* Retrieved on 4th July 2013.

[22] Israel, G.D, 1992: *Determining Sample Size.* Program Evaluation and Organizational Development, IFAS, University of Florida. PEOD-6.

[23] Kakunted, P., 2008: *Nutrition Education Material to Address Iron deficiency Anaemia in Kenya.* A Master's of Science Thesis, Faculty of the Department of Nutrition, Food Science, and Packaging, San Jose State University. UMI Number: 1459707.

[24] Kapur, D., Sharma, S., and Agarwal, K.N., 2003: *Effectiveness of Nutrition Education, Iron Supplementation or both on Iron Status in Children.* Indian Pediatrics. 2003; 40; 1131-1144.

[25] Kasiulevicius, V., Sapoka, V., and Filipaviciute, R. *Sample Size Calculation in Epidemiological Studies*. Theory and Practice. Gerontologija. 2006. 7 (4) 225-231.

[26] Kaya, M., Pehlivan, E., Aydogdu, I., Genc, M., Gunes, G., Kaya, E., and Kuku, I., 2006: *Iron Deficiency Anaemia among Students of Two Primary Schools at Different Socio-Economic Conditions in Malatya, Turkey.* Inonu Universitesi Tip Fakultesi Lojmanlari. 2006. 13 (4) 237-242.

[27] Lachin, J.M. *Introduction to Sample Size Determination and Power Analysis for Clinical Trials.* Controlled Clinical Trials. 1981. 2 (2) 93-113.

[28] Langinger, N. *School Feeding Programs in Kenya: Transitioning to a Homegrown Approach.* Standard Journal of International Relations. 2011. X111 (1) 30-37.

[29] Lyons, L., 2013: *Nepal Children- Agriculture in Schools.* 2000-2013 Global Giving Foundation, NW, Washington, http://www.etc-nepal.org.

[30] Mbithe, D.D., Kimiywe, J.O., Waudo, J.N., and Orodho, J.A. *Promotion of Nutrition Education Interventions in Rural and Urban Primary Schools in Machakos District, Kenya.* A Journal of Applied Biosciences. 2008. 6; 130-139.

[31] Ministry of Agriculture, 2009a: *Thika District Annual Report*. 3-4.

[32] Ministry of Agriculture, 2009b: *Nutrition Information & Recipe Book for People Living with HIV/Aids.* 1.

[33] Ministry of Agriculture, 2007: *Nutrition Training Manual.* Njaa Marufuku Programme; in Collaboration with Ministries of Health and Education. 126.

[34] Ministry of Public Health and Sanitation, 2011: *Kenya National Nutrition Plan of Action (2011-2017) Draft*. 4-58.

[35] Morbidity and Mortality Weekly Report (MMWR), 1996: *Recommendations and Reports, Guidelines for School Health Programs to Promote Lifelong Healthy Eating.* June 14, 1996/Vol. 45/No. RR-9 U.S. Department of Health and Human Services, Public Health Service Centers for Disease Control and Prevention (CDC) Atlanta, Georgia. 2-18.

[36] Mugenda, A.G., 2008: *Social Science Research: Theory and Principles.* Nairobi: Applied Research and Training Services. 186.

[37] Munyanga, M., Jayne, T.S., and Burke, W.J., 2010: *Pathways into and out of Poverty: a Study of Rural Household wealth Dynamics in Kenya.* Egerton University WPS 39/2010. Tegemeo Institute of Agricultural Policy and Development, Nairobi, Kenya. http://www.tegemeo.org. 1-15.

[38] Ramakrishnan, U., and Huffman, S.L., 2008: *Multiple Micronutrient Malnutrition. Nutrition and Health in Developing Countries*. Department of International Health, Rolling School of Public Health, Emory University, Atlanta, GA and Global Alliance for Improved Nutrition, Geneva Switzerland. 531.

[39] Ramzan, M., Ali, I., and Salam, A., 2009*: Iron Deficiency Anemia in School Children of Dera Ismail Khan, Pakistan.* Pakistan Journal of Nutrition. 2009. 8 (3) 259-263.

[40] Reddy, M.B.; Hurrell, R.F., and Cook, J.D. *Meat Consumption in a Varied Diet Marginally Influences Nonheme Iron Absorption in Normal Individuals.* Journal of Nutrition. 2006. 136 (3) 576-81.

[41] Reicks, M., 2006: *Iron Absorption and Coffee/Tea Intake*. University of Minnesota-Extension, Department of Food Science and Nutrition, St. Paul, MN. http://fscn.cfans.umn.edu.

[42] Sangeetha, N. and Premakumari, S., 2010: *Effect of Micronutrient Supplementation on the Nutritional and Immune Status of School Going Children with Iron Deficiency Anaemia*. International Journal of nutrition and metabolism. 2010. 2 (3) 45-55.

[43] Stake, R., 2000: *Case Studies.* Handbook of Qualitative Research. 2nd Edition, In: Denzin, N. & Lincoln, Y. (eds), Sage Publications Inc., Thousand Oaks. 435-54.

[44] Sifri, Z., Darnton-Hill, I., Baker, S.K., Bendech, M., Aguayo, V.M. *A Concise Overview of Micronutrient Deficiencies in Africa and Future Directions.* African Journal of Food & Nutritional Sciences. 2002. 2 (2)

[45] Tatala, S.R., Ash, D., Svanberg, U., and Ndosi, G. *Impact of Dietary Iron Intake on Anaemia in Tanzanian School Children.* South African Journal of Clinical Nutrition. 2004. 17 (3) 94-100.

[46] Taylor, A.K., 2011: *School Feeding Program in Kenya*. NGO Focused On Community Based Conservation Projects. Global Child Nutrition Foundation. National Harbor, MD.

[47] Turyamwijuka, S., 2007: *The Role of Peer-to-Peer Education in Overcoming Cultural Barriers to HIV Prevention Communication Approaches: Experiences from Primary Schools in Uganda.* Uganda Program for Human and Holistic Development (UPHOLD).

[48] United Nations Children Education Fund, 2008: *The Kenya National Technical Guidelines for Micronutrient Deficiency Control.* Micro-nutrient Initiative. Ministry of Public Health and Sanitation, Kenya. 49-64.

[49] United Nations Children Education Fund, Government of Kenya, 1998: *Situation Analysis of Children and Women in Kenya, Nairobi.* 101-110.

[50] USAID, 2013: *Applied Basic Agri-Nutrition Resource Manual for Trainers.* USAID/KHCP, MOA & MOPHS. 13.

[51] Vasishta, S.K., and Patel, N.M., 2002: *Learning Science for Standard 5*, Latest Revised Syllabus. Malimu Publications. Printed by: English Press Limited, Nairobi. 59-119.

[52] World Food Programme, United Nations Children Education Fund, 2006: *Global Framework for Action. Ending Child Hunger and Undernutrition Initiative (Draft*). WFP via C.G. Viola 68, Parco de' medici, Rome Italy.

[53] World Health Organisation, 2007: *Micronutrient Deficiencies*. World Health Organization Website 2007.

[54] World Health Organisation, 2005: *Worldwide Prevalence of Anemia 1993-2005.* WHO Global Database on Anemia. Centers for Disease Control and Prevention. Atlanta, Georgia. 1.

[55] European Peer Training Organisation, 2013: *Together, We learn, We Change*. Retrieved on 5th November 2013. www.epto.org.

[56] School Food Trust, 2012: *School Food Trust, Eat Better Do better, Iron Rich Recipes and Tips-Recipes for Success*. Registered Charity Number 1118995. 1-20. www.schoolfoodtrust.org.uk Retrieved May 2012.

[57] For College Women, 2007: *College Women's Health.* 2. www.4collegewomen.com, Retrieved on 5th April 2008.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 1, pp. 143-154, Article ID Med-281
ISSN 2348-5140

Cloud Publications

Research Article **Open Access**

Aging Differentially Effects Diet-Induced Obesity and Central Leptin Sensitivity in Rats

Lynda M. Brown[1], Keshia R. Ladmirault[2] and Paula T. Cooney[2]

[1]Food and Nutritional Sciences Program, Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University, Greensboro, North Carolina, USA
[2]Department of Nutrition, University of North Carolina at Greensboro, Greensboro, North Carolina, USA

Publication Date: 22 February 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-281

Abstract In this study we examined whether sex differences in central leptin sensitive young rats disappears in middle-aged rats. As animals age, many gain visceral fat and develop leptin resistance, making them more susceptible to inflammation. Middle-aged rats were fed low-fat (LF) or high-fat (HF) diets for 2 months and during this time were given intra-3rd-ventricular (i3vt) leptin injections in a range of doses. Females had a dose dependent decrease in food intake (FI) in response to i3vt leptin. Males reduced FI after i3vt injection of 5.0 µg leptin but not at any other dose. There was also a higher expression of hypothalamic cytokines that are part of the inflammation cascade in males including IL6, TNFα and XBP1. Females remained sensitive to i3vt leptin and had lower hypothalamic cytokine expression than males. The female rats in both diets had visceral fat percentages similar to that of the males which may mean that age increases fat in this depot in rats. These data indicate that middle-aged rats are in a transition period in terms of hormonal sensitivity that may serve as a model to study age-associated changes. Response patterns in female rats that are cycling but have not reached persistent estrous may be suggestive for explanations of physiological changes in perimenopausal women. These findings are important because aging represents a time when health is impacted by diet, body fat distribution and estrogen levels.
Keywords *Sex Differences; Estradiol; High-Fat Diet; Inflammation; Long Evans Rats; Visceral Fat*

1. Introduction

There are several factors that interact in obesity that are part of the current experimental design including sex, diet and age. Consuming high-fat (HF) foods is one of the most important environmental factors leading to obesity [1]. Ingestion of a HF diet leads to hypothalamic leptin and insulin resistance [2, 3]. Obesity is accompanied by chronic inflammation, which is causally linked to leptin and insulin resistance [4-6] and may result in obesity.

When a diet high in saturated fat is consumed free fatty acids (FFAs) increase inflammation by activating toll-like receptor 4 (TLR4) [7]. Chronic activation of pro-inflammatory pathways may be at least partly responsible for obesity-induced insulin resistance and diabetes [4-6]. Pro-inflammatory

cytokines like tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) are elevated in individuals with insulin resistance and diabetes [8, 9]. Despite this, female rats in some experiments have lower levels of inflammatory cytokines than their male controls [10-12].

If the sex differences observed in young rats results from the levels of estrogen in young rats, then aging and the possible increase in inflammation with aging could be a result of lower estrogen levels as women age. One way to test this hypothesis is to measure a known paradigm from young rats like central leptin response in older animals. In a previous experiment sex differences in central leptin demonstrated that female rats were more sensitive to central leptin than their age-matched male controls [13]. Long Evans rats live about 2 years and middle age in our terms would be around 10-12 months old and rats enter estrous senescence around 15 months of age [14]. The rats in this study were 10 -11 months old at the end of the study.

The aim of this study was to determine if diet-induced obesity and central leptin sensitivity remain in middle-aged female and male Long Evans rats. Additionally, because HF diet-induced inflammation leads to central leptin resistance and estrogen is a known anti-inflammatory agent, we sought to determine if middle-aged female rats fed a HF diet will have less hypothalamic inflammation than male rats.

We hypothesized that middle-aged rats will be more susceptible to hypothalamic inflammation that can result in both leptin resistance and diet-induced obesity compared to younger rats. In addition, sex differences observed in younger rats will become less evident in middle age due to declining estrogen levels.

2. Materials and Methods

2.1. Animal Care

All laboratory work was performed at laboratory facilities at the University of North Carolina at Greensboro (UNCG). The UNCG Institutional Animal Care and Use Committee approved all protocols for this experiment.

Age-matched male and female Long Evans rats (8-9 months old, Harlan Labs; Harlan, IN) were individually housed in Plexiglas tubs and maintained on a 12:12-h light-dark cycle in a temperature-controlled vivarium. Upon arrival, they were given 1 week to acclimate to the facility before introduction to sex-specific colony rooms. Rooms were temperature (22 ± 2°C) and humidity controlled and kept on a 12:12 light/dark cycle (lights on at 0400 h). Prior to the start of the experiment, 30 females and 30 males were maintained on a standard laboratory chow (17% fat and 3.1 kcal/g, Harlan Teklad #7012; Indianapolis, IN). Rats had free access to food and water unless otherwise noted. At the start of the experiment, 15 rats of each sex were switched to a high-fat diet (40% fat and 4.54 kcal/g, Research Diets #D03082706; New Brunswick, NJ). Feeding data were converted to kilocalories (kcal) to compare intake between groups.

2.2. I3vt Cannulation

Seven days after arrival, rats were anesthetized with 0.1 ml/100g body weight intraperitoneal (ip) injections of a ketamine (70 mg/kg)/xylazine (2 mg/kg) mixture. Subsequently, 22-gauge guide cannulas (Plastics One Inc., Roanoke, VA) were stereotaxically implanted in the intra- 3rd-cerebral ventricle (i3vt). Briefly, bregma and lambda were situated at the same vertical coordinate and cannula tips were positioned on the midline, 2.2 mm posterior to bregma and 7.5 mm ventral to the dura mater. The cannulas were then fixed to the skull using dental acrylic and anchor screws. Obturators extending 0.5 mm beyond the cannula tract were inserted. When rats returned to their pre-surgical

body weights, cannula placement was confirmed by i3vt infusion of 10 µg of Neuropeptide Y (NPY) in 1 µl normal saline 3 h prior to the onset of dark and monitoring the rat's food intake over a 60-min period. Animals that did not eat at least 2 g of chow within 60 min were excluded from the study.

2.3. I3vt Injection Protocol

The leptin dose-response consisted of i3vt injections leptin (1.5, 3.5, 5.0 and 7.5 µg/ 1 µl; Human Leptin; CalBiochem, San Diego, CA) or 1 µl vehicle. Using a within-subjects design, each rat randomly received each of the 4 leptin doses and vehicle with a 7 day washout period. On each experimental day, food hoppers were removed from the cages and the animals were weighed 3 h prior to the onset of dark which is the start of the experiment (0 h). At 45 min prior to the onset of the dark, body weights were again recorded and rats received an i3vt injection of vehicle or leptin which was slowly infused with a Hamilton syringe. Food hoppers were returned at the onset of dark and the animals were weighed again the next morning which is the 24 h time.

2.4. Leptin Dose Schedule

The changes in 24 h food intake (FI) measured in kcals and change in body weight (BW) change at 24 h were assessed by diet (LF vs. HF), sex (male and female) for the 4 doses of leptin and saline vehicle. The leptin dose response consisted of i3vt injections leptin (1.5, 3.5, 5.0 and 7.5 µg/ 1 µl volume) or 1 µl saline vehicle. Using a within-subjects design, each rat randomly received each of the 4 leptin doses and vehicle with a 7 day washout period. It took 6-7 weeks to complete the experiment from the time of cannulation.

2.5. Body Composition

After euthanasia, the skin and subcutaneous fat (pelt) was dissected from the muscle wall and visceral fat (carcass) as previously described [15]. The fat above the muscles measured in the pelt was used as a measure of subcutaneous fat while the fat under the abdominal muscles was used as a measure of visceral fat (VAT). DEXA measurement was performed using a GE Lunar Prodigy Advanced System (GE Healthcare; Milwaukee, WI) and the data were analyzed by Encore 2007 Small Animal software (version 11.20.068). The system was calibrated according to manufacturer's instructions each day samples were run. Samples were scanned in duplicate to determine fat mass (FM) and lean body mass (LBM) using a protocol previously described [16].

2.6. Plasma Analysis

Trunk blood was collected in heparinized tubes and centrifuged immediately following decapitation. Aliquots of plasma were collected and stored at -80°C until analyzed. Plasma was packed on dry ice and sent to the University of Texas Southwestern for specific radioimmunoassay of estradiol (Quest Diagnostics, Inc.; Nichols Institute Diagnostics, San Juan Capistrano, CA).

2.7. Gene Expression

The medial basal hypothalamus was preserved in RNAlater and stored for 24 h at 4°C and then stored at -80°C until processed. RNA was isolated using QIAGEN RNAeasy kits (Qiagen, Inc.; Valencia, CA) according to the manufacturer instructions. RNA concentration and purity was assessed by Nanodrop spectrophotometer (Thermo Scientific, ND-1000; Wilmington, DE). 2 ng of RNA for each sample was combined with RNase free H2O and master mix solution (Applied Biosystems; Foster City, CA) and run in a Thermocycler (Applied Biosystems; Foster City, CA) for 2.5 h to obtain cDNA. Then the cDNA was used to determine gene expression via quantitative RT-PCR for TNFα, SOCS3, IL6, and XBP1 using primers from Applied Biosystems.

2.8. Statistical Analysis

Statistical analysis was performed using SPSS (version 18.0) for Windows. Treatment effects and interactions were tested using two-way ANOVA and individual group differences were tested using Dunnett's T3 post hoc analysis. Data are presented as means with corresponding standard error of the means (SEM) and significance set at $p \leq 0.05$.

3. Results

3.1. LF Diet: 24 h FI/BW Change

Male rats reduced FI and BW when given the 5.0 µg leptin i3vt injection and reduced BW only when given the 1.5 and 7.5 µg doses (Figure 1). There was no change after the 3.5 µg injection. In contrast, female rats reduced FI and BW following the 1.5 and 7.5 µg injections of leptin but only reduced FI following the 3.5 and 5.0 µg leptin doses. That means female rats responded at every dose of leptin, while males were not as consistent. While the amount of weight lost in 24 h was similar in males and females, females (vehicle mean 72.97 kcal) ate fewer kcals than male rats (vehicle mean 101.45 kcal). After central leptin female rats consumed only about 50 kcal in 24 h.

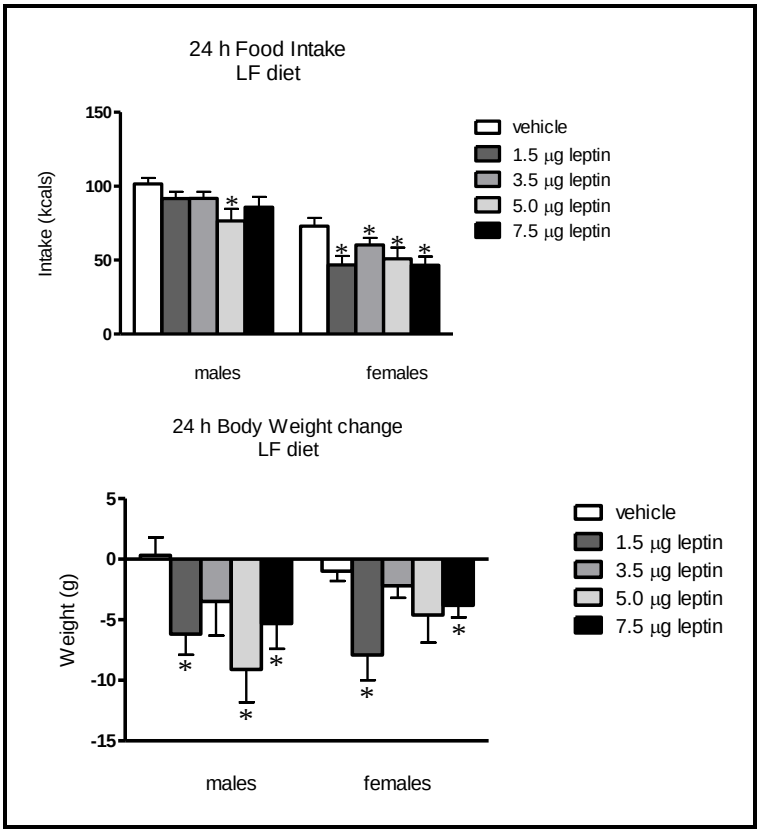


Figure 1: *Leptin Dose-Response -LF Diet Groups*

Food intake (FI) and change in body weight (BW) measured 24 h after i3vt leptin or vehicle injections are graphed for male (n= 15) and female (n=15) rats fed the LF diet (*p< 0.05). In this figure the dose response of male rats can be compared to that of the female rats fed the LF diet.
Abbreviations: LF = low fat

Compared to the results from a previous study using 3-month old males and females, the middle-aged females remained sensitive to central leptin [15]. Three month old males were less sensitive to i3vt

leptin than females and that sex difference remains in this study. The lowest effective dose in our middle-aged males was the 5.0 µg dose which is higher than reported in young males [15] whose lowest effective dose was at the 3.5 µg dose. So while middle-aged males responded to i3vt leptin by losing BW, they lost some sensitivity in that there was no decrease in FI.

3.2. HF Diet: 24 h FI/BW Change

When fed the HF diet, males did not reduce FI at any dose of leptin, and reduced BW after the 3.5 and 5.0 µg leptin doses (Figure 2). However, males reduced BW when given the 1.5 and 7.5 µg doses. Surprisingly, females had a clear leptin response on the HF diet, reducing FI after all doses, and reducing BW after the 5.0 µg dose. This resulted in a main effect for sex for FI at 24 h ($p<0.05$). Generally, rats given a HF diet become leptin resistant. In this protocol, rats were switched to the HF diet on the surgery day and remained on it until sacrifice. Two weeks after surgery, the leptin injections began and were given weekly. That means rats had been fed the diet for 3-7 weeks during the time of the dose response curve. The sex difference remains in that females were more responsive to central leptin, but the kcals eaten were similar in male and female fed the HF diet. Females and males ate the same amount in 24 h (vehicle means 82.57 and 83.69 kcal, respectively) and females reduced this significantly in response to i3vt leptin injection. These data may indicate that middle-aged females fed a HF diet are protected from leptin resistance and that there is some response in males.

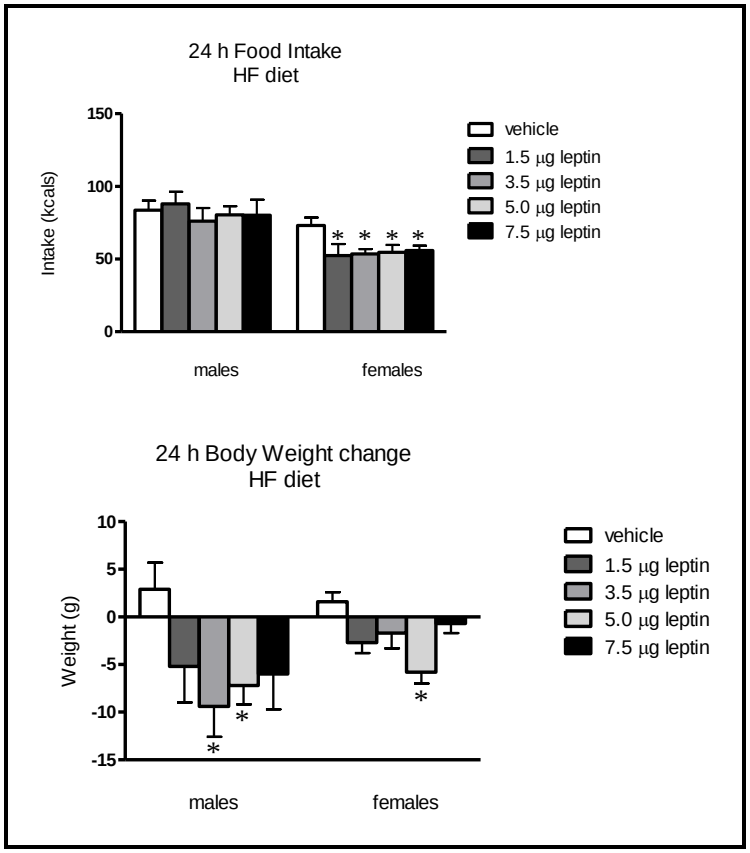


Figure 2: *Leptin Dose-Response -HF Diet Groups*

Food intake (FI) and change in body weight (BW) measured 24 h after i3vt leptin or vehicle injections are graphed for male (n=15) and female (n=15) rats fed the HF diet (*$p< 0.05$). In this figure the dose response of male rats can be compared to that of the female rats fed the HF diet.
Abbreviations: HF = high fat

3.3. Body Composition and Body Fat Distribution

Lean Body Mass (LBM) and fat were measured by DEXA (Table 1) in the carcass and pelt. After euthanasia the pelt which has the skin and its underlying fat is separated from the carcass so that the subcutaneous skin can be measured. The fat that lies under the skin is the subcutaneous adipose tissue (SCAT). The carcass has the muscles and the fat located under the abdominal muscles is the visceral adipose tissue (VAT). The pelt and carcass are separately scanned by DEXA as a method to measure each fat depot.

In the carcass, males had higher LBM than females ($p< 0.05$). Groups fed the HF diet increased their FM which then reduced carcass LBM. Carcass fat in males was two times that of females on the LF diet. Males fed the HF diet increased carcass fat 50% while females doubled carcass fat when fed the HF diet to a level that matched the LF diet males. Males fed the HF diet increased fat by 50% while females doubled their carcass fat when placed on the HF diet. These data indicate that middle-aged females gained body fat at a rate higher than that of males and that they have higher VAT than male rats. Similarly, males had twice as much fat as females in the pelt area. Pelt LBM was similar in male groups which was three times higher than that of HF females. LF diet females had a small amount of LBM that was only 5% of that of males with averages of 42.67 and 2.11 g respectively.

Body fat was measured in the pelt and carcass, and those values were added to get whole body data (Table 2). Age-matched males weighed 200-300 g more than females at the end of the experiment. When males were fed the HF diet, carcass and pelt body fat increased 50%; conversely females fed the HF diet more than doubled their whole body fat, which incidentally was approximately at the same level of fat in LF males. This same pattern occurred for both pelt and carcass fat, males increased both by 50% and females doubled the fat in each depot when fed the HF diet. Males fed the HF diet increased their % FM by 12%, whereas females increased about 20%. The final two columns (shaded gray) in Table 2 give the location of % FM between the depots. This allows direct comparison of the fat distribution for each group since the transformation to % is similar to the transformation of FI to kcals. Males had more fat in the carcass (VAT) than in the pelt by a 55:45 ratio. This was not changed by the HF diet. Females had more fat in the VAT than males (63% vs. 56%) and this was not changed significantly by the HF diet.

3.4. Plasma Estradiol (E2)

Plasma E2 was measured from blood taken after euthanasia. Female rats were phased biweekly to assure they were still cycling, but were not phased on the day of euthanasia, so the result represent various days of the estrous cycle. We were interested in whether the HF diet would alter E2 values, and it did. Rats fed the LF diet 42.36 pg/ml had higher E2 levels than rats fed the HF diet (32.84 ± 2.61 pg/ml ($p=0.049$)).

3.5. Quantitative RT-PCR

After euthanasia, the medial basal hypothalamus was dissected and processed for expression of inflammatory cytokines IL6, TNFα and SOCS3 and a marker of intracellular stress, XBP1 by quantitative RT-PCR (Figure 3).

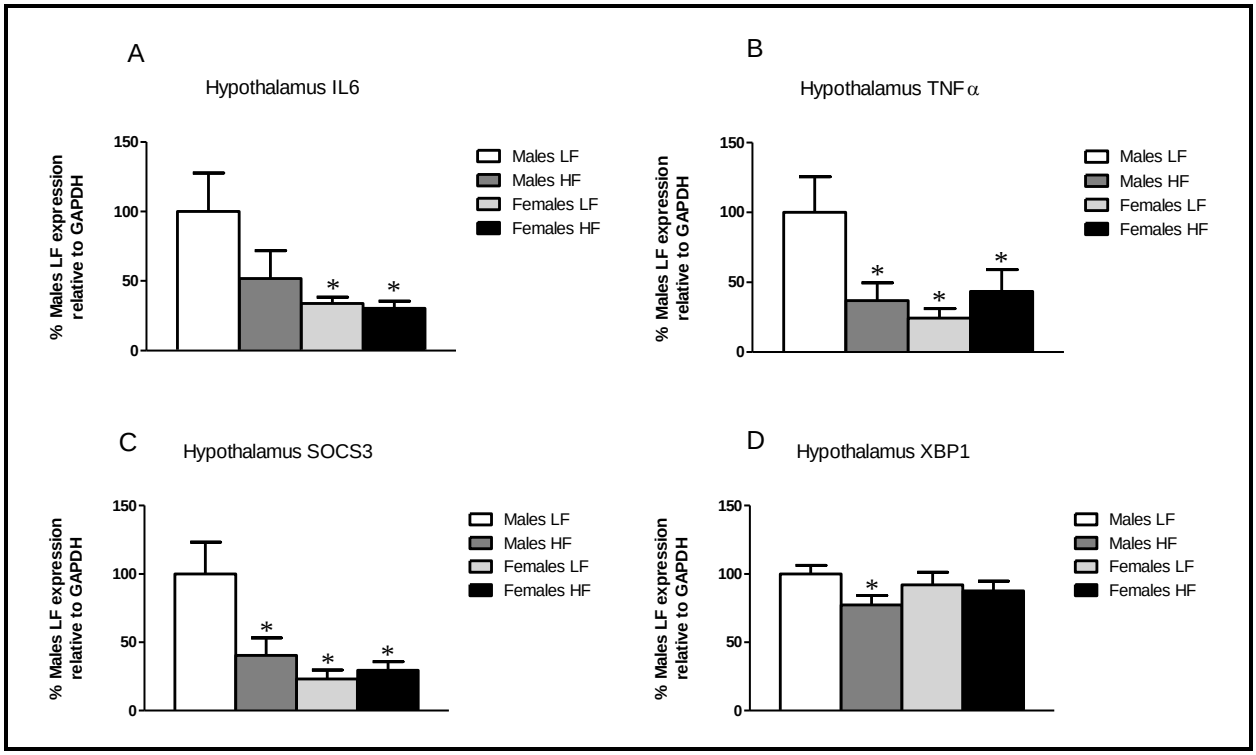


Figure 3: *Hypothalamus qPCR- Middle-aged Rats*

After euthanasia, the medial basal hypothalamus was dissected and processed for expression of inflammatory cytokines IL6, TNFα and SOCS3 and a marker of intracellular stress, XBP1 by quantitative RT-PCR. Statistical differences between groups were performed using Dunnett's T3 post-hoc analysis in SPSS version 18.0 for Windows. (*p< 0.05).

Abbreviations: low fat (LF), high fat (HF), interleukin 6 (IL6), tumor necrosis factor α (TNFα), suppressor of cytokine signaling 3 (SOCS3), x-box binding protein 1 (XBP1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

4. Discussion

Central leptin injections reduce food intake and body weight; effects that are dependent upon sex, diet and age. When young-adult (3-month old) Long Evans rats are given i3vt leptin, males respond at higher doses than females indicating that males are less sensitive to central leptin than females [15, 16]. When young rats are put on a HF diet, they become less sensitive to the ability of leptin to reduce food intake [16]. In this experiment, middle-aged male rats fed the LF diet were slightly less sensitive to i3vt leptin than females which was consistent with findings in younger rats. When the rats were fed a HF diet, the females remained sensitive to i3vt leptin.

Males fed the HF diet became leptin resistant, consistent with the literature [17]. This is an important observation because it indicates that the increased caloric intake and gain of body fat in the middle-aged females fed the HF diet occurred in spite of maintained leptin sensitivity. Since plasma E2 levels were normal in the females, the interaction of the leptin and E2 may prevent central leptin resistance. Additive effects could occur since E2 activates intracellular kinases including MAPK, PI3K, Src-K which phosphorylate the STAT3 transcription factor which overlaps leptin's intracellular cascade [18].

In contrast, males reduced FI after i3vt 5.0 µg leptin and did not reduce FI at any other dose. This is higher than the 3.5 µg effective dose published for young males [15], indicating that age reduced sensitivity to central leptin in male rats. Combined with the higher expression of hypothalamic cytokines in males this may indicate that diet-induced inflammation is not restrained in males as it is in females.

Female groups had lower IL6 expression than males (Figure 3A). This resulted in a main sex effect ($p<0.026$). Diet did not change this further. There was a diet x sex interaction for TNFα ($F(1,24)=5.82$, $p<0.024$; Figure 3B). This resulted from a reduction in TNFα expression in all groups compared to LF males, and an increase in females fed the HF diet when compared to their LF controls. This was similar to what occurred in SOCS3 (Figure 3C). Males fed the LF diet had significantly higher levels of SOCS3 mRNA than all other groups. This resulted in a diet x sex interaction ($F(1,24)=5.18$, $p<0.032$). Males fed the HF diet had lower expression of XBP1 than their LF controls ($p<0.026$; Figure 3D). In contrast, there were no changes to XBP1 in females.

Females had lower expression of IL6, TNFα and SOCS3 than males and this may implicate E2 as a main factor in inflammatory cytokines, regardless of diet. One of the goals of this experiment was to determine whether sex differences observed in younger rats remain in middle-aged rats. They do in the case of inflammatory cytokine expression in the hypothalamus. The genes selected have some control from NFκB since E2 through ERα directly interacts with NFκB.

Retaining central leptin sensitivity with age could explain the persistence of the central effects of leptin working with E2, but the peripheral effects of E2 may have been altered in our study. Rats fed the HF diet started the diet on the day of surgery and remained on it until euthanasia. This was about 2 months. We found that middle-aged females gained more body fat during this time than males. We report for the first time that VAT in our females was similar to that of males, and increased with HF diet. This age-related effect is similar to what is observed in aging women. In young men, excess fat is more likely in the apple pattern (increased VAT) while women tend to gain fat below the waist in the pear shape (increased SCAT) [19]. As women enter middle age and E2 levels decrease, this pattern changes and women gain fat above the waist in the apple pattern [19].

Shown in Table 2, the %FM in males and females increased for the HF diet groups, but females gained 20% more FM in the two months that they were on the HF diet showing a sensitivity to diet-induced obesity. Also shown in Table 2, all groups have approximately 60% of their fat in the visceral depot and only 40% in the SCAT. That is significant because our aged females now have a male fat distribution, regardless of diet. This differs from what is reported in young rats, where males have more VAT than females [15]. However, this age difference is consistent with what occurs in postmenopausal women who accrue more VAT as estrogen levels decline [19]. We interpret this as an age-related change and an indication that estrogen signaling in the fat depot is decreasing.

While plasma E2 levels were not lower than that measured in comparable younger females, the estrogen receptor population and intracellular estrogen signaling could be altered. Rats in the current study were 10-11 months old at the time of euthanasia. According to the literature, female Long Evans rats are still cycling at this age [20] but cycles could be irregular. The females were cycling in our experiment but without a longer history of estrous cycles, we are unable to determine whether the cycles were irregular. Irregular cycle may mean transitioning from 4 d to 5 d cycles. We phased all females in two time points to be sure they were cycling, but did not phase daily. Thus we are only able to report that estrous cycles were regular (4 d length) at those times but do not know if they were irregular at some point during the study.

Estrogen by structure is a steroid hormone and thus can be a factor in decreasing inflammation. This is significant because inflammation, in particular diet-induced inflammation with a saturated fat diet, is a causal factor in central insulin resistance and obesity [3, 15, 17]. The transcription factors measured are controlled by a master controller of inflammation NFκB. NFκB is important for sex differences because 17β-estradiol can block its activation, and the transcription of IL-6, TNFα, and SOCS3 which are all increased by NFκB [21-23]. XBP-1 interacts with E2 and was added to give a snapshot of an additional intracellular cascade. In Figure 3 expression of transcription factors activated by inflammation is presented with the male LF diet group as the control group. Females on both diets

had lower expression IL-6, TNFα, and SOCS3 than the control group by over 50%. This is another indication that central estrogen could have reduced the inflammatory effects of the high-fat diet allowing the females to retain central leptin sensitivity.

There are conflicting data reporting that cytokine expression increases, decreases or remains unchanged with age [24, 25]. There are data reporting increased levels of circulating TNFα and IL-6 [26]. On the other hand, age is associated decreased capacity of the immune system [24]. In addition there is a question about whether increased levels of cytokines, like IL6 is beneficial or detrimental. IL-6 protects neurons against NMDA excitotoxicity *in vitro* and prevents brain from ischemic attacks *in vivo* [27]. However, other studies implicate IL-6 in neurodegenerative disorders [28]. Finally, experiments using neuronal cultures show that IL-6 is both neuroprotective and neurotoxic, depending on concentration [29]. Taken together, these data highlight the importance of context in determining functional significance of inflammatory protein expression. It is beyond the scope of this study to interpret whether the increased and decreased expression of the genes measured represent dysfunction or protection. Our goal was to capture changes with aging, and to illuminate the remaining questions.

The changes that occurred with age were mixed in this study. Males had higher hypothalamic cytokine expression and less central leptin sensitivity than females. However, middle-aged females gained more body fat as a percent of BW than males and it resulted in more VAT in females than males. We interpret this as differences in aging in males and females. Females were more sensitive to diet-induced obesity, yet sensitive to central leptin and had greatly reduced cytokine expression than males. Taken together these data indicate that middle-aged females are in a transitional time when some age changes have occurred, and this is a good model for examining sex differences in ingestive behavior in middle-age rats. Particularly in female rats, this age represents cycling females that have not reached persistent estrous and may be comparable to perimenopausal women.

Acknowledgements

We gratefully acknowledge Debbie Clegg for providing the plasma estradiol data and useful discussions during the preparation of the manuscript. We thank our animal technicians, Tricia Kauffman and Sarah Beaty for their assistance with animal husbandry and collecting data. Special thanks to Colette Miller for animal husbandry, data and statistical analyses. Editorial advice was provided by Ken Gruber and Fern Webb. This work was supported in part by USDA ARS grant NC06871 (LMB), New Faculty Grant from the UNCG Office of Research and Economic Development (LMB) and a NCA&TSU USDA Evans Allen Grant NC.X-289-5-15-170-1 (LMB).

Disclosures

The authors have no conflicts of interest to declare.

Tables

Table 1: *Body Composition*

	LF Males	HF Males	LF Females	HF Females
Carcass Fat (g)	104.22 ± 12.25^{a}	156.22 ± 12.88^{a}	56.44 ± 8.83^{b}	118.15 ± 8.01^{a}
Carcass LBM (g)	312.22 ± 16.49^{a}	270.56 ± 8.55^{a}	169.22 ± 5.32^{b}	155.08 ± 4.76^{b}
Pelt Fat (g)	79.78 ± 10.83^{ac}	128.11 ± 12.57^{a}	32.67 ± 6.87^{b}	72.23 ± 5.05^{c}
Pelt LBM (g)	42.67 ± 3.85^{a}	47.33 ± 6.12^{a}	2.11 ± 1.07^{b}	14.23 ± 2.83^{c}

Body composition measured by DEXA is presented. After euthanasia, the skin and subcutaneous fat (pelt) was dissected from the muscle wall and visceral fat (carcass) so that visceral (carcass) and subcutaneous (pelt) fat could be measured. Samples

were scanned in duplicate to determine mass of fat and lean body mass (LBM). Data are reported as mean ± SEM. Statistics represent differences in SEM across rows. Values sharing a common letter are not different from one another, p<0.05. Abbreviations: low fat (LF), high fat (HF), lean body mass (LBM).

Table 2: *Body Fat Distribution*

	BW (g)	body fat (g)	body % FM	pelt fat (g)	carcass fat (g)	pelt % FM SCAT	carcass % FM VAT
LF Males	574.67	184.00 ± 23.08	32.02 %	79.78 ± 10.83	104.22 ± 12.25	43.36 %	56.64 %
HF Males	634.28	284.33 ± 25.45	44.83 %	128.11 ± 12.57	156.22 ± 12.88	45.06 %	54.94 %
LF Females	303.32	89.11 ± 15.70	29.38 %	32.67 ± 6.87	56.44 ± 8.83	36.66 %	63.34 %
HF Females	387.89	190.38 ± 13.06	49.08 %	72.23 ± 5.05	118.15 ± 8.01	37.94 %	62.06 %

Body composition measured by DEXA. The first three columns represent total values- BW and body fat in grams, and the % of fat in the whole body. The last four columns represent the amount of fat in grams and % fat in the carcass and pelt. Percentage of fat in the pelt and carcass totals 100 % so that the data represent where fat was located for each group, as well as where fat was added as the HF diet groups increased body fat. The pelt % FM is the fat just under the skin or the subcutaneous adipose tissue (SCAT), while the carcass % fat is the fat under the abdominal muscles which is our measure of visceral adipose tissue (VAT). Abbreviations: body weight (BW), fat mass (FM), low fat (LF), high fat (HF).

References

[1] Woods, S.C., D'Alessio, D.A., Tso, P., Rushing, P.A., Clegg, D.J., Benoit, S.C., Gotoh, K., Liu, M. and Seeley, R.J. *Consumption of a High-Fat Diet Alters the Homeostatic Regulation of Energy Balance*. Physiol Behav. 2004. 83 (4) 573-8.

[2] Clegg, D.J., Benoit, S.C., Air, E.A., Jackman, A., Tso, P., D'Alessio, D., Woods, S.C. and Seeley, R.J. *Increased Dietary Fat Attenuates the Anorexic Effects of Intracerebroventricular Injections of MTII*. Endocrinology. 2003. 144; 2941-2946.

[3] Mori, H., Hanada, R., Hanada, T., Aki, D., Mashima, R., Nishinakamura, H., Torisu, T., Chien, K.R., Yasukawa, H. and Yoshimura, A. *Socs3 Deficiency in the Brain Elevates Leptin Sensitivity and Confers Resistance to Diet-Induced Obesity*. Nat Med. 2004. 10 (7) 739-43.

[4] Kahn, B.B. and Flier, J.S. *Obesity and Insulin Resistance*. J Clin Invest. 2000. 106 (4) 473-81.

[5] Wellen, K.E. and Hotamisligil, G.S. *Obesity-Induced Inflammatory Changes in Adipose Tissue*. J Clin Invest. 2003. 112 (12) 1785-8.

[6] Wellen, K.E. and Hotamisligil, G.S. *Inflammation, Stress, and Diabetes*. J Clin Invest. 2005. 115 (5) 1111-9.

[7] Shi, H., Kokoeva, M.V., Inouye, K., Tzameli, I., Yin, H. and Flier J.S. *TLR4 Links Innate Immunity and Fatty Acid-Induced Insulin Resistance.* J Clin Invest. 2006. 116 (11) 3015-25.

[8] de Luca, C. and Olefsky, J.M. *Inflammation and Insulin Resistance*. FEBS Lett, 2008. 582 (1) 97-105.

[9] Wang, P., Mariman, E., Renes, J. and Keijer, J. *The Secretory Function of Adipocytes in the Physiology of White Adipose Tissue*. J Cell Physiol. 2008. 216 (1) 3-13.

[10] Dhar, M.S., Sommardahl, C.S., Kirkland, T., Nelson, S., Donnell, R., Johnson, D.K. and Castellani, L.W. *Mice Heterozygous for Atp10c, A Putative Amphipath, Represent A Novel Model of Obesity and Type 2 Diabetes*. J Nutr. 2004. 134 (4) 799-805.

[11] Gallou-Kabani, C., Vige, A., Gross, M.S., Boileau, C., Rabes, J.P., Fruchart-Najib, J., Jais, J.P. and Junien, C. *Resistance to High-Fat Diet in the Female Progeny of Obese Mice Fed a Control Diet During the Periconceptual, Gestation, and Lactation Periods*. Am J Physiol Endocrinol Metab. 2007. 292 (4) E1095-100.

[12] Payette, C., Blackburn, P., Lamarche, B., Tremblay, A., Bergeron, J., Lemieux, I., Despres, J.P. and Couillard, C. Sex *Differences in Postprandial Plasma Tumor Necrosis Factor-Alpha, Interleukin-6, and C-Reactive Protein Concentrati*ons. Metabolism. 2009.

[13] Clegg, D.J., Riedy, C.A., Smith, K.A., Benoit, S.C. and Woods, S.C. *Differential Sensitivity to Central Leptin and Insulin in Male and Female Rats.* Diabetes. 2003. 52 (3) 682-7.

[14] Sengupta, P. *The Laboratory Rat: Relating Its Age With Human's*. Int J Prev Med. 2013. 4 (6) 624-30.

[15] Clegg, D.J., Brown, L.M., Woods, S.C., and Benoit, S.C. *Gonadal Hormones Determine Sensitivity to Central Leptin and Insulin.* Diabetes. 2006. 55 (4) 978-87.

[16] Miller, C.N., Morton, H.P., Cooney, P.T., Winters, T.G., Ramseur, K.R., Rayalam, S., Della-Fera, M.A., Baile, C.A. and Brown, L.M. *Acute Exposure to High-Fat Diets Increases Hepatic Expression of Genes Related to Cell Repair and Remodeling in Female Rats*. Nutr Res. 2014. 34 (1) 85-93.

[17] Clegg, D.J., Benoit, S.C., Fisher, M.E., Barrera, J.G., Seeley, R.J. and Woods, S.C. *Sex Hormones Determine Body Fat Distribution and Sensitivity to Adiposity Signals*. Appetite. 2003. 40; 324.

[18] Gao, Q. and Horvath, T.L. *Cross-Talk between Estrogen and Leptin Signaling in the Hypothalamus*. Am J Physiol Endocrinol Metab. 2008. 294 (5) E817-26.

[19] Carr, M.C. and Brunzell, J.D. *Abdominal Obesity and Dyslipidemia in the Metabolic Syndrome: Importance of Type 2 Diabetes and Familial Combined Hyperlipidemia in Coronary Artery Disease Risk.* J Clin Endocrinol Metab. 2004. 89 (6) 2601-7.

[20] Fentie, I.H., Greenwood, M.M., Wyss, J.M. and Clark, J.T. *Age-Related Decreases in Gonadal Hormones in Long-Evans Rats: Relationship to Rise in Arterial Pressure*. Endocrine. 2004. 25 (1) 15-22.

[21] Pozzi, S., Benedusi, V., Maggi, A. and Vegeto, E. *Estrogen Action in Neuroprotection and Brain Inflammation*. Ann N Y Acad Sci. 2006. 1089; 302-23.

[22] Vegeto, E., Belcredito, S., Etteri, S., Ghisletti, S., Brusadelli, A., Meda, C., Krust, A., Dupont, S., Ciana, P., Chambon, P. and Maggi, A. *Estrogen Receptor-Alpha Mediates the Brain Antiinflammatory Activity of Estradiol.* Proc Natl Acad Sci USA. 2003. 100 (16) 9614-9.

[23] Vegeto, E., Belcredito, S., Ghisletti, S., Meda, C., Etteri, S. and Maggi, A. *The Endogenous Estrogen Status Regulates Microglia Reactivity in Animal Models of Neuroinflammation.* Endocrinology. 2006. 147 (5) 2263-72.

[24] Frasca, D., Landin, A.M., Riley, R.L. and Blomberg, B.B. *Mechanisms for Decreased Function of B Cells in Aged Mice and Humans.* J Immunol. 2008. 180 (5) 2741-6.

[25] Beharka, A.A., Meydani, M., Wu, D., Leka, L.S., Meydani, A. and Meydani, S.N. *Interleukin-6 Production Does Not Increase with Age*. J Gerontol A Biol Sci Med Sci. 2001. 56 (2) B81-8.

[26] Krabbe, K.S., Pedersen, M. and Bruunsgaard, H. *Inflammatory Mediators in the Elderly*. Exp Gerontol. 2004. 39 (5) 687-99.

[27] Carlson, N.G., Wieggel, W.A., Chen, J., Bacchi, A., Rogers, S.W. and Gahring, L.C. *Inflammatory Cytokines IL-1 Alpha, IL-1 Beta, IL-6, and TNF-Alpha Impart Neuroprotection to an Excitotoxin Through Distinct Pathways.* J Immunol. 1999. 163 (7) 3963-8.

[28] Sawada, M., Imamura, K. and Nagatsu, T. *Role of Cytokines in Inflammatory Process in Parkinson's Disease.* J Neural Transm Suppl. 2006. (70) 373-81.

[29] Li, X.Z., Bai, L.M., Yang, Y.P., Luo, W.F., Hu, W.D., Chen, J.P., Mao, C.J. and Liu, C.F. *Effects of IL-6 Secreted from Astrocytes on the Survival of Dopaminergic Neurons In Lipopolysaccharide-Induced Inflammation.* Neurosci Res. 2009. 65 (3) 252-8.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 1, pp. 188-195, Article ID Med-294
ISSN 2348-5140

Research Article | Open Access

Utilization of Renewable Energy Sources for Drying Medicinal and Nutritional Plants in Panama

Isaias Bermudez[1], Juana Ramos dePerez[1] and Thomas van derHave[2]

[1]Technological University of Panama, School of Science and Technology, Vía Centenario, Ancon, Panama City, Panama
[2]Engineering Technology, Catholic University of Leuven, Ghent, Belgium

Publication Date: 26 February 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-294

Abstract This research study looked at the design, construction, running and evaluation of an artisanal processing plant or factory that included a solar oven to capture the thermal energy for drying medicinal and nutritional plants. The use of renewable resources, especially solar energy plus a minimum of technological equipment and attention to principles of quality, hygiene, and future sustainability, contributed to the success of this engineering project. The factory was located in the rural community of El Cacao, District of Capira, Province of Panama, Republic of Panama, Central America. An indirect solar oven represented the center of the operation of the factory. Tests with locally grown plants including oregano (Origanum vulgare), coriander (Coriandrum sativum), rosemary (Rosmarinus officinalis), balsamino or bitter melon (Momordica charantia) and lemongrass (*Cymbopogon citrullus),* were conducted to measure the heat transfer and conductivity from the oven to the drying room. We also conducted an organoleptic study with community members to assess dried lemongrass *as tea vs. a commercial tea*. Preliminary tests with medicinal plants showed the efficiency of the factory in drying with significantly lower times than those found in the literature, obtaining the recommended humidity percentages. In addition, the organoleptic features of the lemongrass tea were rated as with more acceptable features as compared with the commercial lemongrass tea. The preliminary findings reported here are promising and, from the reception from community members, it is expected that they will accept the products being developed for them. The goal is for this and similar communities to have better and more permanent access to their traditional nutritional and medicinal plants.

Keywords *Drying Time; Humidity Rate; Organoleptic Characteristics; Solar Energy; Temperature Control*

1. Introduction

Since ancient times, man has learned to dehydrate and dry grains, fruits, meat and herbs to ensure the availability of food and medicinal products and thus to have them available all year and to survive in times of scarcity. There is a global movement towards the use of natural and healthy products, among which are medicinal plants used fresh or minimally processed, for example, dehydrated.

However, in developing countries such as Panama, artisanal simple technologies to facilitate the use of plants for nutritional and medicinal purposes are absent in most rural communities. However, in this country, the use of indigenous plants by rural communities is quite common but mainly used as fresh products. As an example, previous work by the authors of this paper revealed that in a rural community of Panama, approximately 150 local plants were identified as with high cultural value because of their medicinal and nutritional attributes, with most of those plants used as fresh herbs [1].

The study reported here was aimed at the planning, building and use of a small processing plant or factory. It included a solar oven that provided the energy needed for drying traditional plants in a rural region of the Republic of Panama. This Central American country has long periods of sunshine equivalent to a monthly average of 1,967 hours/sun and a daily average of 5.4 hours/sun [2].

The capacity of the factory for drying locally produced plants was tested with favorable results as reported in this paper. The results evidenced the potential for the use and application of renewable, low-cost technologies that can contribute to improving innovative production systems and economic capacity of rural populations.

2. Materials and Methods

2.1. Study Area

The rural factory for the artisanal processing and drying of medicinal and nutritional plants was built in the rural community of El Cacao, district of Capira, Province of Panama, Panama. El Cacao is a rural, traditional community where the use of locally grown plants, for nutritional and medicinal purposes, is still widely practiced by its inhabitants [1].

This community is located 75 kilometers from Panama City, capital of the Republic of Panama, at a height of 700 meters above sea level in the highlands near the Trinidad mountains, with Latitude: 8º, 77' N and Longitude: 80º, 02' W (Figure 1).

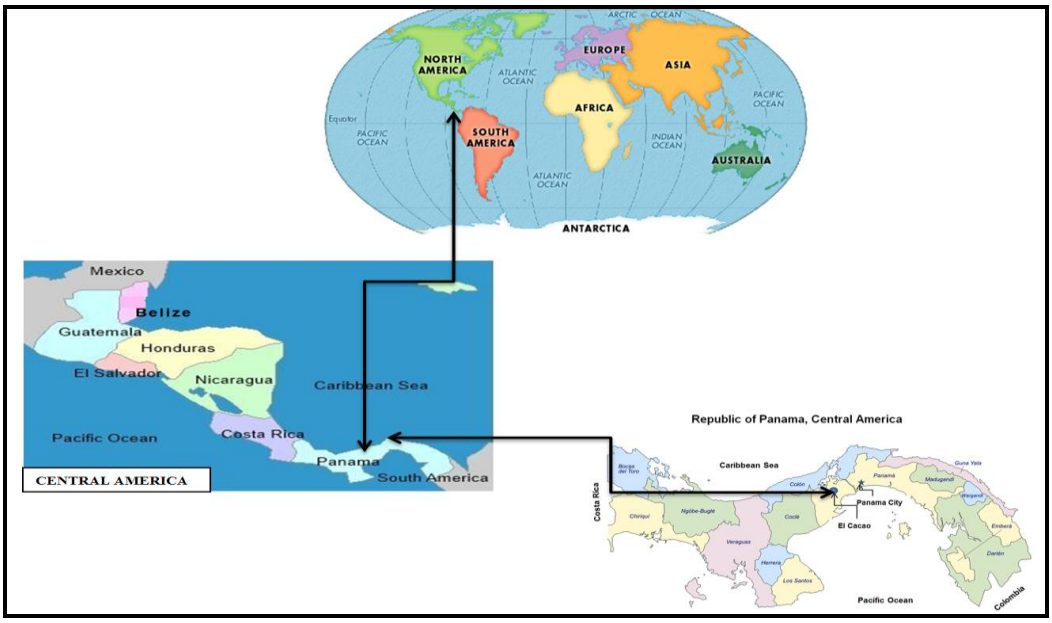


Figure 1: *Localization of the Republic of Panama and the Rural Area of El Cacao in the Globe*

2.2. Design and Construction of the Processing Factory

The land chosen for the project, a two-hectare farm in the community of El Cacao, met all the parameters needed for the building, including drinking water, soil quality, and sufficient rainwater from two creeks and a spring. Local authorities and community leaders gave their approval for this project, and several community members participated in the activities related to the construction of the processing factory and to the planting and harvesting of the plants to be dried at the factory.

An important component of the processing factory was a solar oven. Its construction required to meet the following conditions: dimensions, physical orientation according to the sun path, and conservation of the internal temperature in the processes, plus needed equipment and instrumental. The roof of the oven was built with a semicircle shape using transparent polyethylene sheets to achieve the free passage of the sunlight on the elements for the collection and convection of the heat energy from the sun. The oven floor was built with a concrete slab. Walls and floor were painted dark black to reduce reflection coefficient and increase the absorption coefficient.

2.3. Testing of the Heat Transfer in the Drying Chamber

Once the solar oven was in place, a series of measurements of the different temperatures at critical points of loss of heat transfer were done. These preliminary measurements were performed to find how the heat conduction process occurred, based on temperature gradients and the area to which the heat was transferred. These temperature measurements were aimed at the estimation of the free conduction of heat from the oven to the drying chamber.

2.4. Drying of Nutritional and Medicinal Plants

To test the capability of the processing factory, we conducted a series of tests with local plants to measure the parameters associated with the percentage of dehydration, changes in temperature, time spent in drying and conservation of organoleptic characteristics. For our tests, and based on the literature, we assumed that we started with a percentage of fresh humidity between 70-95% and aimed to reach a humidity of 30-10% dry moisture [3, 4].

For those tests, we used indigenous plants cultivated in a demonstration garden previously established in the premises of the factory. The first series of tests were done during the months of October and November of 2013, with 4.5 hr of solar radiation per day, as average. With oregano (*Origanum vulgare*), coriander (*Coriandrum sativum*), rosemary (*Rosmarinus officinalis)* and balsamino o bitter melon (*Momordica charantia*), which in Panama are commonly used plants as food or as traditional medicine [4, 5].

In December 2013, we continue with additional tests, including drying of lemongrass. The first experiment was to assess drying times. It was done with the leaves cut at different lengths (cm) and separated into four batches of 10 grams each. We processed another batch of lemongrass and packaged the dried leaves in tea bags of 5g, which were used for *an assessment of the organoleptic characteristics of this artisanal tea, as compared to a commercial lemongrass tea.*

*As evaluators, we recruited a group of 15 community residents, who were invited to participate in a tasting session of lemongrass tea. T*he group was asked to assess the sensory attributes of both types of tea with a questionnaire based on 5-item Likert scales. The assessed characteristics included aroma (1= very light, 5= very fragrant), taste (1=very bitter, 5=very sweet), and flavor (1=very mild, 5=very strong) plus overall acceptability, using 5-item Likert scales. We first asked panelists to rate their personal preferences for lemongrass tea (in general) regarding color, aroma and taste. Then we conducted the tasting session where panelists were asked to assess the sensory characteristics of the artisanal and commercial lemongrass teas, which were only identified as tea A and tea B.

3. Results

3.1. The Processing Factory and the Solar Oven

The physical plant was built as a linear building of 4m wide by 17mts long following standard specifications for ceilings, finishes, doors, and windows, plus the specifications in the electrical and plumbing plans. Under the roof, it was placed an insulating sheet to prevent radiation to the areas of production. The physical structure had a horizontal orientation from east to west, taking into account the azimuth, inclination, and orientation of the sun factor, where the sun rises and where it sets. The position of the building gave the orientation of the solar oven, and the photovoltaic solar panels were placed on the roof of the building.

3.2. Heat Transfer in the Drying Chamber

Temperature measurements were performed with a laser thermometer and taken at critical points at the exterior and interior walls of the drying chamber, in the month of October 2013. Seven measurements were registered at the following times: 8:00 am, 10:00 am, 12:00 m, 2:00 pm, 4:00 pm, 6:00 pm and 8:00 pm, with the results observed in Figure 2.

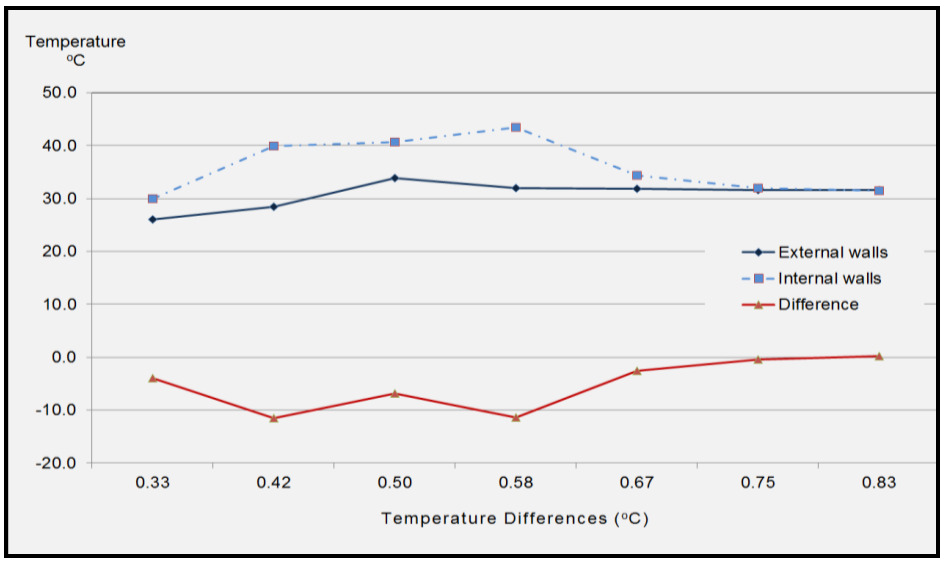


Figure 2: *Values of Critical Points in the Outer and Inner Walls of the Solar Oven*

3.3. Drying of Medicinal and Nutritional Plants

The selection of samples was rigorous, choosing healthy plants at the demonstration garden. Healthy leaves of bright color were selected. All plants were harvested in the early hours of the morning. For washing and cleaning they, clean water with about 10 mg/L of calcium hypochlorite was used. After washing, a manual pre-drying with clean towels was done.

The first round of preliminary drying tests was performed with four lefty plants: coriander, balsamino, rosemary, and oregano, also obtained the demonstration garden. With coriander leaves, two groups of different weights were tested. Average temperatures in the drying chamber were registered as well as the time needed to reach a moisture content ratio (MCR) expressed with the weight in grams as in percentages. The results of the drying of these four products are shown in Table 1. It can be seen that, in a relatively short time, the expected MCRs were reached. These times were shorter than the values recommended in the literature and those reported in traditional processes with drying with direct sunlight.

Table 1: *Results Obtained with Drying of Medicinal Plants during the Months of October and November 2013*

	Coriander (Batch 1)	Coriander (Batch 2)	Balsamino	Oregano	Rosemary
Average Temperature (^{o}C)[1]	33.3	33.3	32.6	32.3	32.2
Drying time (h:min)	18:20	18:20	14:00	9:55	17:15
Yields					
Initial weight (g)	150	175	200	105	227
Dry weight (g)	40	31	78	37	68
MCR (g)	110	144	122	68	189
MCR (%)	73.3	82.3	61.1	64.8	83.2

[1]Recorded average temperature in the drying chamber

We continue with additional testing using another plant, lemongrass, which is commonly use in El Cacao, both as medicinal and as a nutritional herb. Fresh leaves were collected and cleaned as indicated before, and four batches of 10 g with leaves cut at different lengths. Each batch was subjected to the drying process for different periods of time to observe changes in color and aroma as drying continued, until obtaining an expected water loss of at least 60%. The batches were weighed at intervals of time at 1, 2, 3, 4 and 21 hours when dehydration, estimated as the MCR expressed in percentages, was achieved, as presented in Table 2. It was observed that, with the four sets of samples, the percentage of water loss (33%) was rapid and constant during the first 4 hours and then the drying process slowed as expected, with a further evaporation of 40% or more achieved in 17 additional hours.

Table 2: *Drying of Lemongrass with Leaves Cut at Different Lengths*

Batch Specification	Batch 1 Leaf ± 12 cm	Batch 2 Leaf ± 6 cm	Batch 3 Leaf ± 3 cm	Batch 4 Leaf ± 1 cm
Drying Time (hr)	(g)	(g)	(g)	(g)
0 hr	10.0	10.1	10.1	10.0
1 hr	7.0	6.8	8.1	7.2
2 hr	6.2	6.3	7.0	6.5
3 hr	5.7	6.0	6.9	5.5
4 hr	5.2	5.7	6.5	5.4
21 hr	2.9	2.8	2.9	2.6
%MCR (%)	71.0 %	71.7%	71.0 %	74.0 %

3.4. Assessment of the Acceptability of Artisanal Lemongrass Tea

To assess the acceptability of the artisanal lemongrass tea, we tested the perceptions of a group of community residents of El Cacao, which included 15 adults (9 men and six women), with ages between 23 – 56 years.

Those naïve panelists were first asked to rate their personal preferences for lemongrass tea, which are detailed in Table 3. We noted that the majority of panelists preferred a moderate color (not too light, not too dark); a strong aroma and a sweet or “just right” (not too sweet, not too bitter) taste.

Table 3: *Ratings of Desirable Organoleptic Characteristics in Lemongrass Tea by Community Evaluators*

Preferences	Ratings	
	N	%
Color		
1. Very light	1	6.7
2. Light	5	33.3
3. Moderate	8	53.3
4. Dark	1	6.7
5. Very dark	0	0.0
Aroma		
1. Very light	0	0.0
2. Faint	0	0.0
3. Moderate	4	26.7
4. Strong	8	53.3
5. Very fragrant	3	20.0
Taste		
1. Very bitter	0	0.0
2. Bitter	1	6.7
3. Just right	6	40.0
4. Sweet	8	53.3
5. Very sweet	0	0.0

Blind assessment of the flavor and taste of the artisanal vs. commercial lemongrass tea were done. The results revealed that the panelists perceived more the floral and lemony aromas instead of the others less desirable for them in the artisanal tea, while they identified an aroma of dried straw in the commercial tea at a higher level as compared to the expected lemony and floral aromas, as seen in Table 4. Panelists indicated that both types of tea tested mainly as lemon, although they found the artisanal tea to have a stronger lemon taste than the commercial one. The panelists also identified a near strong sweet taste in the artisanal tea. Finally, we asked panelists to indicate they preferred tea (artisanal vs. commercial), using a Likert scale of 5 options for preferences, with one as not preferred, and five as strongly preferred. The artisanal tea explicitly came forth as the preferred tea with 14 out of 15 preference votes, with a mean 4.5 (SD=0.6) of acceptability, vs. only one vote for preference of the commercial tea, as seen in Table 5. While the overall acceptability of the commercial tea was rather “neither liked nor disliked” (although a high deviation on the rating), the artisanal tea was assessed as very much liked by all respondents.

Table 4: *Panelists Assessment of the Aroma Characteristics of Artisanal Vs. Commercial Lemongrass Tea*

Aroma	Artisanal Tea (Mean ± SD)	Commercial Tea (Mean ± SD)
Floral	**3.2 ± 0.9**	2.0 ± 1.0
Lemon	**3.4 ± 0.7**	2.0 ± 1.0
Walnut	1.1 ± 0.3	1.4 ± 0.5
Roasted grain	1.3 ± 0.6	1.3 ± 0.6
Brown rice	1.1 ± 0.4	1.3 ± 0.6
Dried straw	2.1 ± 1.0	**2.5 ± 0.9**
Cut grass	2.3 ± 1.1	2.0 ± 0.9
Burnt leaf	1.5 ± 1.0	1.5 ± 1.1
Metallic	1.1 ± 0.4	1.3 ± 0.8

Ratings for Aroma: 1= absent; 2= faint; 3= moderate; 4= strong

Table 5: *Panelists Assessment of the Taste Characteristics of Artisanal vs. Commercial Lemongrass Tea*

Taste	Artisanal Tea (Mean ± SD)	Commercial Tea (Mean ± SD)
Sweet taste	**2.5 ± 0.9**	1.9 ± 0.7
Sour taste	1.7 ± 0.7	1.5 ± 0.5
Bitter taste	1.3 ± 0.6	1.6 ± 0.8
Astringency	1.6 ± 1.1	1.7 ± 1.3
Lemon	**3.4 ± 0.9**	**2.6 ± 1.0**
Orange	2.1 ± 0.7	1.8 ± 0.7
Grapefruit	1.6 ± 0.8	1.4 ± 0.5
Cut grass	2.1 ± 1.1	2.0 ± 0.8
Burnt leaf	1.3 ± 0.9	1.3 ± 0.5
Metallic	1.3 ± 0.8	1.3 ± 0.6

Ratings for Taste: 1= absent; 2= faint; 3= moderate; 4= strong

4. Conclusions

As described in this paper, we successfully planned and built an agro-industrial factory for the processing of nutritional and medicinal plants commonly used in rural Panama, particularly in the community of El Cacao, district of Capira. We assessed the functionality of the factory, including its capacity to utilize solar energy for drying nutritional and medicinal plants, with favorable results.

Preliminary tests with different plants yielded positive results, as it was possible to reduce the moisture content to recommended levels in shorter times than those reported in the literature for similar processes [3, 6]. Conservation of the organoleptic characteristics including taste, aroma and color were maintained at expected levels during the drying process, and comparable or better than similar commercial products found in the area, as determined by sensory evaluations by a panelist group of community residents. These results were very promising, especially because they suggest that the local population would accept their traditional plants processed in their community.

Acknowledgements

Drs. Mahabir Gupta and Enrique Murillo, Professors at the University of Panama, are highly acknowledged for their guidance on the subject.

Thanks to the Research Center in Global Nutrition and Health (CINSAG) of Panama, for providing financial support for this work.

References

[1] Bermudez Isaias and Juana Ramos dePerez. *Ethnobotany in El Cacao, Capira, Panama: Identification and uses of Medicinal Plants (Spanish).* Prisma Tecnologico. 2014. 5 (1) 48-52.

[2] Empresa de Transmisión Eléctrica S.A - ETESA, Duración Media De Brillo Solar U Horas De Sol. http://www.hidromet.com.pa/brillo_solar.php. Accesado Marzo 2015.

[3] Almada, M., et al. Guía De Uso De Secaderos Solares Para Frutas, Legumbres, Hortalizas, Plantas Medicinales Y Carnes. Fundación Celestina Pérez de Almada. Asunción, Paraguay. 2005. 41.

[4] Gupta, M.P. Plantas Medicinales Iberoamericanas. Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED) y Organización del Convenio Andrés Bello. Bogotá, Colombia. 2008. 1003.

[5] Solís, P.N.e., Plantas Medicinales Para La Atención Primaria De Salud. Proyecto Mediano TRAMIL-GEF/UNEP/Enda-Caribe. Centro de Investigaciones Farmacognósticas de la Flora Panameña (CIFLORPAN). Publicado en Panama. 2005. 23.

[6] Cáceres, A. and Samayoa, B. Tamizaje De La Actividad Antibacteriana De Plantas Usadas En Guatemala Para El Tratamiento De Afecciones Grastrointestinales. Centro Mesoamericano de Estudios sobre Tecnologia Apropiada, Universidad de San Carlos de Guatemala. 1989. 138.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 1, pp. 196-202, Article ID Med-295
ISSN 2348-5140

Cloud Publications

Research Article **Open Access**

Keeping Quality of Sorghum Soybean Supplemented Wheat Flour *Ladoos*

Pandey L. and Sangwan V.

Department of Foods & Nutrition, CCS Haryana Agricultural University, Hisar, Haryana, India

Publication Date: 12 April 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-295

Abstract The present study was done to assess the keeping quality of value added *ladoos* developed from newly released wheat varieties (WH-1129 and HD-2967) flours supplemented with sorghum and soybean flours. The control *ladoo*s had mean score of overall acceptability 7.46 whereas all other types of *ladoos* made from combinations of wheat, sorghum and soybean flours had mean scores of overall acceptability ranging from 7.86 to 8.62, which were significantly higher (P≤0.05) than that of control *ladoos*. The protein and fat content in control *ladoo*s were 9.06 and 20.14 per cent, respectively which significantly (P≤0.05) increased in composite flour ladoo of WH-1129 wheat flour to 11.39 and 23.00 per cent, respectively and to 10.89 and 25.02 per cent, respectively in that of HD-2967 flour. The crude fibre and ash contents in control *ladoos* were 1.22 and 1.39 per cent, respectively which significantly (P≤0.05) increased in composite *ladoos* of WH-1129 flour to 4.16 and 1.77 per cent, respectively. All types of *ladoos* were organoleptically acceptable upto 90 days of storage and fell in the category of' 'liked moderately' to 'liked very much'. The total bacterial count of *ladoo* of WH-1129:SGF:SBF (60:30:10) and (40:40:20) varied from 0 to 10×10^2 and 0 to 7×10^2 cfu/g of *ladoo,* respectively while that of HD:SGF:SBF (60:30:10) and (40:40:20) ranged from 0 to 9×10^2 and 0 to 10×10^2 cfu/g of *ladoo*, respectively. The total bacterial count of *ladoo* was within the permissible limit upto 45 days of storage.
Keywords *Composite Flour; Ladoos; Supplementation; Nutritive Value; Keeping Quality*

1. Introduction

Ladoo is a sweet relished equally by people of all age groups and is energy and protein dense food. The basic ingredients of *ladoo* are wheat flour, ghee, grounded sugar and sesame seeds. Wheat flour is an important source of not only energy and protein but also provides substantial amounts of vitamins and minerals in human diets specially low income group people. It has been extensively and widely used for the preparation of various types of value added *sev*, bakery products like biscuits, cakes, cookies, bread etc., traditional products like *ladoo, chapatti* etc., throughout the world (Pandey, 2015). It is commonly known that the main nutritional drawback of cereals is lack of essential amino acid lysine which can be easily compensated by supplementing cereals with oilseeds like soybean. Soybean has great potential as an exceptionally nutritive and very rich protein food. It can supply the

much needed protein to human diets, because it contains above 40 per cent protein of superior quality and all the essential amino acids particularly glycine, tryptophan and lysine, similar to cow's milk and animal proteins. Soybean also contains about 20 per cent oil with an important fatty acid, lecithin and Vitamin A and D. The 4 percent mineral salts of soybeans are fairly rich in phosphorous and calcium. Soybean has great potential in overcoming protein calorie malnutrition because it contains 38 to 40 per cent protein and 18 to 20 per cent fat (Rastogi and Singh, 1989). Incorporation of soybean flour into a staple food like wheat and coarse cereal like sorghum is a feasible means of increasing the nutritive value of people's diet. Sorghum is important crop for food security in semi-arid and arid regions due to their high nutritional quality and low production inputs. Sorghum is gluten free and can be important food source to millions of people who are intolerant to gluten (celiac disease), including diabetic patients, in both developed and developing countries (Masilamani et al., 2012). It is established in literature that cereal, coarse cereal and soybean flours possess ability to prevent cancer, control diabetes, obesity, promote cardiovascular health considered safe for Celiac disease patients, improve digestive health, build strong bones, promote red blood cell development and boost energy and fuel production (Yang et al., 2009; Goerke et al., 2012; Cao et al., 2011; Masilamani et al., 2012). In the present study efforts were made to develop nutrient rich *ladoos* by incorporating wheat, sorghum and soybean flours and were evaluated for organoleptic acceptability, nutritional characteristics and keeping quality.

2. Material and Methods

2.1. Procurement of Raw Material

Two newly released wheat (*Triticum aestivum)* varieties (WH-1129, HD-2967), traditional wheat variety (C-306) and *Sorghum vulgare* (HJ-541) used for product development in the present study were procured in a single lot from the breeders, Department of Genetics and Plant Breeding, CCS Haryana Agriculture University, Hisar. Soybean flour along with other ingredients required for the development of value added *ladoos* were procured from local market.

2.2. Processing of Material

Triticum aestivum (WH-1129, HD-2967 and C-306) and *Sorghum Vulgare* (HJ-541) were subjected to processing before use to remove dust, dirt and other unhygienic foreign materials. The wheat and sorghum grains were cleaned and ground in an electric grinder (Cyclotec, M/s Tecator, Hoganas, Sweden) and flours thus obtained were sieved through a 60 mesh sieve and packed in airtight plastic containers for product development and further analysis.

2.3. Development and Organoleptic Characteristics of Value Added *Ladoos*

The preparation method of value added *ladoos* is presented in Table 1. Using two ratios (60:30:10 and 40:40:20) of each wheat variety flour (WF), sorghum flour (SGF) and soybean flour (SBF) four types of *ladoos* were developed. 100% wheat flour *ladoos* prepared from C-306 were kept as control. The *ladoos* were organoleptically evaluated by a panel of ten judges for sensory parameters like colour, appearance, flavour, texture, taste and overall acceptability using 9 point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9 to like extremely). Between tasting different samples, participants rinsed their mouth with warm water. On the basis of organoleptic acceptability, from each category the *ladoos* rated higher for organoleptic characteristics were selected for further study.

Table 1: *Ingredients and Preparation Method for Development of Ladoos*

Supplementation level (%)	Wheat flour (g)	Sorghum flour (g)	Soybean flour (g)	Ghee (g)	Sugar (g)	Gingelly seeds (g)
Control(100%WF)	100	-	-	55	50	30
WF : SGF : SBF						
60 : 30 : 10	60	30	10	55	50	30
40 : 40 : 20	40	40	20	55	50	30

Method

1. Roasted wheat, sorghum and soybean flours separately in skillet and mixed together.
2. Then added roasted and grounded gingelly seeds and ghee.
3. Allowed the mixture to cool.
4. Added grounded sugar and mixed well and made the *ladoos*.

2.4. Nutritional Characteristics of Value Added *Ladoos*

Proximate composition (moisture, crude protein, crude fat, crude fibre and ash) of one most acceptable ratio of *ladoos* developed from wheat, sorghum and soybean flour blends were estimated by employing the standard method of analysis (AOAC, 2000).

2.5. Keeping Quality of Value Added *Ladoos*

For studying the keeping quality/shelf –life the most acceptable value added *ladoos* were stored for 3 months in air tight plastic containers at room temperature. The *ladoos* were evaluated for sensory parameters using 9 point hedonic scale by a panel of ten judges and total bacterial count at regular intervals of 0, 15, 30, 45, 60, 75 and 90 days.

For the most acceptable were stored for 3 months in air tight plastic containers at room temperature. The *ladoos* were evaluated for at regular intervals of 0, 15, 30, 45, 60, 75 and 90 days.

2.5.1. *Estimation of Total Bacterial Count*

Composition of PCA Media (g/l)

Peptone - 5g
Yeast Extract - 2.5g
Dextrose - 1.0g
Agar - 20.0g
Distil water - 1000ml

Sterilization of Media and Glassware

Plate count agar media was prepared in distilled water and autoclaved at 121.6˚C (15psi) for 15 min. All the glassware were sterilized in hot air oven at 160˚C for 2 hour.

Procedure

One g of sample was dissolved into 9.0 ml of sterilized distilled water blank and shaken thoroughly. One ml of 10^{-1} dilution was taken and dissolved into another 9.0 ml sterilized water blank. This was 10^{-2} dilution. Similarly 10^{-3} dilution was made. 0.1ml of 10^{-1}, 10^{-2} and 10^{-3} dilutions were poured in petri

plate containing PCA media. Plates were incubated at 30±2˚C for 24-48 hours. Numbers of colonies were counted and colony forming unit (cfu) was calculated by using formula:-

No. of colonies × dilution factor × 10 = cfu /g of sample

2.6. Statistical Analysis

The data were statistically analysed in complete randomized design for analysis of variance, mean, standards deviation and critical difference according to the standard method (Sheoran and Pannu, 1999).

3. Results and Discussion

3.1. Organoleptic Characteristics

The mean scores of organoleptic acceptability of value added *ladoos* are presented in Table 2. The control *ladoo*s had mean score of overall acceptability 7.46 whereas all other types of *ladoos* made from combinations of wheat, sorghum and soybean flours had mean scores of overall acceptability ranging from 7.86 to 8.62, which were significantly higher (P≤0.05) than that of control *ladoos*. All types of *ladoos* were organoleptically acceptable and their scores belonged to category 'liked very much'. Our findings were in agreement with those of Singh (2003), Singh and Sehgal (2008), Rajbala (2010), Chandel (2014) and Rana (2015) who reported that on the basis of organoleptic evaluation, the mean scores of overall acceptability of different value added products i.e. l*adoo, sev, chapati, matar, parantha*, biscuit and pasta were higher than that of (100%) wheat flour products.

Table 2: *Mean Score of Organoleptic Acceptability of Value Added Ladoos*

Ladoos	**Colour**	**Appearance**	**Aroma**	**Texture**	**Taste**	**Overall acceptability**
Control (100% WF)	7.40±0.27	7.30±0.30	7.70±0.21	7.50±0.27	7.40±0.34	7.46±0.07
Type I	8.30±0.15	8.20±0.20	7.90±0.23	7.80±0.25	7.80±0.13	8.00±0.10
Type II	8.80±0.13	8.80±0.13	8.50±0.17	8.20±0.20	8.80±0.14	8.62±0.10
Type III	7.90±0.18	7.70±0.15	7.90±0.10	7.60±0.16	8.20±0.20	7.86±0.10
Type IV	8.30±0.15	8.20±0.13	8.20±0.14	7.50±0.15	8.60±0.16	8.16±0.18
CD (P≤0.05)	0.52	0.56	0.50	N.S	0.60	0.36

Type I (WH-1129:SGF:SBF 60:30:10) Type II (WH-1129:SGF:SBF 40:40:20)
Type III (HD-2967:SGF:SBF 60:30:10) Type IV (HD-2967:SGF:SBF 40:40:20)
WF=Wheat flour (WH-1129 and HD-2967). SGF= Sorghum flour. SBF=Soybean flour

3.2. Nutritional Composition of Value Added *Ladoos*

The data pertaining to proximate composition of most acceptable *ladoos* is presented in Table 3. The protein and fat content in control *ladoo*s were 9.06 and 20.14 per cent, respectively which significantly (P≤0.05) increased in composite flour ladoo of WH-1129 wheat flour to 11.39 and 23.00 per cent, respectively and to 10.89 and 25.02 per cent, respectively in that of HD-2967 flour. The crude fibre and ash contents in control *ladoos* were 1.22 and 1.39 per cent, respectively which significantly (P≤0.05) increased in composite *ladoos* of WH-1129 flour to 4.16 and 1.77 per cent, respectively. As for supplemented ladoos prepared from HD-2967 flour the crude fibre content increased to 3.90 per cent but ash content (1.29 %) was almost similar to control. WH-1129 wheat flour *ladoos* contained significantly (P≤0.05) higher amount of protein, crude fibre and ash as compared to HD-2967 flour *ladoos* supplemented with sorghum and soybean flours. HD-2967 wheat flour *ladoos* were found to possess highest fat content as compared to all other types of *ladoos*. These results are in agreement with those of earlier workers (Gupta, 2001; Rani et al., 2008; Punia and Gupta, 2009 and Sangwan

and Dahiya, 2013), who found that proximate composition of value added products were higher than that of control products developed from 100 per cent wheat flour. The difference in proximate composition of value added products developed from two different wheat varieties was basically due to difference in the proximate composition of wheat varieties.

Table 3: *Proximate Composition of Composite Flour ladoos (%, on dry matter basis)*

Supplementation level (%)	Moisture	Protein	Fat	Crude fibre	Ash
Ladoo					
Control (100% WF)	10.22±0.07	9.06±0.04	20.14±0.60	1.22±0.02	1.39±0.03
Type II	11.13±0.05	11.39±0.22	23.00±0.57	4.16±0.03	1.77±0.08
Type IV	10.87±0.05	10.89±0.26	25.02±0.51	3.90±0.05	1.29±0.02
CD(P≤0.05)	0.21	0.71	1.99	0.14	0.18

Type II (WH-1129:SGF:SBF 40:40:20) Type IV (HD-2967:SGF:SBF 40:40:20)
WF=Wheat flour (WH-1129 and HD-2967). SGF= Sorghum flour. SBF=Soybean flour

3.3. Keeping Quality of Value Added *Ladoos*

The effects of storage period on organoleptic acceptability and total bacterial count of value added *ladoos* are shown in Table 4 and 5.

Mean scores of overall acceptability in control, WH-1129: SGF: SBF (60:30:10 and 40:40:20), and HD:SGF:SBF (60:30:10 and 40:40:20) showed gradual decline during storage. The acceptability score for control *ladoos* declined from 7.46 (zero day) to 6.24 (90^{th} day), for WH-1129:SGF:SBF (60:30:10 and 40:40:20), it declined from 8.00 (zero day) to 6.60 (90^{th} day), 8.62 (zero day) to 6.80 (90^{th} day) and for HD:SGF:SBF (60:30:10 and 40:40:20), it declined from 7.86 (zero day) to 6.54 (90^{th} day) and 8.20 (zero day) to 6.76 (90^{th} day), respectively during storage. On the mean basis all types of *ladoos* were organoleptically acceptable upto 90 days of storage and fell in the category of' 'liked moderately' to 'liked very much'.

The total bacterial count of the control *ladoo* from zero to 45^{th} day of storage, varied from 0 to 10×10^2 cfu/g of *ladoo*. The total bacterial count of *ladoo* of WH-1129:SGF:SBF (60:30:10) and (40:40:20) varied from 0 to 10×10^2 and 0 to 7×10^2 cfu/g of *ladoo,* respectively while that of HD:SGF:SBF (60:30:10) and (40:40:20) ranged from 0 to 9×10^2 and 0 to 10×10^2 cfu/g of *ladoo*, respectively. The total bacterial count of *ladoo* was within the permissible limit upto 45 days of storage (Table 5). These findings are in agreement with those of several other workers (Gurusu et al., 1997; Sangwan and Dahiya, 2013; Chandel, 2014; Rana, 2015) who found that the value added products developed from composite flour could be stored upto 90 days.

Table 4: *Effect of Storage Period on Overall Acceptability Scores of Wheat, Sorghum and Soybean Composite Flour Ladoos*

Supplementation Level (%)	Storage Period (days)							
	0	15	30	45	60	75	90	Mean
	Overall Acceptability							
Control (100% WF)	7.46±0.09	7.32±0.13	7.16±0.15	6.96±0.21	6.78±0.22	6.54±0.13	6.24±0.10	6.89±0.12
WH-1129:SGF:SBF (60:30:10)	8.00±0.16	7.90±0.23	7.70±0.21	7.54±0.16	7.36±0.28	7.14±0.21	6.60±0.15	7.44±0.12
WH-1129:SGF:SBF (40:40:20)	8.62±0.17	8.50±0.10	8.30±0.30	7.94±0.20	7.78±0.22	7.42±0.17	6.80±0.12	7.79±0.09
HD-2967:SGF:SBF (60:30:10)	7.86±0.01	7.72±0.10	7.60±0.16	7.42±0.22	7.22±0.20	7.04±0.15	6.54±0.18	7.35±0.10

HD-2967:SGF:SBF (40:40:20)	8.20±0.12	8.00±0.11	7.86±0.18	7.68±0.20	7.50±0.21	7.40±0.15	6.76±0.18	7.58±0.23
Mean	8.03	7.89	7.72	7.51	7.33	7.11	6.59	

Table 5: *Total Bacterial Count (Cfu/G) Of Composite Flour Ladoos at Different Storage Period (On Dry Weight Basis)*

Supplementation Level (%)	Storage Period (days)						
	Total Bacterial Count (cfu/g)						
	0	15	30	45	60	75	90
Control (100% WF)	0	2×10^{2}	7×10^{2}	10×10^{2}	21×10^{2}	41×10^{2}	54×10^{2}
Type II	0	3×10^{1}	4×10^{2}	7×10^{2}	25×10^{2}	33×10^{2}	48×10^{2}
Type IV	0	3×10^{1}	6×10^{2}	10×10^{2}	35×10^{2}	43×10^{2}	52×10^{2}

Type II (WH-1129:SGF:SBF 40:40:20) Type IV (HD-2967:SGF:SBF 40:40:20)
WF=Wheat flour (WH-1129 and HD-2967). SGF= Sorghum flour. SBF=Soybean flour cfu=colony forming unit

4. Conclusion

The utilisation of alternative sources of food specially less utilised coarse cereals and refinement of technology is need of the hour. From the present study it is concluded that coarse cereals like sorghum and protein rich soybean can be utilised for supplementing the wheat flour which is staple diet of the population. The development and utilization of the composite flour *ladoos* on one hand will promote value addition of the products and on the other hand will provide low cost nutritious alternatives specially in poor developing countries for combating malnutrition among children and vulnerable sections of the society. Setting up of small scale industries for production of *ladoos* by rural women will ensure the economic, food and nutrition security and it will also encourage utilisation of low input, sustainable crops, together with staple crops.

References

AOAC, 2000: *Official Methods of Analysis of Association of Official Analytical Chemists*. Washington, D.C.

Cao, Z., Sun, L., Cao, X., and He, Y. *Antioxidant Activity of Soybean Peptides*. Advanced Materials Research. 2011. 233; 854-865.

Chandel, J., 2014: *Development, Acceptability and Nutritional Evaluation of Linseed Supplemented Value-Added Products*. M.Sc. Thesis, CCS Haryana Agricultural University, Hisar, India.

Gupta, S., 2001: *Utilization of Vegetable Powder for Value Added Baked Product.* M.Sc. Thesis, CCS Haryana Agricultural University, Hisar, India.

Gurusu, O., Earcan, R. and Denli, E. *Effect of the Addition of Soyflour on Quality and Shelf-Life of Biscuit*. Gida. 1997. 22 (2) 95-103.

Goerke, M., Eklund, M., Sauer, N., Rademacher, M., Piepho, H.P., and Mosenthin, R. *Standardized Ileal Digestibilities of Crude Protein, Amino Acids, and Contents of Antinutritional Factors, Mycotoxins, and Isoflavones of European Soybean Meal Imports Fed to Piglets*. Journal of Animal Science. 2012. 90; 4883-4895.

Masilamani, M., Wei, J., and Sampson, H.A. *Regulation of the Immune Response by Soybean Isoflavones.* Immunological Research. 2012. 54; 95-110.

Pandey, L., 2015: *Nutritional Evaluation of Wheat (Triticum Aestivum) Varieties and Development of Value Added Products Using Wheat-Sorghum-Soybean Flour Blends.* M.Sc. Thesis, CCS Haryana Agricultural University, Hisar, India.

Punia, D. and Gupta, M. *Sensory Characteristics, Nutrient Composition and Storage Studies of Value Added Products for Children*. Journal of Nutrition and Food Science. 2009. 39 (5) 503-510.

Rajbala, 2010: *Improvement in Nutritional Status of School Children through Micronutrient Rich Dietary Supplements.* Ph.D. Thesis, CCS Haryana Agricultural University, Hisar, India.

Rana, N., 2015: *Nutritional Evaluation and Acceptability of Value Added Products Based on Composite Flour (Pearl Millet-Sorghum-Mungbean-Marwa).* Ph.D. Thesis, CCS Haryana Agricultural University, Hisar, India.

Rani, V., Grewal, R.B., and Khetarpaul, N. *Sensory and Nutritional Evaluation of Soy Supplemented Nutritious Baked Products.* Journal of Dairying Foods and Home Sciences. 2008. 27 (3/4) 209-215.

Rastogi, A. and Singh, G. *Effect of Addition of Full Fat Soyflour of Different Varieties on Quality Characteristics and Bread Making Quality of Wheat Flour*. Bull. Grain Technology. 1989. 27; 26-34.

Sangwan, V. and Dahiya, S. *Physico-Chemical and Nutritional Properties of Wheat-Sorghum-Soybean Composite Flours and Their Biscuits*. Asian Journal of Dairy and Food Research. 2013. 32 (1) 65-70.

Sheoran, O.P, Tonk, D.S., Hasija, R.C., and Pannu, R.S., 1998: *Statistical Software Package for Agricultural Research Workers in Recent Advances in Information Theory, Statistics and Computer Application.* Hooda D.S. and Hasija R.C.(eds.) 139-143.

Singh, G., 2003: *Development and Nutritional Evaluation of Value Added Products from Pearl Millet (Pennisetum glaucum).* Ph.D. Thesis, CCS Haryana Agricultural University, Hisar, India.

Singh, G. and Sehgal, S. *Nutritional Evaluation of Ladoo Prepared from Popped Pearl Millet.* Journal of Nutrition and Food Science. 2008. 38; 310-315.

Yang, L., Browning, J.D., and Awika, J.M. *Sorghum 3-Deoxyanthocyanins Possess Strong Phase II Enzyme Inducer Activity and Cancer Cell Growth Inhibition Properties.* Journal of Agricultural and Food Chemistry. 2009. 57 (5) 1797-804.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 2, pp. 226-233, Article ID Med-316
ISSN 2348-5140

Cloud Publications

Research Article **Open Access**

Effect of Maternal Nutritional Status on Birth Outcome

Urvi M. Gala[1], Meena L. Godhia[2] and Yogeshwar S. Nandanwar[3]

[1]Department of Food Science & Nutrition, SNDT Women's University, Juhu Tara Road, Mumbai, Maharashtra
[2]Department of Food Nutrition & Dietetics, Sir Vithaldas Thackersey College of Home Science, Juhu Tara Road, Mumbai, Maharashtra
[3]Department of Obstetrics/Gynecology, L.T.M.M.C & G.H., Sion, Mumbai, Maharashtra

Publication Date: 13 July 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-316

Abstract The present cross-sectional descriptive study was conducted to assess the nutritional status, maternal haemoglobin concentration, anthropometric details and its association with neonatal anthropometry. 200 pregnant women aged 18-37 years in the gestational age of 27-41 weeks, without any co-morbidity and having a complete medical record were included in the study. Pregnant women who were in labour in the maternity ward and had visited the tertiary centre in Mumbai for antenatal checkups were enrolled for the study. Predesigned, pretested questionnaire was used to obtain socio-demographic and pregnancy details along with 24-hour dietary recall taken prior to delivery. Maternal and neonatal anthropometry was measured by trained personnel using standardized techniques. Haemoglobin concentration prior to delivery and postpartum, birth weight and length was obtained from the hospital record. Analyses were performed using SPSS software (version 16.0) to determine the effect of nutritional status on birth outcome. P-value <0.05 was considered to be statistically significant. The mean maternal anthropometric details were height-153.13±10.39cm, postpartum weight-57.02±11.57kg, postpartum BMI-24.29±3.54kg/m^2, haemoglobin concentration prior to delivery-11.19±1.78g/dL and post-partum-9.97±1.68g/dL. The mean neonatal birth weight was 2.77±0.50kg though 22.3% neonates had low birth weight (<2.5kg). The mean neonatal anthropometric details were length-45.72±1.14cm, MUAC-10.48±1.14cm, ponderal index-2.88±0.43g/cm^3 and MUAC/Head circumference-0.31±0.03. However, women (≥28 years) were older (+2.46 years), weighed more both pre- and post-partum and also had a higher interpregnancy interval (+1.3 years) and gravida as compared to women (≤27 years) ($p<0.05$). Birth outcome was influenced by maternal height, weight, postpartum BMI, haemoglobin levels prior to delivery, gestational age and nutrient intake particularly energy, protein, vitamin C and calcium. Maternal diet prior to pregnancy and during pregnancy along with maternal anthropometry, haemoglobin concentrations prior to delivery and gestational age greatly influences birth outcome. Thus, attention has to be given to nutrition of an adolescent girl by proper nutrition education, pre-pregnancy counseling which will help in better pregnancy outcome.

Keywords *Diet; Maternal Anthropometry; Neonatal Anthropometry; Pregnancy Outcome*

1. Introduction

Nutritional status of the mother before and during pregnancy is the most critical for optimum growth and development of the foetus. Maternal nutrition greatly influences not only foetal nutrition but also is a determinant of the child's nutritional status, growth and development after birth, productive and reproductive health of the nation [1]. Poor maternal nutritional status has been related to adverse birth outcomes including preterm delivery, low birth weight, restricted foetal growth and which can have lifelong consequences for development, quality of life and healthcare costs [2, 3]. Poor nutritional status could be due to inadequate diet, lack of availability of food or inadequate absorption of nutrients which leads to various deficiencies, lifestyle behaviours e.g. smoking, alcohol consumption, cultural and traditional fads which lead to the elimination of certain food groups and thereby leads to poor nutritional status and further health implications [3].

The prevalence of obesity has increased overtime; increasing BMI is associated with greater risk of pregnancy complications like induction of labour and cesarean delivery; while underweight women had better pregnancy outcomes than women with normal BMI [4]. Moreover, anaemia which is highly prevalent among preschool-age children, pregnant women and non-pregnant women of child bearing age in developing countries [5] could be due to poor nutritional status which can further affect the maternal and neonatal health.

Numerous studies in India have shown that in chronically undernourished women subsisting on unchanged dietary intake, pregnancy has an adverse effect on maternal nutritional status [6]. Thus, in order to improve foetal growth and reduce the risk of adverse birth outcome, a balanced-protein-energy supplement along with micronutrients should be given to expectant women [7-9].

Nationwide nutrition intervention programmes have been in operation over two decades, this is a nutrition transition stage with coexistence of under and over-nutrition. The purpose of this study is to assess the anthropometry and nutritional status of the pregnant women, their interdependence with neonatal anthropometry and hence its effect on birth outcome. Furthermore, we hypothesized that various maternal factors like anthropometry, diet, haemoglobin prior to delivery are associated with neonatal anthropometry.

2. Materials and Methods

A cross-sectional descriptive study was conducted in a tertiary centre in Mumbai among 200 pregnant women during November-December 2015. This sample size was calculated to achieve a statistical power of 0.88. The pregnant women who had visited the tertiary centre for antenatal checkups and were in labour in the maternity ward were enrolled in the study. Two mothers delivered twins thus data on 202 has been presented in the current study.

Inclusion criteria were pregnant women between the age of 18-37 years and in the gestational age of 27-41 weeks. Exclusion criteria were pregnant women suffering from conditions such as asthama, chronic hypertension, tuberculosis, sickle cell anaemia, malignancy, foetal anomaly and diabetes and those who smoke.

Institutional Ethics Committee clearance was taken before the commencement of the study and prior permissions were obtained from Internal Ethics Committee of Lokmanya Tilak Municipal Medical College (L.T.M.M.C.) to interview the pregnant women (21.08.2015). Written informed consent was also taken from the pregnant women after explaining to them the objectives of the study in their own language and only those willing to participate were interviewed for data collection.

Predesigned, pretested questionnaire was used to obtain socio-demographic and pregnancy details along with 24-hour dietary recall taken prior to delivery. Maternal and neonatal anthropometric details were measured by trained personnel using standard measurement techniques [10] and the BMI and neonatal anthropometric indices like MUAC/ Head circumference, the ponderal index was calculated. Neonatal Z-scores were also calculated using WHO Anthro software and compared with the WHO growth standards [11]. Haemoglobin concentration prior to delivery and postpartum, birth weight and length was obtained from the hospital records.

Analyses were performed using SPSS software for Windows (version 16.0, 2007, SPSS Inc, Chicago, IL). For purposes of analysis, women were divided into 2 age groups ≤ 27 years and ≥28 years of age. Many studies suggest that pregnancy-related complications increase from the age of 28 years and hence this threshold was used to make groups. Data are presented as Mean ± SD. The frequency distributions were tabulated according to age groups and were compared using cross-tabulations and chi-square test. Independent sample T-Test was used to analyze the difference in various parameters according to age groups. Pearson was used to assess the correlation between maternal anthropometry and dietary intake with neonatal outcome. P-value <0.05 was considered to be statistically significant.

3. Results and Discussion

The mean age of the pregnant women was 25.93±4.36 years with most of them being Hindus, 30.7% being Muslims and 2% were either Christians or Buddhists. Also, the majority of them lived in joint family and 46% lived in the nuclear family.

The socioeconomic status of the families was classified according to Kuppuswamy's Classification (2015) on the basis of educational status, work profile and family monthly income [12]. Nearly, 69.3% of them were in the upper lower socio-economic group (scores: 5-10) and 12.4% were in the lower middle socio-economic group (scores: 11-15) whereas the upper middle socio-economic group (scores: 16-25) constituted of about 6.4%. About 10.4% were in the lower socio-economic group (scores: <5) whereas only 1% was in the upper socio-economic group (scores: 26-29).

In the study, an attempt was made to categorize the study group into women ≤27 years of age (n=160) and ≥28 years of age (n=62) as it is known that chances of pregnancy-related complications increase as the age increases. Hence, all comparisons are discussed based on this classification. The mean height of the mothers was 153.13±10.39cm while mean pre-pregnancy weight was 52.58±11.73kg. 105 mothers could remember their body weights at first visit (antenatal checkup). The mean weight at first visit was 55.84±10.00kg while, postpartum weight was 57.02±11.57kg and postpartum BMI was 24.29±3.54kg/m^2. The mean age at marriage of the study group was 20.56±3.76 years while the mean inter-pregnancy interval was 2.93±2.25 years.

Older mothers (≥28 years) married later were taller (+2.05cm), had higher pre pregnancy weight (+4.62kgs) and post pregnancy BMI though not statistically significant. However, there was a significant difference ($p<0.001$) in interpregnancy interval (+1.3 years) and age at marriage (+2.46 years). The weight at the first visit and post pregnancy weight were also significantly associated ($p<0.05$).

According to World Health Organization (WHO, 2004), classification of BMI for Asians [13] more than half of the subjects were normal prior to delivery, however, the percentage declined postpartum as the percentage of overweight and obesity increased (Figure 1). This could be because the mothers belonged to the lower socioeconomic status and also due to the weight gain during pregnancy but a

gradual weight loss occurs postpartum which can help them to reach the normal BMI range.

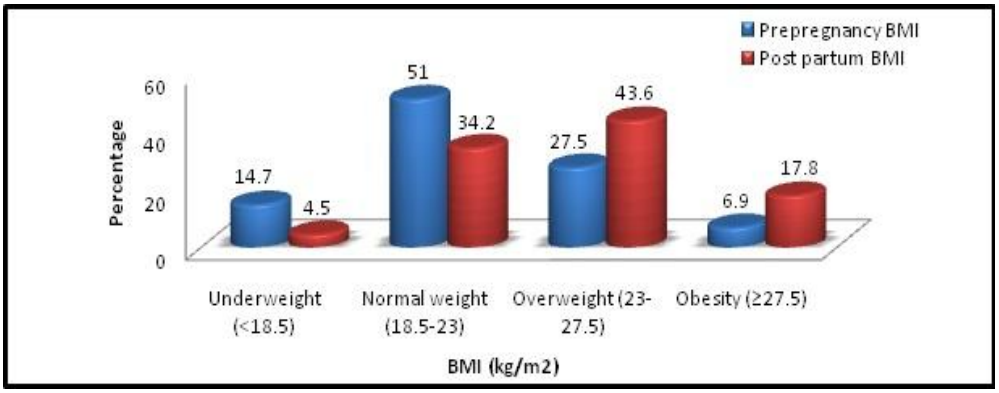


Figure 1: *Maternal pre-pregnancy BMI (n=102) and postpartum BMI (n=202)*

Furthermore, according to World Health Organization (WHO, 2011) classification for anaemia during pregnancy [14], majority of the mothers had a normal haemoglobin concentration prior to delivery which is in contrast to the study conducted in 2009 which said that nearly half the pregnant women suffer from varying degree of anaemia, with the highest prevalence in India and also has the highest number of maternal deaths in the Asian region [7]. However, this percentage reduced postpartum as the percentage for anaemia increased which could be due the pregnancy-related complications and/or excessive blood loss during delivery (Figure 2).

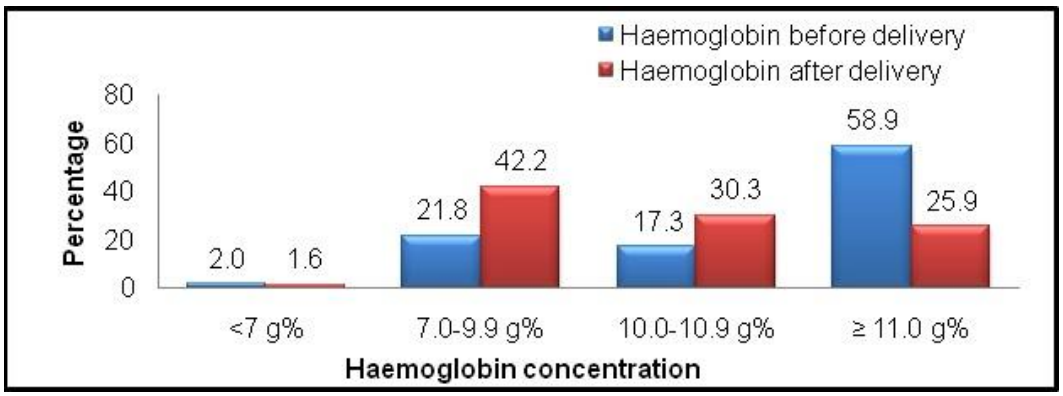


Figure 2: *Maternal haemoglobin before delivery (n=202) and after delivery (n=185)*

For 48.6% of the mothers who were ≤27 years of age, it was their primipara whereas, multipara (gravid 2-6) was seen in the majority (88.7%) of the older women (≥28 years) thereby showing a significant positive correlation ($\chi 2=40.238$; p=0) between maternal age and gravida.

From the total, 97% neonates were born alive and the remaining 3% were either stillborn or had intrauterine foetal death (IUFD). However, in a review article, the figures were used from government data after statistical extrapolation and it was found out that India tops half of the chart with 22 still births per 1,000 births as compared to the global rate of stillbirths at 19 for every 1,000 births [15]. From the 196 neonates born alive, only one had an early neonatal death. Out of the total 202 newborns, 112 (55.4%) were males and 90 (44.6%) were females. Furthermore, 78% of the pregnancies were term pregnancies while 21% were born pre-term with higher incidence (25.8%) among women ≥28 years as compared to women ≤27 years. Also, the incidence of C-section (78.7%)

was higher among the subjects as the study was conducted in a tertiary centre where 60% referral is for high risk or only for operative delivery.

The mean birth weight of the newborns (2.77±0.50kg) was within the normal range of the reference standard (2.7-2.9kg) [16] but had a lower mean neonatal length (45.72±1.14cm) when compared to reference (50cm) [16]. Of the total 202 newborns, 74.7% had normal birth weight while 22.3% had low birth weight (<2.5kg) and 3% had very low birth weight (<1.5kg). While globally, more than 20 million infants are born with low birth weight. According to UNICEF & WHO, 2004 the incidence of low birth weight in India alone accounts for 40 percent of the cases in the developing world and more than half of those in Asia [17]. The mean neonatal head circumference was 33.45±1.53cm which was within the normal range of the reference standard (33-35cm) [16] and chest circumferencewas 31.44±1.56cm. However with an increase in maternal age, birth weight, MUAC, ponderal index and MUAC/ head circumference also increased, though statistically not significant (p>0.05). According to BMI-for-age Z-score, 49.5% of the newborns were in the normal range whereas, 17.3% were mildly undernourished, 14.9% were slightly overweight and 4% were severely undernourished.

A study conducted in 2011 reported that the optimum cut-off point for MUAC/ head circumference ratio is 0.303 [18] which indicate wasting. However, in this study majority (62%) of the neonates had a ratio of ≥0.303.

Furthermore, ponderal index (PI) of 2.5 g/cm^3 is considered normal whereas PI <2.0 g/cm^3 is classified as a low PI (malnourished) and PI between 2 to 2.5 g/cm^3 is considered hypo-plastic [19]. However, the majority (86.6%) of the neonates had PI ≥2.5g/cm^3 whereas 10.4% were hypoplastic and the rest (2.5%) were malnourished.

As seen in Table 1, maternal anthropometric parameter such as height, weight and post-partum BMI significantly influences neonatal anthropometric indices and Z-scores. This is because when the maternal height and weight is appropriate the better is the growth of the foetus and better is the birth outcome.

Table 1: *Association between maternal and neonatal anthropometric indices and neonatal Z-scores (n=202)*

Neonatal Characteristics	Mothers height (cm)	Pre-pregnancy weight (kg)	Weight at the first visit (kg)	Post-partum weight (kg)	Post-partum BMI (kg/m^2)
Birth weight (kg) (n=202)	0.119	0.238*	0.209*	0.283*	0.274*
Length (cm) (n=202)	-0.021	0.14	0.087	0.143*	0.210**
MUAC (cm) (n=195)	0.152*	0.152	0.132	0.202**	0.148*
Ponderal Index (n=202)	0.153*	0.236*	0.220*	0.288**	0.250**
MUAC/Head circumference (n=195)	0.146*	0.174	0.098	0.174*	0.129
Weight-for-length	0.184*	0.181	0.19	0.250**	0.191**
Length-for-age	0.013	0.147	0.134	0.167*	0.208**
Weight-for-age	0.119	0.240*	0.213*	0.286**	0.277**
BMI-for-age	0.124	0.237*	0.203*	0.281**	0.265**
Head circumference-for-age	0.078	0.016	0.138	0.141*	0.098

Data presented Pearson coefficient r value
* indicate p<0.05 of significant correlation
**indicate p< 0.01 of significant correlation

According to the existing evidence low haemoglobin levels during pregnancy lead to reduced iron stores, causing infantile anaemia before the age of six months [20], reduce the oxygen supply to the foetus [21, 22] and can also adversely affect the immune system thereby increasing the host susceptibility to genital tract infections leading to poor pregnancy outcome [7]. Furthermore it is said that maternal anemia during pregnancy is associated with reduced birth weight, perinatal, maternal and infant mortality as well as higher risk of premature delivery [1]. However, in the study, mothers had a better haemoglobin level during pregnancy which greatly influenced the neonatal anthropometry especially the birth weight ($p<0.05$) and length ($p<0.01$) (Table 2). Also, the more the amount of time available for the growth of the foetus the better will be the growth and birth outcome. Hence, gestational age significantly influences the neonatal anthropometry and Z-scores ($p<0.01$) (Table 2).

Table 2: *Association of haemoglobin levels before delivery and gestational age with neonatal anthropometry and Z-scores (n=202)*

Neonatal Characteristics	Haemoglobin before delivery (g/dl)	Gestational age (weeks)
Birth weight (kg)	0.149*	0.570**
Length (cm)	0.227**	0.607**
Head circumference (cm)	-0.019	0.253**
Chest circumference (cm)	0.022	0.266**
MUAC (cm)	-0.053	0.206**
Weight-for-length	0.005	0.252**
Length-for-age	0.205**	0.600**
Weight-for-age	0.157*	0.605**
BMI-for-age	0.136	0.578**
Head circumference-for-age	-0.019	0.269**

Data presented Pearson coefficient r value
* indicate $p<0.05$ of significant correlation
**indicate $p< 0.01$ of significant correlation

Based on the 24-hour dietary recall taken prior to delivery, energy, macronutrients and selected micronutrient intake were calculated using values from Nutritive Value of Indian Foods (ICMR) [23] (Table 3). Though the mean maternal calcium intake was 75.1% of the recommended dietary allowance (RDA), it showed a positive significant correlation with neonatal length and Z-score for length-for-age and weight-for-age ($p<0.05$). This is because calcium enhances foetal growth, improves neonatal bone density & prolongs gestation thereby better neonatal anthropometry. Furthermore, the mean maternal phosphorus intake was 85.7% of the RDA; it was positively correlated with neonatal length.

Table 3: *Mean Maternal Nutrient Intake of the subjects (n=202)*

Nutrients	Mean ± SD
Energy (kcals)	1578.10±360.82
Carbohydrates (g)	192.08±41.89
Protein (g)	45.11±12.87
Fat (g)	42.11±12.22
Calcium (mg)	901.23±398.13
Phosphorus (mg)	1028.65±283.05
Iron (mg)	16.52±11.21
Dietary folate (mcg)	141.03±101.85
Vitamin B12 (mcg)	0.56±1.15
Vitamin C (mg)	68.15±45.14
Zinc (mg)	4.23±1.18

Moreover, vitamin C being an antioxidant, helps in the formation of collagen, connective tissue, cartilage, muscles and the lowest layer of skin thereby helps in the growth of the foetus and improves the pregnancy outcome. Thus, vitamin C was significantly positively correlated ($p<0.05$) with birth weight, neonatal length, Z-scores for weight-for-age, length-for-age, BMI-for-age. Furthermore, the positive significant association was observed between birth weight, neonatal length, length-for-age and weight-for-age with percentage RDA of calcium and vitamin C ($p<0.05$) whereas, BMI-for-age was significantly correlated with percentage RDA of vitamin C ($p<0.05$).

The neonatal length was also significantly positively correlated with percentage RDA of energy, and protein intake ($p<0.05$). However, due to low maternal protein and zinc intake as per the RDA, they were negatively correlated ($p<0.05$) with neonatal MUAC and MUAC/Head circumference.

4. Conclusion

Maternal height, pre-pregnancy weight, post-partum weight and BMI were associated with neonatal anthropometry. The strong association with maternal height and post-partum weight with newer neonatal anthropometric indices like MUAC/Head circumference and ponderal index indicates that maternal anthropometry greatly influences neonatal anthropometry. Moreover, birth outcome was strongly influenced by maternal diet particularly energy and the maternal nutrient intake of protein, calcium and vitamin C. Also, maternal haemoglobin concentration prior to delivery and gestational age influenced birth outcomes. Hence, in order to reduce the adverse pregnancy outcomes awareness should be created among pregnant women and women of child bearing age about the factors that can improve the nutritional status of the women prior to conception and during pregnancy.

Acknowledgement

The authors are grateful to the L.T.M.M.C. Institutional Ethics Committee for providing ethical consent to conduct the research. We also thank Dr. Neha Sanwalka for helping us in statistical analysis and interpretation.

References

[1] Agarwal, A. and Udipi, S.A., 2014: *Nutrition and Dietary Considerations at Different Life Stages*. Textbook of Human Nutrition. Jaypee Brothers Medical Publishers. 365-425.

[2] Kalanda, B. *Maternal Anthropometry and Weight Gain as Risk Factors for Poor Pregnancy Outcomes in a Rural Area of Southern Malawi.* Malawi Medical Journal. 2007. 19 (4) 149-153.

[3] Abu-Saad, K. and Fraser, D. *Maternal Nutrition and Birth Outcomes.* Epidemiologic Reviews. 2010. 32 (1) 5-25.

[4] Wallace, J.M., Bhattacharya, S., Campbell, D.M., & Horgan, G.W. *Inter-pregnancy Weight Change Impacts Placental Weight and is Associated with the Risk of Adverse Pregnancy Outcomes in the Second Pregnancy.* BMC Pregnancy Childbirth. 2014. 14 (1) 40.

[5] Kaur K. *Anaemia 'A Silent Killer' among Women in India: Present Scenario*. European Journal of Zoological Research. 2014. 3 (1) 32-36.

[6] National Nutrition Monitoring Bureau. *1979-2002: NNMB Reports*. National Institute of Nutrition, Hyderabad.

[7] Muthayya, S. *Maternal Nutrition and Low Birth Weight - What is Really Important?* Indian Journal of Medical Research. 2009. 130; 600-608.

[8] Shaikh, F., Zeeshan, F., Hakeem, R., Basit, A., Fawwad, A. and Hussain, A. *Maternal Dietary Intake and Anthropometric Measurements of Newborn at Birth*. The Open Diabetes Journal. 2014. 7 (1) 14-19.

[9] Chong, M.F., Chia, A., Colega, M., Tint, M., Aris, I.M., Chong, Y. and Lee, Y.S. *Maternal Protein Intake during Pregnancy is Not Associated with Offspring Birth Weight in a Multiethnic Asian Population*. Journal of Nutrition. 2015. 145 (6) 1303-1310.

[10] CDC/NCHS, 2009: National Health and Nutrition Examination Survey: Anthropometry Procedures Manual.

[11] WHO. WHO Anthro (version 3.2.2, January 2011) and macros. Retrieved from http://www.who.int/childgrowth/software/en/

[12] Sharma, R. *Online Interactive Calculator for Real-Time Update of the Kuppuswamy's Socioeconomic Status Scale*. Available at www.scaleupdate.weebly.com. Accessed on: 26/07/2015.

[13] WHO Expert Consultation. *Appropriate Body-Mass Index for Asian Populations and Its Implications for Policy and Intervention Strategies*. The Lancet. 2004. 363; 157-163.

[14] WHO, 2011: *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity* VMNIS (Vitamin and Mineral Information System) WHO/NMH/NHD/MNM/11.1.

[15] Koshy, J.P. *India has Highest Number of Stillbirths*. The Lancet Medical Journal. 2011.

[16] Sharma, M., 2013: *Nutritional Assessment of Children.* Pediatric Nutrition in Health and Disease. Jaypee Brothers Medical Publishers. 24-42.

[17] United Nations Children's Fund and World Health Organization, Low Birthweight: Country, Regional and Global Estimates. UNICEF, New York, 2004.

[18] Sajjadian, N., Shajari, H., Rahimi, F., Jahadi, R., and Barakat, M.G. *Anthropometric Measurements at Birth as Predictor of Low Birth Weight*. Health. 2011. 03 (12) 752-756.

[19] Elizabeth, K.E., 2009: *Nutrition and Child Development.* 3rd ed. Hyderabad: Paras Publishing. 43.

[20] Allen, L.H. *Anemia and Iron Deficiency: Effects on Pregnancy Outcome.* American Journal of Clinical Nutrition. 2000. 71; S1280-S1284.

[21] Durrani, A. and Rani, A. *Effect of Maternal Dietary Intake on the Weight of the Newborn in Aligarh City, India*. Nigerian Medical Journal. 2011. 52 (3) 177-181.

[22] Mishra, V., Thapa, S., Retherford, R.D., and Dai, X. *Effect of Iron Supplementation during Pregnancy on Birthweight: Evidence from Zimbabwe*. Food Nutrition Bull. 2005. 26 (4) 338-347.

[23] Gopalan, C., Rama Sastri, B.V., and Balasubramaniam, S.C., 2012: *Nutritive Value of Indian Foods*. National Institute of Nutrition, ICMR, Hyderabad.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 2, pp. 212-218, Article ID Med-317
ISSN 2348-5140

Cloud Publications

Research Article **Open Access**

Infant and Young Child-Feeding Practices, Indicators and Index, and Role of Socio-economic Status

Khusboo Karwa[1], Meena L. Godhia[2] and Alka R. Jadhav[3]

[1]SNDT Women's University, Juhu Tara Road, Mumbai, Maharashtra
[2]Sir Vithaldas Thackersey College of Home Science, Juhu Tara Road, Mumbai, Maharashtra
[3]L.T.M.M.C & G.H., Sion, Mumbai, Maharashtra

Publication Date: 4 July 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-317

Abstract The present cross-sectional descriptive study was conducted at the paediatric OPD of a government and a private clinic to assess the indicators of Infant-and Young Child-feeding (IYCF) practices and compare them among lower middle and upper middle socioeconomic groups. The study enrolled 200 mother-child pairs with children aged 18-36 months, classified using Modified Kuppuswamy scale into Lower Middle Socioeconomic Group (LMSEG) (score: 11-15) and Upper Middle Socioeconomic Group (UMSEG) (score:16-25). IYCF indicators formed the basis of the questionnaire used to interview the mothers. This calculated the children's dietary diversity score, meal frequency and IYCF index. Statistical analyses were performed using SPSS software (version 16.0) to determine the effect on socio-economic status on IYCF practices. P-value <0.05 was considered to be statistically significant. Only 52.5% (n=105) infant were exclusively breastfed for the first six months of life with significant difference between the two socioeconomic strata, with 64% (n=64) from LMSEG and 41% (n=41) from UMSEG (p=0.01). 67% (n=67) infants from LMSEG received breast milk till two years in contrast to 44% (n=44) infants from UMSEG (p=0.01). However, a higher frequency of food intake (p=0.03) and higher minimum dietary diversity score (p=0.012) was observed among UMSEG as compared to LMSEG. UMSEG mothers had greater prevalence of using bottles to feed their children as compared to LMSEG (n=51 vs n=27). Besides, it was found that educational qualification of the mothers had a positive impact on IYCF practices (p=0.015) but the mother's employment or the child's birth order had no significant effect. IYCF practices differed significantly among the two socioeconomic groups. LMSEG have better qualitative practices and UMSEG showed better quantitative factors. Health education as well as digital media may serve as important intervention programs to spread awareness to protect, promote and sustain optimal IYCF practices in Indian context.

Keywords *Breastfeeding; Complementary Feeding; Dietary Diversity; Meal Frequency*

1. Introduction

An appropriate diet is critical in the growth and development of children, especially in the first two years of life [1]. The World Health Organisation (WHO) and United Nations Children Fund (UNICEF) have articulated a global strategy for infant- and young child-feeding which recommends exclusive breastfeeding for the first six months of life with early initiation and continuation of breastfeeding for two years or more together with nutritionally-adequate, safe, age-appropriate complementary feeding starting at six months [2]. These optimal infant- and young child-feeding (IYCF) practices are crucial for nutritional status, growth, development, health, and ultimately the survival of infants and young children [3, 4].

There have been various indicators used to assess IYCF practices, the major ones being early initiation of breastfeeding, exclusive breastfeeding under 6 months, continued breastfeeding at 1 year, introduction of solid, semi-solid or soft foods, minimum dietary diversity, minimum meal frequency, minimum acceptable diet, bottle feeding and consumption of iron-rich or iron-fortified foods [5]. Some of these indicators construct the Infant and Young Child Feeding Index (IYCI), which is an age-specific scoring system that gives points for positive practices such as breastfeeding, avoiding use of bottle for feeding, meal frequency and dietary diversity [6]. Therefore, the study aimed to assess and quantify the IYCF practices for children enrolled in the study.

IYCF practices have been significantly associated with standard of living index (SLI) and per-capita income, indicating poor CF practices in low socioeconomic group compared to high socioeconomic group [7]. Therefore, the study also aimed to determine the association of IYCF practices with socioeconomic status of the mothers by comparing the indicators among Upper Middle and Lower Middle socio-economic groups classified as per the Kuppuswamy Scale [8].

2. Methodology

A cross-sectional descriptive study was conducted in two hospitals- Lokmanya Tilak Municipal Government Hospital (L.T.M.G.H.), Sion (a government hospital) and Kashyap Nursing Home, Dadar (a private paediatric clinic) among children aged 18-36 months during November-December 2015. With the help of Kuppuswamy scale, mother-children pairs were classified into Lower Middle Socioeconomic Group (LMSEG) (score: 11-15) and Upper Middle Socioeconomic Group (UMSEG) (score: 16-25) of 100 pairs each using purposive sampling.

Institutional Ethics clearance was taken before the commencement of the study and prior permission was obtained from Internal Ethics Committee of Lokmanya Tilak Municipal Medical College to interview the mothers (21.08.2015). Written informed consent were also taken from the mothers after briefing them the objectives of the study in their own language and only those willing to participate were interviewed for the data collection.

The interviews were conducted with the help of a questionnaire based on the 10 key indicators of IYCF [5]. Retrospective data was taken on breastfeeding practices and introduction of complementary food while current information was taken about the child's diet which included the food items and the number of times they were consumed. The food items selected from various groups for the child in the last 24 hours calculated the dietary diversity score of the child and the number of food groups given in the entire week to the child added up to the "weekly food group frequency." These scores were added along with breastfeeding and bottle feeding scores to evaluate IYCF index (IYCI) as shown in Table 1 [6]. The maximum score that could be obtained from the index was 9. Therefore, scores lesser than 9 were compared to selected mother-child factors like mother's education, employment and child's birth order to study the association between them (if any).

Analyses were performed using SPSS software for Windows (version 16.0, 2007, SPSS Inc, Chicago, IL). Data were presented as Mean ± SD or frequency (percentage). The frequency distributions were tabulated according to socioeconomic groups and were compared using cross tabulations and chi-square test. Independent sample T Test was used to analyse the difference in various parameters according to income groups. Correlation of IYCF with various parameters like education, working status and birth order were determined using Spearman Correlation & Kendall Tau b Correlation respectively. P-value <0.05 was considered to be statistically significant.

Table 1: *Variables and scoring system used to construct IYCF index*

Variables	IYCF Score 12-24 months
Breastfeeding	Yes-1 No- 0
Bottle feeding	Yes- 0 No- 1
Dietary diversity score (24-h recall)	0-2 food group- 0 3 food group- 1 ≥4 food groups- 2
Food group frequency score (past 7d-food frequency questionnaire)	0-3 food groups- 0 4 food groups- 1 ≥5 food groups-2
Feeding frequency	0-2 times- 0 3 times- 1 4 times- 2 ≥5 times- 3

Source: Lohia and Udipi, 2014

3. Results and Discussion

The mean age of the mothers enrolled for the study was 29.3±4.9 years. Most of them were literate, 72% being graduate or postgraduate. This is similar to the Mumbai Census Report [9] which shows female literacy as 86.45%. However, educational qualification was observed to be significantly associated with socioeconomic status as 62% (n=62) mothers from UMSEG had graduation or post-graduation certificates in contrast to 16% (n=16) of LMSEG who were found to be illiterate. Similarly, greater number of mothers from UMSEG (n=32) were involved in full time employment as compared to 88% mothers from LMSEG who reported staying back at home for looking after their family.

The average age of children enrolled in the study was 27.8±6.71 months. More than half of them (n=129) were males, depicting a high child sex ratio, similar to the Mumbai Census Report [9] which reported child sex ratio to be 914. A majority of children (n=109) were of first birth order.

Almost all the mothers (n=200), irrespective of the socio-economic status, had visited antenatal clinics for pre-natal check-ups. However, their frequency of visits ranged from less than 3 to more than 9 times during pregnancy. However, most of them (n=160) had check-ups more than 6 times, as recommended to be ideal [10].

It was observed that with respect to the IYCF practices, only 31.5% (n=63) mothers had started with initial breastfeeding within the first half an hour of delivery. However, no significant difference was found with respect to the socio-economic status (p=0.101). This was very low as compared to another study in Mumbai which reported 82.3% rate of initial breastfeeding [11]. Although some mothers (n=9) preferred breast bank milk during this time, but others went for formula milk as a substitute to initial breastfeeding, in the study.

Table 2: *Frequency of EBF Practice up to 6 months in LMSEG and UMSEG (n=200)*

Characteristic	LMSEG (n=100)	UMSEG (n=100)	χ2	P value
Exclusive Breastfeeding	64 (64)	41 (41)	10.607	0.001

*Figures in parenthesis indicate percentages

In the study, only 52.5% (n=105) mothers followed exclusive breastfeeding for six months of age. This was higher in comparison to a study in urban slums of Kolkata which observed EBF being practised at the rate of 33% [12] and similar to a study in south coastal India where 57.9% of mothers had exclusively breast fed their child up to six months [13]. More mothers from LMSEG followed EBF as compared to UMSEG (Table 2). Mothers when asked for reasons of not breastfeeding exclusively gave reasons like the child was hungry (n=60) or other things would make the child healthy (n=76).

Only 7.5% of the mothers enrolled in the study continued breastfeeding till one year, 40.5% continued till two years of age and 15% continued for longer which shows a lower practice than a study carried out in West Bengal which reported 55.7% rate of continued breastfeeding (12-15 months) [14]. This figure also differed significantly between the two groups with LMSEG continuing for longer periods as compared to UMSEG (p=0.01).

The mothers participating in the study reported different ages for the introduction of complementary foods. Out of 200 mothers, most of them (n=154) started at 6-12 months, i.e. the recommended age, however, there were cases for early as well as late initiation of complementary foods too. 0.5% of mothers (n=1) started with complementary feeding at 0-1 months, 4% (n=8) started at 1-3 months and 15% (n=30) at 3-6 months. This was better than the NFHS-4 Maharashtra report which stated timely introduction of complementary food in only 43.3% children [15].

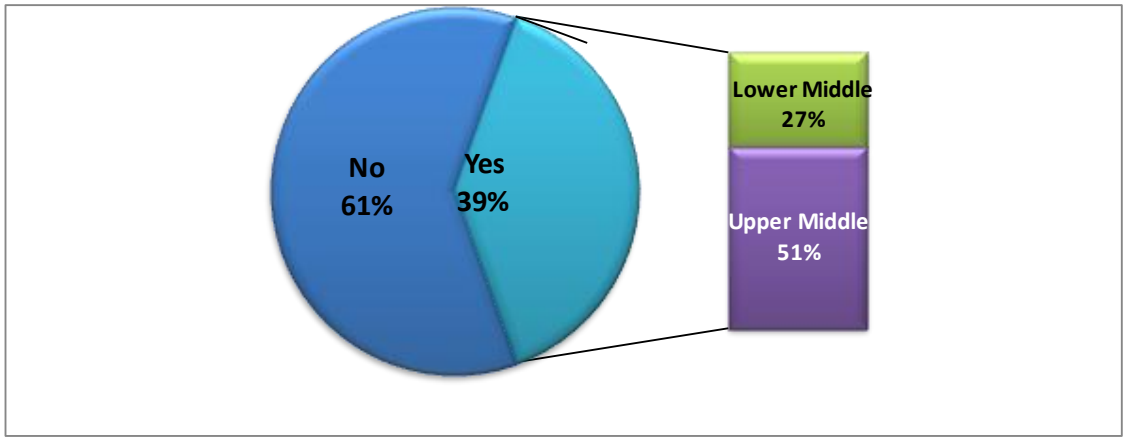


Figure 1: *Frequency of Bottle Feeding among LMSEG and UMSEG (n=200)*

Bottle feeding was found to be a common practice in the present study with about 39% (n=78) of mothers following it, which is much greater than an earlier study which stated bottle feeding to be practised by only 22% children in India [12]. This prevalence of bottle feeding was also observed to be dependent on their socioeconomic status, with 27% cases (n=27) from LMSEG versus 51% (n=51) from UMSEG, showing a significant difference between the groups (p value= 0.001) (Figure 1).

Table 3: *Age-wise Dietary Diversity Score, Weekly Food Group Score and IYCF Score of LMSEG and UMSEG (n=200)*

Characteristic	Age Groups	LMSEG (n=100)	UMSEG (n=100)	p-value
Dietary Diversity Score	18-24 months	4.37±0.99	4.84±1.14	0.012
	24-30 months	4.21±1.16	4.81±1.01	
	30-36 months	4.71±1.16	4.78±0.76	
Weekly Food Group Frequency Score	18-24 months	2.71±0.46	2.84±0.37	0.04
	24-30 months	2.92±0.28	2.78±0.43	
	30-36 months	2.76±0.49	2.91±0.28	
IYCF Score	18-24 months	6.03±1.73	6.34±1.38	0.06

Dietary Diversity Score, which is the sum total of number of food groups consumed by the child the previous day, out of (i) Milk and Milk Products; (ii) Eggs; (iii) Flesh Foods (Meat, Fish, etc.); (iv) Vitamin-A Rich Fruits and Vegetables; (v) Other Fruits and Vegetables; (vi) Grains; and (vii) Nuts and Legumes [5], was found to have a mean of 4.64±0.99, as compared to ideal score of 5-6. There was a significant difference in DDS between the 2 groups, with UMSEG having better scores due to better availability of resources, as seen in Table 3. This was in line to another study which reported that minimum dietary diversity was achieved by 54.75% in urban population and only 19.47% in rural areas [16]. Weekly food group frequency score also showed similar findings where UMSEG had an upper hand (Table 3).

According to existing studies, about 39.3% of mothers give three or more feeds per day to their children [1]. However, in the present study, it was observed that 71% (n=142) children were fed more than 3 times a day. This data was significantly different for the two socioeconomic groups with p=0.03, demonstrating that UMSEG families had more feeding frequency as compared to the LMSEG families.

Mothers enrolled in the study were enquired when they give meals to the child and when do they stop it. Most of the mothers gave food at some scheduled time (n=168) and stopped feeding when the baby refrained to eat (n=116). However, there were cases of force eating also seen in the interviews where mothers said that they fed their children till the complete food was fed (n=76). This finding was found to be similar in both the socioeconomic groups (p=0.312). Most parents also reported feeding to be accompanied by activities like watching television, playing toys or using phones, which when removed, affected the intake. However, this habit was more seen in UMSEG as compared LMSEG (p=0.000). Similarly, 91% mothers (n=182) gave outside food to their children, which consisted mostly of biscuits, wafers, chocolates, bread, farsans, noodles, paw bhaji, cake, etc.

The mothers enrolled in the study were enquired if they gave any iron, calcium or multivitamin supplements to their children in lines to the WHO recommendations [2]. However, the trends of supplementation was seen to be moderate in UMSEG and low among the LMSEG (p=0.000).

Based on these indicators, IYCF index (IYCI) was calculated for children below 24 months (as per table 1), which although did not show any significant difference between the two groups (Table 3) but had values greater than a previous study in Mumbai [6] which reported mean IYCI value 5.9 ± 1.9, showing better practices. This index was also found to be significantly associated with educational qualification of the mothers (p=0.015), however did not have any significant relation with the working status of the mothers (p=0.7) or the birth order of the child (p=0.07).

4. Conclusion

The study reveals areas of both similarities and few distinct differences in IYCF practices in relation to the indicators between the two socioeconomic groups. LMSEG had better practices with respect to

early initiation of breastfeeding, exclusive breastfeeding till 6 months and continued breastfeeding till 2 years, but UMSEG had sub-optimal practices. On the other hand, UMSEG had better dietary diversity and frequency than the LMSEG, but lagged behind in the avoidance of bottle feeding. Therefore, unlike the earlier studies which indicated poor complementary feeding practices in low socioeconomic group compared to high socioeconomic group [7], the present study reports sub-optimal IYCF practices for both the lower middle as well as the upper middle socioeconomic groups.

The study also shows a positive correlation between education and IYCF practices, and since the coverage of digital media is increasing, health education as well as digital media may serve as important intervention programs to spread awareness to protect, promote and sustain optimal IYCF practices in both the socioeconomic groups.

Acknowledgement

The authors are grateful to the L.T.M.M.C. Institutional Ethics Committee for providing ethical consent to conduct the research. We also thank Dr. M.R. Lokeshwar who permitted us for data collection in his private clinic. We thank Dr. Neha Sanwalka for helping us in statistical analysis and interpretation.

References

[1] Aggarwal, A., Verma, S., Faridi, M., and Dayachand. *Complementary Feeding-Reasons for Inappropriateness in Timing, Quantity and Consistency.* Indian Journal of Pediatrics. 2008. 75; 49-53.

[2] World Health Organisation, 2003: *Global Strategy for Infant and Young Child Feeding.* Accessed on: 03/01/2016. http://www.who.int/nutrition/publications/infantfeeding/infant_feeding

[3] Saha, K.K., Frongillo, E.A., Alam, D.S., Ariffen, S.E., Persson, L.A., and Rasmussen, K.M. *Appropriate Infant Feeding Practices Result in Better Growth of Infants and Young Children in Rural Bangladesh.* American Journal Clinical Nutrition. 2008. 87; 1852-9.

[4] Bhutta, Z.A., Ahmed, T., Black, R.E., Cousens, S., Dewey, K., and Giugliani, E. What Works? *Interventions for Maternal and Child Undernutrition and Survival*. Lancet. 2008. 371; 417-40.

[5] World Health Organisation, 2010: *Indicators for Assessing Infant and Young Child Feeding Practices Part 2.* Accessed on: 06/10/15. http://www.unicef.org.

[6] Lohia, N. and Udipi, S.A. *Infant and Child Feeding Index Reflects Feeding Practices, Nutritional Status of Urban Slum Children.* BMC Pediatrics. 2014. 14; 290.

[7] Garg, A. and Chadha, R. *Index for Measuring the Quality of Complementary Feeding Practices in Rural India.* Journal of Health, Population and Nutrition. 2009. 27 (6) 763-771.

[8] Sharma, R. *Online Interactive Calculator for Real-Time Update of the Kuppuswamy's Socioeconomic Status Scale.* Accessed on: 26/07/2015. www.scaleupdate.weebly.com

[9] Census of India, 2011: *Mumbai City District Population Census 2010*. www.censusindia.gov.in

[10] Indian Academy of Pediatrics. *Infant and Young Child Feeding Guidelines: 2010.* Indian Pediatrics. 2010. 47; 995-1004.

[11] Parekh, C., Bavdekar, S.B., and Shaharao, V. *Study of Infant Feeding Practices: Factors Associated With Faulty Feeding.* Journal of Tropical Pediatrics. 2004. 50; 306-308.

[12] Roy, S., Dasgupta, A., and Pal, B. *Feeding Practices of Children in an Urban Slum of Kolkata*. Indian Journal of Community Medicine. 2009. 34; 362-363.

[13] Kumar, N., Unnikrishnan, B., Rekha, T., Mithra, P., Kulkarni, V., Papanna, M.K., Holla, R., and Jain, A. *Infant Feeding And Rearing Practices Adapted by Mothers in Coastal South India.* International Journal of Collaborative Research on Internal Medicine & Public Health. 2011. 4 (12) 1988-1999.

[14] Sinhababu, A., Mukhopadhyay, D.K., Panja, T.K., Asit, B.S., Nirmal, K.M., and Akhil, B.B. *Infant- and Young Child-feeding Practices in Bankura District, West Bengal, India.* Journal of Health, Population and Nutrition. 2010. 28 (3) 294-299.

[15] International Institute for Population Sciences, 2016: *National Family Health Survey-4 (NFHS-4), 2015-2016, Mumbai.* International Institute for Population Sciences. 267-86.

[16] Ashwini, S., Katti, S.M., and Mallapur, M.D. *Comparison of Complementary Feeding Practices among Urban and Rural Mothers–A Cross Sectional Study.* Al Ameen Journal of Medical Science. 2014. 7 (4) 257-264.

Cloud Publications
International Journal of Advanced Nutritional and Health Science
2016, Volume 4, Issue 2, pp. 203-211, Article ID Med-318
ISSN 2348-5140

Cloud Publications

Review Article | Open Access

Sirtuins and Aging: is there a Role for Resveratrol?

Caterina Carollo[1], Alberto Firenze[2], and Gregorio Caimi[1]

[1]Department of Internal and Specialistic Medicine, University of Palermo, Italy
[2]Department of Hygiene and Preventive Medicine, University of Palermo, Italy

Publication Date: 30 June 2016

Article Link: http://medical.cloud-journals.com/index.php/IJANHS/article/view/Med-318

Abstract Prolonged human life duration is consequently associated with a higher incidence of chronic diseases. Aging is a very complex process in which genetic, environmental and cellular pathways are involved. Along with aging, longevity has been linked with Sirtuins. Sirtuin enzymes are a family of highly conserved protein deacetylases that have been linked with calorie restriction and aging by modulating energy metabolism, genomic stability and stress resistance. Aim of this brief review is to describe Sirtuins' influence on the conditions that worsen the physiological aging. We will also report the beneficial effects of the polyphenol resveratrol on these molecules and the possible therapeutical perspectives.
Keywords *Resveratrol; Aging; Sirtuins*

1. Introduction

The growing interest of scientists and researchers about aging may be also explained if we consider that the prolonged human life duration is consequently associated with increasing costs of public health due to the higher incidence of chronic diseases like diabetes, kidney failure, hypertension, neurodegenerative diseases, osteoporosis and cancer. From this point of view the future health challenge is to provide people with a better aging without excessive health costs.

At the date, if you type on PubMed screen the query "aging", more than 330000 results will be shown. Modern societies, expecially the western ones, are aging due to the prolonged life span, the reduced birth rates and the technological and scientific advances.

Different hypotheses have been postulated to explain aging and its underlying mechanisms. We actually know that it is a very complex process in which genetic, environmental and cellular pathways are involved. Among these, oxidative stress was widely investigated for its tight implications in vascular aging that plays a key role in this process. In fact, cardiovascular and cerebrovascular events are significantly more frequent in aging adults [1].

Along with aging, longevity was largely focused. Caloric restriction (CR) is a nutritional technique used in different models as a healthy method to prolong lifespan [2] through a reduction of calorie intake by 30-40% but avoiding malnutrition. This method's effects could be mediated by Sirtuins.

2. Sirtuins

Sirtuin enzymes are a family of highly conserved protein deacetylases that depend on nicotinamide adenine dinucleotide (NAD+) for their activity. Sirtuins catalyze the removal of acetyl groups from lysine residues. They may promote different post translational modifications in a wide range of proteins so they are actually defined as deacylases [3].

Seven Sirtuins have been actually identified in mammals, listed from 1 to 7. Each of them exhibits a catalytic domain present in all Sirtuins whilst different N- and C- ends give to these proteins their characteristical biological properties.

They have distinct subcellular localizations: SIRT1, 6 and 7 are nuclear; SIRT2 is cytosolic whilst SIRT3, 4 and 5 are primarily located in mitochondria [4]. Among these proteins, only SIRT4 has no a known deacetylation substrate [5].

Sirtuins were originally investigated in yeast and they have been linked with calorie restriction and aging by modulating energy metabolism, genomic stability and stress resistance [6]. SIRT4 and SIRT6 also exhibit ADP-ribosyl-transferase activity [7].

For each of the formerly mentioned chronic diseases, a direct or indirect role of one or more Sirtuins has been demonstrated.

Aim of this brief review is to describe Sirtuins' influence on the conditions that worsen the physiological aging. We will also report the beneficial effects of the polyphenol resveratrol on these molecules and the possible therapeutical perspectives.

3. Sirtuins, Glucose Metabolism and Kidney Disease

In animal models, SIRT1 influences glucose-dependent insulin production in pancreatic beta-cells [8, 9] whose proliferation is negatively regulated by the same Sirtuin. Mitochondrial SIRT4 inhibits insulin secretion in response to aminoacids. In insulinoma cells an overexpression of SIRT4 has been demonstrated, thus leading to a decreased insulin synthesis as a reaction to blood glucose concentration [3].

In the liver SIRT1 promotes gluconeogenesis and blunts glycolysis via deacetylation of PGC-1α (PPAR-γ coactivator-1α) [10].

The same deacetylase enhances insulin sensitivity by modulating insulin signalling [11], inhibits fat storage and stimulates fatty acids release in white adipose tissue [12].

Transgenic overexpression of SIRT1 not only prevents diabetes in animal models, but also dulls diabetes that occurs during normal aging [13, 14]. Similarly, chemical activation of SIRT1 has antidiabetic and other beneficial effects. SRT1720 is one of potential SIRT1 activators being examined in clinical trials.

Recently also SIRT6 has been proved to be involved in metabolic control: in fact, its absence is likely related to an enhancement of insulin signalling with consequent hypoglycemia [15]. With its histone

deacetylase activity, Sirt6 regulates the glucose levels through the blockade of different glycolytic genes [16].

This is more interesting if we consider the high incidence of diabetes and its complications among which diabetic nephropathy plays a leading role as a major determinant of morbidity and mortality [17]. A protective effect of Sirtuins in kidney is widely demonstrated by different mechanisms: they blunt hypoxia, reduce fibrosis, inhibit apoptosis and inflammation, reduce autophagy and modulate blood pressure [18]. For what concerns the last item, SIRT1 acts through the regulation of vascular tone and the handling of renal sodium in the collecting duct [19, 20].

Moreover, SIRT3 attenuates lipotoxicity and ROS-related inflammation in proximal tubular cells, thus underlining the protective role of these molecules against oxidative stress in its multiple tissue expression [21]. SIRT3 is also able to regulate fatty acids metabolism and promotes lipid catabolism by deacetylating various mitochondrial proteins [22].

4. Sirtuins, DNA Stability, Oxidative Stress and Cancer

Aging is accelerated by DNA damage. Sirtuins rescue this damage. SIRT1, one of the most investigated in this field, can deacetylate several factors involved in DNA reparation and genomic stability [23].

Also SIRT6 plays a role in genome stabilization, gene expression and DNA repair. Its deacetylation activity reduces chromosomal instability which is a fundamental feature of human cancer cells [24]. On the other hand, SIRT6 allows repair factors to reach chromatin thus minimizing DNA damage [25]. SIRT6 chromosomal locus is frequently broken in human acute myeloid leukemia [26]. In addition, the same deacetylase influences transcriptional activity through the inhibition of NF-κB target genes, especially those associated with aging [27]. It is known that NF-κB is a central factor for aging, inflammation, immunity and cell proliferation; for many authors this is the link between all these conditions and aging.

Oxidative stress, along with other factors, is involved in chronic obstructive pulmonary disease (COPD) which is associated, on its turn, with the premature lung senescence. SIRT6 is significantly decreased in lung of patients with severe COPD [28]. In this clinical condition, NF-κB regulates the expression of genes for proinflammatory molecules [29].

We know that ROS may damage nucleic acids, thus eliciting the onset of cancer (whose incidence grows in elder). In animal models the lack of SIRT3 is associated with greater genomic instability and increased sensitivity to oncogenic transformation if compared with controls [30].

The protective role of SIRT3 is also suggested by the observation that several human neoplastic tissues exhibit reduced SIRT3 levels when compared with healthy tissues [30]. Overexpression of the same Sirtuin suppresses cancer proliferation by inhibiting the activity of Hypoxia Inducible Factor-1-α (HIF-1-α) [31, 32]. It is interesting to underline that this factor is activated and stabilized by ROS thus strengthening the role of oxidative stress.

From the study of different human tumours, SIRT3 emerges as a powerful, cell-specific and very complex tumor suppressor [33].

As a further demonstration of its antioxidant activity, in mice SIRT3 delayed the age-related hearing loss by enhancing mitochondrial antioxidant defenses [34].

As mentioned above, SIRT1 is the most investigated about its possible relationship with cancer, but only SIRT7 has been seriously linked with different cancer types. In fact, it has been found to be overexpressed in tumours originating from thyroid, breast, bladder, liver and colon, thus leading to the working hypothesis that it could be a potential oncogene [35].

5. Sirtuins and Neurodegenerative Diseases

In light of the former considerations, it's easy to understand that Sirtuin enzymes are potential therapeutic targets in several human diseases including cancer, diabetes, inflammatory disorders and neurodegenerative diseases.

In fact it is reasonable to hypothesize that a selective modulation of a single Sirtuin could beneficially affect different clinical conditions. For example, SIRT6 is an attractive target for the prevention and treatment of inflammatory, cardiovascular and pulmonary diseases [5].

Modulation of Sirtuin activity has been shown to impact the course of several aggregate-forming neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and spinal and bulbar muscular atrophy.

Lewy bodies are frequently found in many of these disorders and consist of protein inclusions whose major constituent is α-Synuclein (α-Syn), a presynaptic neuronal protein that besides the brain is widely expressed in other tissues. This protein has been pointed out to be a target of cellular protein quality control whose activity is impaired by aging thus causing neurodegeneration. On the other hand, the same protein could influence the protein quality control system. Sirtuins can influence the progression of neurodegenerative disorders by modulating transcription factor activity and directly deacetylating proteotoxic species, expecially the ones involved in autophagy degradation pathway [36].

6. Sirtuins and Resveratrol

Further research is needed but on our opinion Sirtuins could become an interesting and powerful target to treat many human diseases.

At this regard we would like to underline the potential role of resveratrol, a natural Phytoalexin compound, which was extensively studied for its antioxidant and radicals' scavenging properties. More recently, this polyphenol has been shown to interact also with Sirtuins and from these complex (direct and indirect) interactions a putative therapeutical role for many of the above mentioned diseases could arise.

It is a common observation that a healthy diets associated with a delayed onset of stroke and neurological diseases. Regular consumption of fruit, vegetables and fish reduces the risk of cognitive decline in the elderly population. Old people who regularly drink red wine exhibit a reduced (up to 50%) risk of developing dementia [37]. These neuroprotective effects of resveratrol could be partially mediated by SIRT1 activation and scavenging activity. Moreover Resveratrol could influence amyloid generation and clearance [38]. However, Resveratrol's poor absorption and availability (less than 1%) in human it is well known, besides its fast metabolism [39]. Biotechnology is actually working to develop different formulations (e.g. nanocapsules) that could provide a higher dose of resveratrol without eliciting its side effects (frequently nausea and diarrhea) [40]; daily doses less than 1g are generally considered safe. Resveratrol is able in lowering blood glucose levels via an increase in GLP-1 production in mice. More recently, in type 2 diabetic subjects supplemented with a daily resveratrol dose at 10 mg, Brasnyo and coworkers [41] observed an improvement of insulin sensitivity, a reduction in oxidative stress and in postprandial glucose spike. In subjects with metabolic

syndrome treated with resveratrol for six months improved flow-mediated dilation was observed and this beneficial effect disappeared when resveratrol treatment was stopped [42]. In diabetics resveratrol supplement for three months was associated with a better fasting blood glucose, blood pressure, triglycerides and LDL cholesterol levels if compared with subjects with a standard antidiabetic treatment [43].

For a better understanding of resveratrol effects in humans, we have to say that trials are not really comparable for sample size, aims, methods and resveratrol dose but we can say that resveratrol is a powerful SIRT1 activator, may be directly. This activation, on its turn, induces deacetylation and suppresses the activity of the Foxo1 transcription factor which is involved in insulin signaling due to its inhibitory role in glucose uptake and utilization in skeletal muscle [44]. This is interesting if we consider that aging and diabetic subjects usually exhibit a significant reduction in skeletal muscle mass and strength thus worsening the sedentary way of life that is typically observed in these people and that contributes to make these subjects more prone to obesity and its complications.

The same SIRT1/Foxo1 axis has been recently postulated to be involved in cardiac aging. In fact heart performance decreases with age and Foxo1-related apoptotic signalling increases. A long term treatment with resveratrol improves cardiac function in senescent mice also by reducing age-induced deposition of collagen fibers [45].

Besides the well-known antioxidant effects, resveratrol could be a promising natural approach to many of the age-related disorders.

In our previous papers we suggested that a moderate and regular wine consumption, in the wide frame of Mediterranean diet, could be useful to prevent and treat cardiovascular, metabolic and renal disorders [46-49].

Recently, Russo and coworkers [51] proposed Sirtuins-resveratrol axis as a model to deeply investigate the beneficial effects of the Mediterranean diet: in fact this food regimen positively influences microbioma composition and stem cell function by means of the activity of many useful components, resveratrol included.

Many efforts are needed to better understand these molecular mechanisms but it is reasonable to hypothesize that a similar advice could be suggested to delay the onset of aging and its complications.

7. Conclusion

Many efforts are needed to better understand these molecular mechanisms but it is reasonable to hypothesize that a similar advice could be suggested to delay the onset of aging and its complications. From this point of view, dietary and lifestyle interventions could be useful instead of drugs thus significantly saving public resources.

References

[1] Camici, G.G., Savarese, G., Akhmedov, A., and Lüscher, T.F. Molecular *Mechanism of Endothelial and Vascular Aging: Implications for Cardiovascular Dise*ase. Eur Heart J. 2015. 36 (48) 3392-403.

[2] Minor, R.K., Allard, J.S., Younts, C.M., Ward, T.M., and de Cabo, R. Dietary Interventions to Extend Life Span and Health Span Based on Calorie Restriction. J Gerontol A Biol Sci Med Sci. 2010. 65 (7) 695-703.

[3] Verdin, E., Hirschey, M.D., Finley, L.W., and Haigis, M.C. *Sirtuin Regulation of Mitochondria: Energy Production, Apoptosis, and Signaling.* Trends Biochem Sci. 2010. 35 (12) 669-75.

[4] Kiran, S., Anwar, T., Kiran, M., and Ramakrishna, G. *Sirtuin 7 in Cell Proliferation, Stress and Disease: Rise of the Seventh Sirtuin!* Cell Signal. 2015. 27 (3) 673-82.

[5] Beauharnois, J.M., Bolívar, B.E., and Welch, J.T. *Sirtuin 6: A Review of Biological Effects and Potential Therapeutic Properties*. Mol Biosyst. 2013. 9 (7) 1789-806.

[6] Guarente, L. *Sir2 Links Chromatin Silencing, Metabolism, and Aging*. Genes Dev. 2000. 14 (9) 1021-6.

[7] Lavu, S., Boss, O., Elliott, P.J., and Lambert, P.D. *Sirtuins-Novel Therapeutic Targets To Treat Age-Associated Diseases*. Nat Rev Drug Discov. 2008. 7 (10) 841-53.

[8] Moynihan, K.A., Grimm, A.A., Plueger, M.M., Bernal-Mizrachi, E., Ford, E., Cras-Méneur, C., et al. *Increased Dosage of Mammalian Sir2 in Pancreatic Beta Cells Enhances Glucose-Stimulated Insulin Secretion in Mice*. Cell Metab. 2005. 2 (2) 105-17.

[9] Bordone, L., Motta, M.C., Picard, F., Robinson, A., Jhala, U.S., Apfeld J., et al. *Sirt1 Regulates Insulin Secretion by Repressing UCP2 in Pancreatic Beta Cells.* PLoS Biol. 2006. 4 (2) e31.

[10] Morris, B.J. *Seven Sirtuins for Seven Deadly Diseases*. Free Radic Biol Med. 2013. 56; 133-71.

[11] Rodgers, J.T., Lerin. C., Haas, W., Gygi, S.P., Spiegelman, B.M., and Puigserver, P. *Nutrient Control of Glucose Homeostasis through a Complex of PGC-1alpha and SIRT1.* Nature. 2005. 434 (7029) 113-8.

[12] Liang, F., Kume, S., and Koya, D. *Sirt1 and Insulin Resistance*. Nat Rev Endocrinol. 2009. 5 (7) 367-73.

[13] Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., et al. *Sirt1 Promotes Fat Mobilization in White Adipocytes by Repressing PPAR-gamma*. Nature. 2004. 429 (6993) 771-6.

[14] Bordone, L., Cohen, D., Robinson, A., Motta, M.C., van Veen, E., Czopik, A., et al. *SIRT1 Transgenic Mice Show Phenotypes Resembling Calorie Restriction.* Aging Cell. 2007. 6 (6) 759-67.

[15] Banks, A.S., Kon, N., Knight, C., Matsumoto, M., Gutiérrez-Juárez, R., Rossetti, L., et al. *SirT1 Gain of Function Increases Energy Efficiency and Prevents Diabetes in Mice.* Cell Metab. 2008. 8 (4) 333-41.

[16] Sanchez-Fidalgo, S., Villegas, I., Sanchez-Hidalgo M., and de la Lastra, C.A. *Sirtuin Modulators: Mechanisms and Potential Clinical Implications.* Curr Med Chem. 2012. 19 (15) 2414-41.

[17] Zhong, L, D'Urso, A., Toiber, D., Sebastian, C., Henry, R.E., Vadysirisack, D.D., et al. *The Histone Deacetylase Sirt6 Regulates Glucose Homeostasis via Hif1alpha*. Cell. 2010. 140 (2) 280-93.

[18] Tancredi, M., Rosengren, A., Svensson, A.M., Kosiborod, M, Pivodic, A, Gudbjörnsdottir, S, et al. *Excess Mortality among Persons with Type 2 Diabetes*. N Engl J Med. 2015. 373 (18) 1720-32.

[19] Kitada, M., Kume, S., Takeda-Watanabe, A., Kanasaki, K., and Koya, D. *Sirtuins and Renal Diseases: Relationship with Aging and Diabetic Nephropathy.* Clin Sci (Lond). 2013. 124 (3) 153-64.

[20] Haase, V.H. *Hypoxia-Inducible Factors in the Kidney.* Am J Physiol Renal Physiol. 2006. 291 (2) F271-81.

[21] Mattagajasingh, I., Kim, C.S., Naqvi, A., Yamamori, A., Hoffman, T.A., Junget, S.B. et al. *SIRT1 Promotes Endothelium-Dependent Vascular Relaxation by Activating Endothelial Nitric Oxide Synthase.* Procl Nat Acad Sci USA. 2007. 104 (37) 14855-60.

[22] Koyama, T., Kume, S., Koya, D., Araki, S., Isshiki, K., Chin-Kanasaki M., et al. *SIRT3 Attenuates Palmitate-Induced ROS Production and Inflammation in Proximal Tubular Cells.* Free Radic Biol Med. 2011. 51 (6) 1258-67.

[23] Yuan, Z., Zhang, X., Sengupta, N., Lane, W.S. and Seto, E. *SIRT1 Regulates The Function of the Nijmegen Breakage Syndrome Protein*. Mol Cell. 2007. 27 (1) 149-62.

[24] Negrini, S., Gorgoulis, V.G., and Halazonetis, T.D. *Genomic Instability-An Evolving Hallmark of Cance*r. Nat Rev Mol Cell Biol. 2010. 11 (3) 220-8.

[25] Wilson, D.M. and Bohr, V.A. *The Mechanics of Base Excision Repair, and its Relationship to Aging and Disease.* DNA Repair (Amst). 2007. 6 (4) 544-59.

[26] Mahlknecht, U. and Voelter-Mahlknecht, S. *Fluorescence in Situ Hybridization and Chromosomal Organization of the Sirtuin 4 Gene (Sirt4) in the Mouse.* Biochem Biophys Res Commun. 2009. 382 (4) 685-90.

[27] Kawahara, T.L., Rapicavoli, N.A., Wu, A.R., Qu, K., Quake, S.R. and Chang, H.Y. *Dynamic Chromatin Localization of Sirt6 Shapes Stress- and Aging-Related Transcriptional Networks*. PLoS Genetics. 2011. 7 (6) e1002153.

[28] Natoli, G. *When Sirtuins and NF-kappaB Collid*e. Cell. 2009. 136 (1) 19-21.

[29] Christman, J.W., Sadikot, R.T. and Blackwell, T.S. *The Role of Nuclear Factor-kappa B in Pulmonary Diseases.* Chest. 2000. 117 (5) 1482-7.

[30] Kim, H.S., Patel, K., Muldoon-Jacobs, K., Bisht, K.S., Aykin-Burns N., Pennington J.D., et al. *SIRT3 is A Mitochondria-Localized Tumor Suppressor Required for Maintenance of Mitochondrial Integrity and Metabolism During Stress*. Cancer Cell. 2010. 17 (1) 41-52.

[31] Bell, E.L., Emerling, B.M., Ricoult, S.J. and Guarente, L. *SirT3 Suppresses Hypoxia Inducible Factor 1α and Tumor Growth by Inhibiting Mitochondrial ROS Production.* Oncogene. 2011. 30 (26) 2986-96.

[32] Hirschey, M.D., Shimazu, T., Goetzman, E., Jing, E., Schwer, B., Lombard, D.B., et al. *Sirt3 Regulates Mitochondrial Fatty Acids Oxidation by Reversible Enzyme Deacetylation.* Nature. 2010. 464 (7285) 121-5.

[33] Finley, L.W., Carracedo, A., Lee, J., Souza, A., Egia, A., Zhang, J., et al. *SIRT3 Opposes Reprogramming of Cancer Cell Metabolism through HIF1α Destabilization.* Cancer Cell. 2011. 19 (3) 416-28.

[34] Han, C. and Someya, S. *Maintaining Good Hearing: Calorie Restriction, Sirt3, and Glutathione*. Exp Gerontol. 2013. 48 (10) 1091-5.

[35] Lombard, D.B. and Zwaans, B.M.M. *Sirt3: as Simples as It Seems?* Gerontology. 2014. 60 (1) 56-64.

[36] Sampaio-Marques, B. and Ludovico, P. *Sirtuins and Proteolytic Systems: Implications for Pathogenesis of Synucleinopathies*. Biomolecules. 2015. 5 (2) 735-57.

[37] Orgogozo, J.M., Dartigues, J.F., Lafont, S., Letenneur, L., Commenges, D., Salamon, R., et al. *Wine Consumption and Dementia in the Elderly: A Prospective Community Study in the Bordeaux Area*. Rev Neurol. 1997. 153 (3) 185-92.

[38] Ho, L., Chen L.H., Wang J., Zhao W., Talcott S.T., Ono K., et al. *Heterogeneity in Red Wine Polyphenolic Contents Differentially Influences Alzheimer's Disease-Type Neuropathology and Cognitive Deterioration.* J Alzheimer Dis. 2009. 16 (1) 59-72.

[39] Brown, V.A., Patel, K.R., Viskaduraki, M., Crowell, J.A., Perloff, M., Booth, T.D., et al. *Repeat Dose Study of the Cancer Chemopreventive Agent Resveratrol in Healthy Volunteers: Safety, Pharmacokinetics, and Effect on the Insulin-Like Growth Factor Axis.* Cancer Res. 2010. 70 (22) 9003-11.

[40] Walle, T. Bioavailability of Resveratrol. Ann NY Acad Sci. 2011. 1215; 9-15.

[41] Brasnyó, P., Molnár, G.A., Mohás, M., Markó, L., Laczy, B., Cseh, J., et al. *Resveratrol Improves Insulin Sensitivity, Reduces Oxidative Stress and Activates the Akt Pathway in Type 2 Diabetic Patients.* Br J Nutr. 2011. 106 (3) 383-9.

[42] Fujitaka, K., Otani, H., Jo, F., Jo, H., Nomura, F., Iwasaki, M., et al. *Modified Resveratrol Longevinex Improves Endothelial Function in Adults with Metabolic Syndrome Receiving Standard Treatment*. Nutr Res. 2011. 31 (11) 842-7.

[43] Bhatt, J.K., Thomas, S., and Nanjan, M.J. *Resveratrol Supplementation Improves Glycemic Control in Type 2 Diabetes Mellitus.* Nutrition Res. 2012. 32 (7) 537-41.

[44] Sin, T.K., Yung, A.P., Yung, B.Y., Yip, S.P., Chan, L.W., Wong, C.S., et al. *Modulating Effect of Sirt1 Activation Induced by Resveratrol on Foxo1-Associated Apoptotic Signaling in Senescent Heart.* J Physiol. 2014. 592 (12) 2535-48.

[45] Sin T.K., Yung B.Y., and Siu P.M. *Modulation of SIRT1-Foxo1 Signalling Axis by Resveratrol: Implications in Skeletal Muscle Aging and Insulin Resistance*. Cell Physiol Biochem. 2015. 35 (2) 541-52.

[46] Caimi, G., Carollo, C., and Lo Presti, R. *Diabetes Mellitus: Oxidative Stress and Wine*. Curr Med Res Opin. 2003. 19 (7) 581-6.

[47] Caimi, G., Carollo, C., and Lo Presti, R. *Wine and Endothelial Function*. Drugs Exp Clin Res. 2003. 29 (5-6) 235-42.

[48] Carollo C., Lo Presti R., and Caimi G. *Wine, Diet and Arterial Hypertension*. Angiology. 2007. 58 (1) 92-6.

[49] Lo Presti, R., Carollo, C., and Caimi, G. *Wine Consumption and Renal Diseases: New Perspectives*. Nutrition. 2007. 23 (7-8) 598-602.

[50] Caimi, G., Carollo, C., and Lo Presti, R. *Chronic Renal Failure: Oxidative Stress, Endothelial Dysfunction and Wine.* Clin Nephrol. 2004. 62 (5) 331-5.

[51] Russo, M.A., Sansone, L., Polletta, L., Runci, A., Rashid M.M., De Santis E., et al. *Sirtuins and Resveratrol-Derived Compounds: A Model for Understanding the Beneficial Effects of the Mediterranean Diet.* Endocrin Metab Immun Disord Drug Targets. 2014. 14 (4) 300-8.