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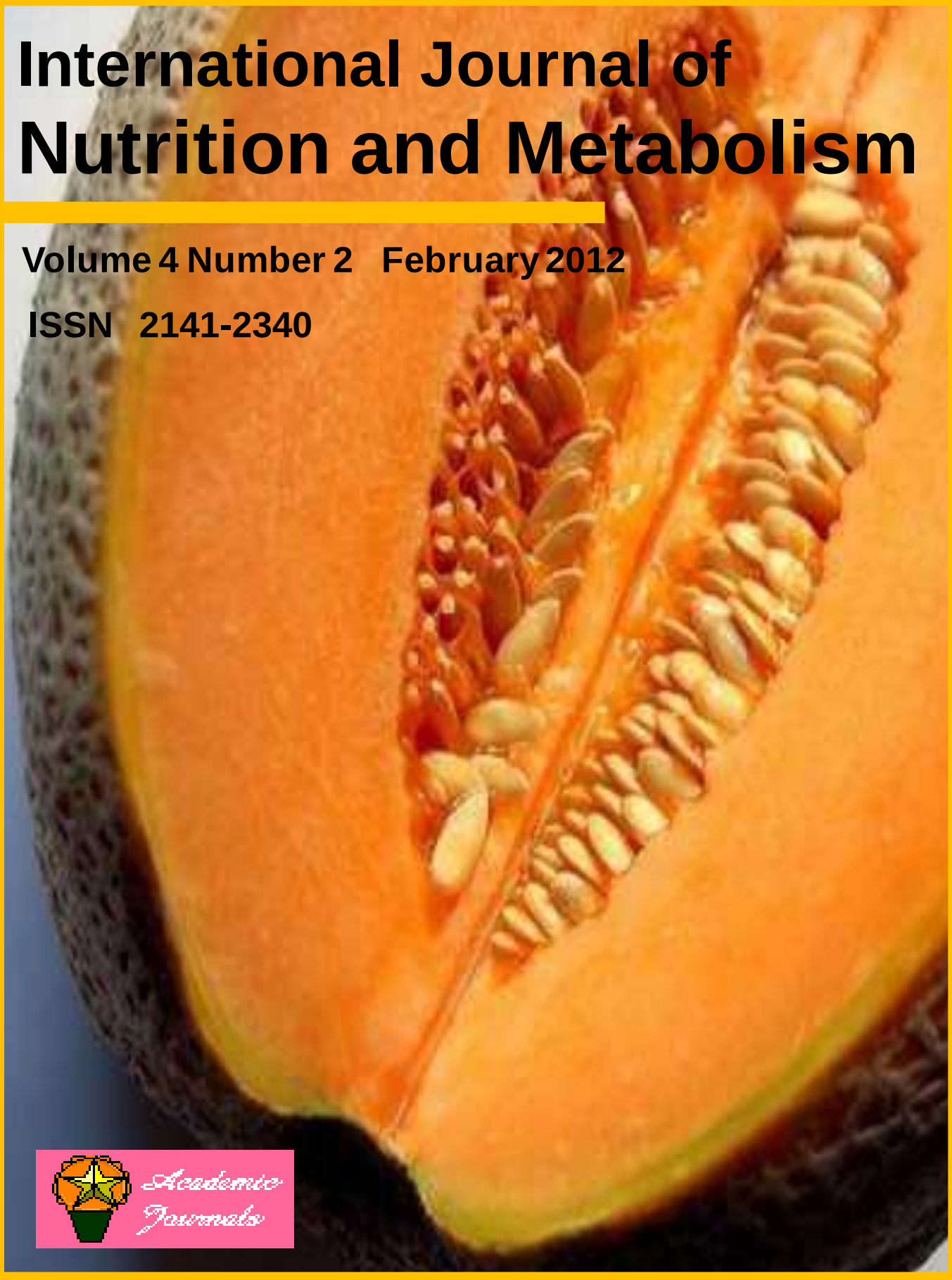

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research

Dietary diversity, micronutrient intake and their variation among black women in informal settlements in South Africa: A cross-sectional study

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This study assessed dietary diversity, its relation to micronutrient intake and variability between age-groups among women 19–69 years from informal settlements of Gauteng province, South Africa. The study was cross-sectional, involving 260 women. Dietary intake was obtained from 24-hour recall data, and Dietary Diversity Score (DDS) from Food Frequency Questionnaire, calculated based on 9 food groups and 80 foods. Intake of eleven micronutrients was assessed as Nutrient Adequacy Ratios (NAR) and Mean Adequacy Ratios (MAR), using Estimated Average Requirements and Adequate Intakes of these micronutrients, following Institute of Medicine recommendations. On average, 26 foods and 7 food groups were consumed. There was a strong significantly positive relationship ($p<0.001$) between the NAR of respective micronutrients (as well as MAR) with the DDS, the relationship was negative with food variety and diversity within food groups; except for vitamin C. Age-group comparisons revealed the older age groups (36-years and older) being at-risk of low micronutrient intake; particularly in calcium, vitamins C and A. In conclusion, dietary diversity was above reference, there was a strong relationship between DDS and micronutrient intake, and there is a significant variation in intake between age-groups, with women 36-years and older being more at-risk of low micronutrient intake.

Key words: Dietary diversity, informal settlements, micronutrients, nutrient adequacy, nutrition education.

INTRODUCTION

Micronutrient malnutrition remains a serious nutritional concern, of which vitamin A, iodine and iron deficiency are highly reported (World Health Organization, 2002). While these deficiencies can have a number of causes not necessarily related to nutrition, a high proportion results from nutritional inadequacies (Kennedy et al., 2010; Maunder et al., 2001). Dietary diversification is one of the four main strategies advocated internationally for the improvement of micronutrient intake and status, especially in undernourished individuals (Maunder et al., 2001). Many studies in several age groups have shown that an increase in individual dietary diversity score is related to increased nutrient adequacy of the diet. Dietary diversity scores have been positively correlated with increased mean micronutrient adequacy of complementary foods (Swindale and Bilinsky, 2006), micronutrient adequacy of the diet in adolescents (Mirmiran et al., 2004) and adults (Ogle et al., 2001; Foote et al., 2004). Indicators of micronutrient status (iron, vitamin A and zinc) among women in South Africa indicate that women's micronutrient status is un-satisfactory. Recent reports (Development Bank of Southern Africa DBSA, 2008) indicate that while, iodine and folic acid status appear to be adequate uniformly throughout the country, almost one third of women are anaemic and 1 out of 4 women have a poor vitamin A status. Trends in the food intake of south Africans (10+

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years) based on reports (Nel and Steyn, 2002; Department of Health, 2003), indicate that starchy foods are the most commonly consumed, with wheat and maize based foods topping the list, contributing between 62 to 71% of total energy intake especially among the black African rural dwellers (Steyn et al., 2006a; b). Among others, energy, calcium, iron, zinc and vitamin A are consumed at less than 67% of the Recommended Dietary Allowances (RDAs), which compromises intake of micronutrients such as several B–vitamins, vitamin E, magnesium and zinc. Sugar intake is also high (84%) (Nel and Steyn, 2002), with low intakes of vegetables, roots/tubers and fruit (40, 64 and 70%, respectively).

The Department of Health has been successful regarding the implementation of fortification schemes to eliminate micronutrient deficiencies in the South African population. The iodization of salt became compulsory in 1995, and the fortification of maize and wheat flour (with vitamin A, thiamine, riboflavin, niacin, folic acid, vitamin B6, iron and zinc) in October 2003, which are done so as to deliver 33% of the RDA per serving at the point of consumption (National Food Fortification Task Group, 1998; 2002). Other programmes adopted include use of the food-based dietary guidelines, to encourage dietary diversity and increased consumption of fruit and vegetables (Love et al., 2001). These guidelines include a number of recommendations, with a focus on healthy eating habits, rather than individual nutrients. However, the precise number of foods or food groups that one should strive to consume over any given period is not commonly mentioned in any dietary guidelines, those of South Africa inclusive. Japan advises consumption of 30 different food items per day (Truswell, 1987), and the US advocates consumption of a variety of nutrient dense foods and beverage within and among five basic food groups, with an item from each food group consumed daily (USDA, 2005). Despite many nutritional guidelines recommending consumption of a variety of foods, the use of dietary diversity as an indicator of adequate nutrient intake remains under evaluation.

Women, one of the most vulnerable groups in society, and; in particular those from poor populations including informal settlers have problems of micronutrient deficiencies, a greater risk of infectious disease (Maunder et al., 2001) and have a low dietary variety (Labadarios et al., 1999). World Health Organization (WHO, 2001) notes that the prevalence of many of these deficiencies vary greatly according to age, gender, physiological, pathological and socio-economic conditions, and yet women's dietary intake receives insufficient attention in many developing countries; South Africa inclusive. More over, numerous localized studies in South Africa have indicated high prevalence of iron deficiency (Kruger et al., 1994; Dannhauser et al., 1999) and of vitamin A deficiency (Kennedy-Oji et al., 2001; Visser et al., 2003) in women. Further, there are no nationally representative data on women's dietary diversity and food intake in South Africa. Available dietary intake information is fragmented. The rationale for this investigation is based on the fact that Oldewage-theron et al. (2005) reported poor diets among these informal settlers, the diet consisting overwhelmingly of refined carbohydrates.

The top 10 food items consumed were: stiff and soft maize meal porridge, brewed rooibos and leaf tea, coffee, mabela, white bread, crumbly maize porridge, carbonated cold drink and mageu.

Interventions currently available for women have been limited to promoting the health of, in particular; pregnant and lactating women. Providing this kind of information may guide in the design of new interventions that can help uplift the situation of women in this category. Food and Agriculture Organization (FAO, 1992) states that programmes that address hunger, malnutrition and disease among communities significantly improve their living conditions, a reason why this study can be valuable. The purpose of this study was therefore to assess the dietary diversity and its relation to micronutrient intake among women of different age groups, ranging from 19-69 years in informal settlements of Gauteng province, South Africa. It is expected that these findings will provide useful information for planning interventions for the women and other groups in informal settlements; and target at-risk groups accordingly.

MATERIALS AND METHODS

Study area and design

Data were collected from women aged 19–69 years, residing in informal settlements of Alexandra, Boipatong and Eaton side, who were randomly sampled from the list of women in the study areas. Data were collected from 2004 to 2007. The study used a cross sectional design involving 260 participants, who were selected following informed consent after a meeting that was convened by the local leaders in the study areas. These communities were identified from a participatory planning workshop involving all stakeholders (community leaders, local and district government officials, delegates from departments of health and education) as being in need of support (as measured by their poverty levels) and could therefore benefit from implementation of this project. The results of the strategic participatory planning workshop with all these stakeholders also indicated that 100% of the participants agreed that a need existed for this project, for purposes of uplifting food and nutrition security, care of vulnerable groups such as mothers and children, and for improvement of essential human services including health, education, water, environmental sanitation and housing.

According to the national census of 2001, Gauteng is home to about 19.7% of South Africa's population (Provide project, 2005). Measured by its total current income, Gauteng is the richest province in South Africa. In terms of per capita income, the province also ranks first (Stats SA, 2003a). Despite its relatively fortunate position, the province is nevertheless marred by high poverty rates, inequalities in the distribution of income between various population sub-groups and unemployment, although not to the same degree as are other regions in South Africa. Using a computer program G-power version 3.1.2, and considering an alpha level of 0.05, a desired statistical power of 0.95, an anticipated effect size (f^2) of

0.15 and a total of 10 predictors, the minimum sample size for this study was 74 participants.

In each site, a simple random sample was taken from all women who were eligible to participate, after stratifying them by 10-year age interval (except for the 19-25 year age-group where 7 year interval was applied due to the exceptionally high demands of the group). For practical and logistical reasons, 310 women (from 310 households) were randomly selected to participate in the study. Women were eligible to participate if they were 19 and above, and were not pregnant or breastfeeding at the time. Of these women, 34 lacked clear age records and 17 had other incomplete information, leaving 260 women. The response rate was therefore 83.6%, which we feel was quite representative of the original sample we began with. A description of the age-groups and numbers that participated shows that; 19-25 (n=46); 26-35 (n=67); 36-45 (n=56); 46-55 (n=41); and 56+ (n=50) years. The study was approved by the Witwatersrand University's medical ethics committee for research on human subjects and the protocol was submitted in accordance with the existing policy for research in the institution. The guidelines of the Medical Research Council (SA) for research on human subjects were adhered to.

Dietary assessment and nutrient intake

Assessment of nutrient intake was done quantitatively using 24 hours recall data, to measure the quantity consumed over a one-day period. This assessment was repeatedly done for 3 non-consecutive days, to allow for better estimation of food intake. Methods of data collection were similar for all the different sites in the study area. As most of the women participated in cooking meals at home, it was assumed that they had a good ability to remember foods and estimate portion sizes as reported by Mirmiran et al. (2006). The 24 hours recall was validated based on Oldewage-Theron et al. (2005). A list of meals, dishes and all food items and beverages consumed in the last 24 hours were recorded by trained interviewers, and the place of preparation/consumption noted. Participants were asked for a full description of ingredients in mixed dishes, and amounts eaten were estimated using household measures and models. Participants were also prompted for specific foods such as snacks, and drinks. They were not however prompted for use of supplements.

Standard reference tables (Langenhoven et al., 1991) were used to convert household portions to grams for calculation. The data from the dietary recalls were analyzed by a registered dietician using FoodFinder® version 3 soft ware program (MRC, 2004), a computer software application developed by the Nutritional Intervention Research Unit (NIRU) and Biomedical Informatics Research Division (BIRD) of the South African Medical Research Council (MRC), based on the South African food composition tables (Langenhoven et al., 1991), for quantification of nutrient intake. Mean nutrient intake was calculated as the average of the three 24 hours recalls. For the purpose of this paper, the list of micronutrients that were included for assessment of adequacy included; calcium (ca); iron (Fe); zinc (Zn); vitamin A (vit A); thiamin; riboflavin; Niacin; vitamin B6 (vit B6); folate; vitamin B12 (vit B12) and vitamin C (vit C). The micronutrients were chosen based on their public health relevance and the likely availability of nutrient values in food composition tables. Vitamin D and iodine were excluded due to incomplete information from food composition tables on these micronutrients. Requirement distributions for individual nutrients were defined by estimated average requirements (EARs) (Institute of Medicine IOM, 2002).

Calcium is a nutrient of public health concern (IOM, 1997), for which no EAR is available. An adequate intake (AI) has been set, but no SD specified. To include an evaluation of calcium intake in the analyses, intake levels were compared to the AI. In the calculation of nutrient intake for the 24 hours, food composition values used were for the foods in their natural state, no fortification was considered.

Dietary diversity scores

Dietary diversity scores were obtained qualitatively using food frequency questionnaires, which were designed to capture information over a period of one week. For this study, the nine food groups, recommended by Food and Agriculture Organization (FAO, 2007b) and as used in an earlier study on children (Steyn et al., 2006) including; flesh food group, egg group, dairy group, vegetable group, cereal and root/tuber group, legume group, vitamin A rich vegetable group, fruit group, fat/oil group were used. Nine varieties of foods were included in the flesh group, one for egg, nine for dairy, seventeen for vegetable, sixteen for cereal, four for legume, seven for vitamin A rich vegetable, fifteen for fruits and four for fat/oil groups, which gave a total of 80 varieties of foods (Table 1). The individual varieties of foods consumed were recorded into the 9 food groups, and calculation of DDS was done by summing the number of unique food groups consumed by the women in the period of the study.

The estimated average requirements (EARs) were used to assess the nutrient adequacy of micronutrient intake (NAR) as illustrated in Ruel (2002). Given typical dietary patterns in black informal settings in south Africa, characterized by few animal source foods and high phytate content, low levels of bioavailability were assumed for iron (5%) and zinc (25%), according to international guidelines (WHO/FAO, 2004; Brown et al., 2004). Iron and zinc intakes were derived by multiplying the amounts in the 24 hours period by percent absorption. The nutrient adequacy ratio was calculated for each individual nutrient by dividing the intake of the nutrient by the EAR or AI, then multiplied by 100 (Table 4). When intake exceeded the requirement, the value was capped at 100, indicating 100% adequacy.

For calcium, adequacy of intake was assessed through the method used by Foote et al. (2004). The AI for calcium is 1000 mg/d for women aged 19 years and above. Following Foote et al. (2004), intakes between 0–250 mg/d were given 0% nutrient adequacy, those between 251–500 mg/d were given 25%, those between 501–750 mg/d were given 50%, those between 751–1000 mg/d were given 75% and those above 1000 mg/d were given 100% nutrient adequacy. The mean adequacy ratio of micronutrient intake (MAR) was calculated by summing up each of the nutrient adequacy ratios for the respective micronutrients and dividing by the 11 micronutrients in the analysis.

Data was analyzed using the Statistical Package for Social Scientists (SPSS, version 18.0). Descriptive statistics for frequencies of food groups consumed and DDS; means and standard deviations of nutrient intakes;

Table 1. Food groups and food varieties obtained from food frequency data. The foods in each food group are listed in order of how most frequently they were consumed.

Food group	Food varieties
Flesh foods	Chicken, fish/ tinned fish, processed meat, beef, Tripes/offal/organ meats, sausage, mutton, goat, pork.
Eggs	Eggs (all types)
Dairy	Powdered milk, whole/full cream milk, cheese, custard, yoghurt, Inkomazi (sour milk), Ice cream, sour cream, evaporated milk, skim/low fat milk.
Vegetables	Beet root, cabbage, onion, green beans, tomatoes, rhubarb, lettuce, peas (fresh), green pepper, cauliflower, garlic, sweet corn, mushroom, chilli (red), gem squash, baby marrow, turnips.
Cereals, roots and tubers	Maize meal, bread, pasta, sorghum (Mabella), Rice, stamp, scones, breakfast cereal, biscuits, maize/rice mixture, oats, fat cakes, Mageu (cereal drink), Traditional beer.
Legumes	Nuts/seeds/peanut butter, soya, peas, dried beans.
Vitamin A rich vegetables and fruits	Morogo, carrot, spinach, butternut, pumpkin, mango, apricots.
Fruits and fruit juices	Peaches, grapes, apple, pineapple, pears, banana, Naartjies (Tangerines), Plum, Fruit juice, avocado, water melon, orange, guava, lemon, Kiwi fruit.
Fats and Oils	Margarine, sun flower, salad oil, lard.
Total 9	80

mean nutrient adequacy for individual nutrients; and contribution of different food groups to nutrient adequacy were generated to characterize the nutrient intake of the women. Correlation tests for strength of the relationship between DDS and nutrient adequacy were calculated using spearman's correlation tests. A p–value of 0.05 was regarded as the level at which tests were considered significant.

RESULTS AND DISCUSSION

Food group and dietary diversity

The sample size comprised of 260 women, of average age 41.0 ± 15.74 years, ranging from 19.0–69.0 years. Tables 1 and 2 present the food groups and mean intake for the nine food groups that were considered for this study. Over all, there were 80 foods and nine food groups considered (Table 1). Currently, there is no international consensus on which food groups to include in the scores to create dietary diversity scores, although work is underway to determine the best set of food groups as indicators of adequate micronutrient intake (FAO, 2007b). In general, cereals and tubers was the most highly reported food group (99.2%) and had a mean consumption rate of 5.00 ± 4.19; followed by flesh foods (93.1%) and a mean consumption rate by the group of 3.49 ± 2.31. Fats/oils (91.2%) and dairy (87.3%) were also highly reported by the participants (Table 2). Eggs, vegetables, fruits and vitamin A rich vegetables were consumed in moderation (by slightly above half the participants). Looking at the mean intake of the food groups (by age-group), there was a slightly higher intake (mean ± SD) among the older adults (56+ years) compared to the younger ones (19–25 years) for all food groups (Table 2).

On average, the women consumed 26.15 (± 21.12) food varieties and 6.70 (± 2.22) food groups (Table 3). Some local studies have reported average DDS values of 4.02 (Labadarios et al., 2011) and 3.41 (Oldewage-Theron and Kruger, 2008a) for South Africans; and 4.9, 4.6, 5.2 and 3.3, in other developing countries including Filipino, Burkina Faso, Laos and Northern Uganda, respectively (Kennedy et al., 2009a;b). The mean for the current group was high (6.7) and the possible reason may be associated with mis-reporting of intake.

From the flesh group, chicken was the most highly consumed (81.5%), with tinned fish following the list (57.7%) (Table 3). Less than 50% of the participants reported consuming other varieties in the group. In the milk group, powdered milk was the most highly reported (60.8%), with others consumed either in quantities less than 50%. Cabbage, beet root, onion and green beans

Table 2. Food groups and varieties, mean (±SD) of consumption of foods from the groups. Data provided are for both the whole study group and disaggregated by age-group (n=260).

Variable (food groups)	Age group (years)	Mean intake (±SD)
Flesh group (9)	Overall	3.49 ± 2.31
	19–25 (n=46)	3.41 ± 2.41
	26–35 (n=67)	3.54 ± 2.20
	36–45 (n=56)	3.36 ± 2.42
	46–55 (n=41)	2.90 ± 2.07
	56+ (n=50)	4.14 ± 2.36
Egg group (1)	Overall	0.59 ±0.49
	19–25 (n=46)	0.57 ±0.50
	26–35 (n=67)	0.60 ± 0.49
	36–45 (n=56)	0.55 ± 0.50
	46–55 (n=41)	0.59 ± 0.50
	56+ (n=50)	0.64 ±0.48
Dairy group (9)	Overall	3.13 ± 2.50
	19–25 (n=46)	3.28 ± 2.60
	26–35 (n=67)	3.30 ± 2.40
	36–45 (n=56)	2.68 ± 2.22
	46–55 (n=41)	2.93 ± 2.45
	56+ (n=50)	3.42 ± 2.84
Vegetable group (17)	Overall	4.43 ± 4.97
	19–25 (n=46)	5.15 ± 5.19
	26–35 (n=67)	4.03 ± 4.32
	36–45 (n=56)	3.20 ± 4.54
	46–55 (n=41)	3.66 ± 4.77
	56+ (n=50)	6.30 ± 5.69
Cereal and Root/tuber group (14)	Overall	5.00 ± 4.19
	19–25 (n=46)	5.13 ± 4.33
	26–35 (n=67)	5.23 ± 3.95
	36–45 (n=56)	4.14 ± 4.22
	46–55 (n=41)	4.44 ±4.21
	56+ (n=50)	5.98 ± 4.24
Legume/Nuts group (4)	Overall	1.26 ± 1.25
	19–25 (n=46)	1.35 ± 1.33
	26–35 (n=67)	1.27 ± 1.21
	36–45 (n=56)	0.93 ± 1.00
	46–55 (n=41)	1.05 ± 1.20
	56+ (n=50)	1.70 ± 1.42
Vitamin A vegetable rich group (7)	Overall	2.61 ± 2.59
	19–25 (n=46)	2.98 ± 2.74
	26–35 (n=67)	2.64 ± 2.42
	36–45 (n=56)	1.93 ± 2.48
	46–55 (n=41)	2.10 ± 2.56
	56+ (n=50)	3.40 ± 2.66
Fruit and fruit juice group (15)	Overall	3.75 ± 4.44
	19–25 (n=46)	4.30 ± 4.79
	26–35 (n=67)	3.60 ± 4.06

Table 2. Contd.

	36–45 (n=56)	2.50 ± 3.82
	46–55 (n=41)	3.02 ± 4.07
	56+ (n=50)	5.46 ± 5.08
Fat/oil group (4)	Overall	1.90 ± 1.04
	19–25 (n=46)	1.80 ± 1.00
	26–35 (n=67)	1.91 ± 1.07
	36–45 (n=56)	1.93 ± 0.93
	46–55 (n=41)	1.61 ± 1.12
	56+ (n=50)	2.16 ± 1.02

Table 3. Food groups, food varieties, number of women (%) who consumed the foods and mean intake of the food varieties and food groups measured by FFQ (n=260).

Food group	Food varieties	Number (%)	Food varieties	Number (%)
Flesh foods	Chicken	216 (81.5)	Sausage	82 (30.9)
	Fish/tinned fish	153 (57.7)	Mutton	77 (29.1)
	Processed meat	120 (45.3)	Goat	58 (21.9)
	Beef	106 (40.0)	Pork	14 (5.3)
	Tripes /Offal/ organ meats	82 (30.9)		
Eggs	Eggs (all types)	153 (58.8)		
Dairy	Powdered milk	161 (60.8)	Inkomazi (sour milk)	78 (29.4)
	Whole/full cream milk	123 (46.4)	Ice cream, sour cream	66 (24.9)
	Cheese	101 (38.1)	Evaporated milk	58 (21.9)
	Custard	88 (33.2)	Skim/low fat milk	54 (20.4)
	Yoghurt	84 (31.7)		
Vegetables	Beet root	129 (48.7)	Cauliflower	54 (20.4)
	Cabbage	128 (48.3)	Garlic	48 (18.1)
	Onion	124 (46.8)	Sweet corn	44 (16.6)
	Green beans	106 (40.0)	Mushroom	42 (15.8)
	Tomatoes	80 (30.2)	Chilli (red)	40 (15.1)
	Rhubarb	76 (28.7)	Gem squash	36 (13.6)
	Lettuce	74 (27.9)	Baby marrow	26 (9.8)
	Peas (fresh)	68 (25.7)	Turnips	20 (7.5)
	Green pepper	56 (21.1)		
Cereals	Maize meal	244 (92.1)	Breakfast cereal	76 (28.7)
	Bread	132 (49.8)	Biscuits	72 (27.2)
	Pasta	124 (46.8)	Maize/rice mixture	70 (26.4)
	Sorghum (Mabella)	98 (37.0)	Oats	66 (24.9)
	Rice	96 (36.2)	Fat cakes	66 (24.9)
	Stamp	84 (31.7)	Mageu (cereal drink)	54 (20.4)
	Scones	82 (30.9)	Traditional beer	36 (13.6)
Legumes/ Nuts	Nuts/Seeds/Peanut butter	151 (57.0)	Peas	64 (24.2)
	Soya	66 (24.9)	Dried beans	46 (17.4)
Vitamin A rich vegetables and fruits	Morogo	122 (46.0)	Pumpkin	94 (35.5)
	Carrot	122 (46.0)	Mango	78 (29.4)
	Spinach	116 (43.8)	Apricots	52 (19.6)

Table 3. Contd.

	Butternut	94 (35.5)		
Fruits and fruit juices	Peaches	120 (45.3)	Fruit Juice	58 (21.9)
	Grapes	102 (38.5)	Avocado	50 (18.9)
	Apple	90 (34.0)	Water melon	48 (18.1)
	Pineapple	86 (32.5)	Orange	38 (14.3)
	Pears	84 (31.7)	Guava	32 (12.1)
	Banana	80 (30.2)	Lemon	30 (11.3)
	Naartjies (Tangerines)	68 (25.7)	Kiwi fruit	24 (9.1)
	Plum	66 (24.9)		
Fats and Oils	Margarine	176 (66.4)		
	Sun flower	130 (49.1)		
	Salad oil	119 (44.9)		
	Lard	68 (25.7)		
Mean of food varieties consumed	26.15 (21.12)[a.]			
Mean of food groups consumed	6.70 (2.22)[b.]			

[a.] Mean food variety intake (± SD) is the average number of food items consumed in the study period (out of 80 foods). [b.] Mean Food group intake (± SD) is the average number of food groups consumed in the study period (out of 9 food groups).

were the top most consumed vegetable foods; however, less than 50% of the participants reported consumption of these foods. In the cereal group, almost all the participants (92.1%) reported consumption in the period of the study; all the other foods in the group were less reported (less than 50% consumption). Nuts/seeds topped the list of legumes. Vitamin A rich vegetable and the fruit/juice groups were reported by less than 50% of the group, indicating low/ inadequate consumption of these food groups. In the fat group, Margarine was the highest reported variety in the group (66.4%), other varieties in the group were less reported. The patterns of food intake among the communities in the present study do agree with reports (Nel and Steyn, 2002; Steyn et al., 2006c; Oldewage-Theron and Kruger, 2008a) that cereals and starchy foods, especially maize based foods top the list of foods consumed by South Africans, especially the black populations.

The usual diet of the women was composed largely of cereals/roots/tubers, flesh, fats/oils and dairy foods. Seven types of foods including; chicken, fish, eggs, powdered milk, maize meal, nuts, and margarine, constituted nearly two thirds of intake, of which two (maize meal, 92% and chicken, 82%) were top most. Using a mean DDS of 4 to define poor dietary intake and because of lack of national dietary data on adults, the findings of this study revealed that 19.6% of the women had a dietary score (DDS) less than four indicating a poor dietary diversity, while 55.4% consumed less than the average number of foods consumed by the group. The nutrient intake of the women (Table 4) indicated that the mean energy intake (8521±5420 kj), was less than the Estimated Energy Requirements (8 820 Kj) for the group. Mean protein (71.02 ± 77.22 g/d) and fat (62.98 ± 58.32 g/d) intake were above recommendations of Institute of Medicine (IOM, 2005). Inadequate vitamin A, C, B12, riboflavin, folate and thiamin was also prevalent. Considering the Importance of these nutrients, and the fact that the group of study was women, a majority of whom were at reproductive age; this was an important finding that supports the increased need for strategies to increase intake of these nutrients including food fortification and nutrition education. For most nutrients (except for energy), group mean intake must exceed the reference values in order to achieve an acceptably low prevalence (IOM, 2000; Willet, 1998). Although the mean calcium intake was less than the adequate intake (AI), it was not possible to infer conclusions regarding this particular nutrient with regard to inadequacy. However, because only 7.7% of the study group reported calcium intakes more than the AI, it is highly probable that there is a high proportion of inadequacy for this particular nutrient and this finding supports the need for intervention programs to improve intake of this nutrient. High phytate content reportedly affects calcium bioavailability (IOM, 2011). Accordingly, there was high inadequate intake of this nutrient from food recorded for both the group (92.3%) and within age-groups (Table 4), the worst affected being the 46–55 year old, where 97.6% of participants consumed less than the requirement. Examination of food sources of calcium for the group showed that the major sources of calcium were milk and milk products, and grain products. All other groups of foods (which were less consumed) including eggs,

Table 4. The mean intake (±SD) measured by 24-h recall, estimated average requirements, nutrient adequacy ratios and number (%) of women below the requirements for the respective micronutrients (n=260).

Micronutrients	Age group (Years)	Mean Intake (±SD)	EAR [a, b, c, d.]	NAR (±SD) [§.]	Number (%) below requirement
Calcium (mg/d)	Overall	395.32 ±339.71	1000[c.]	26.15 ± 30.89	240 (92.3)
	19–25 (n=46)	477.41 ±368.83		31.52 ± 30.48	42 (91.3)
	26–35 (n=67)	469.44 ±398.52		33.96 ± 35.54	58 (86.6)
	36–45 (n=56)	354.36 ± 292.22		23.21 ± 28.55	53 (94.6)
	46–55 (n=41)	296.13 ± 228.04		17.07 ± 22.67	40 (97.6)
	56+ (n=50)	347.70 ± 326.40		21.50 ± 30.73	47 (94.0)
Iron (mg/d)	Overall	11.75 ± 7.57	8.1[d.]	88.24 ± 22.76	83 (31.9)
	19–25 (n=46)	13.47 ± 8.46		93.10 ± 16.17	12 (26.1)
	26–35 (n=67)	12.58 ± 8.43		88.49 ± 23.61	20 (29.9)
	36–45 (n=56)	10.62 ± 6.89		84.94 ± 24.85	20 (35.7)
	46–55 (n=41)	12.01 ± 7.94		88.76 ± 21.99	14 (34.1)
	56+ (n=50)	10.12 ± 5.40		86.71 ± 24.92	17 (34.0)
Zinc (mg/d)	Overall	17.75 ±18.68	6.8[d.]	89.89 ± 20.34	80 (30.8)
	19–25 (n=46)	18.93 ± 16.87		93.94 ± 14.46	10 (21.7)
	26–35 (n=67)	16.16 ± 15.73		88.36 ± 22.74	21 (31.3)
	36–45 (n=56)	23.85 ± 24.71		93.90 ± 16.24	14 (25.0)
	46–55 (n=41)	18.92 ± 19.51		92.04 ± 15.05	11 (26.8)
	56+ (n=50)	10.99 ± 12.61		81.94 ± 26.68	24 (48.0)
Vitamin A (RE) µg/d	Overall	945.33 ±1981.97	500[d.]	73.49 ± 30.29	151 (58.1)
	19–25 (n=46)	1 254.61 ± 2 487.02		79.18 ± 24.49	27 (58.7)
	26–35 (n=67)	1 198.75 ± 1 860.55		80.72 ± 29.25	27 (40.3)
	36–45 (n=56)	1 125.21 ± 2 869.70		67.85 ± 34.00	35 (62.5)
	46–55 (n=41)	508.69 ± 66.99		70.84 ± 30.90	26 (63.4)
	56+ (n=50)	477.77 ± 633.81		67.05 ± 29.71	36 (72.0)
Thiamin (mg/d)	Overall	1.15 ±0.75	0.9[d.]	85.14 ± 22.32	117 (45.0)
	19–25 (n=46)	1.47 ± 0.96		91.35 ± 16.06	14 (30.4)
	26–35 (n=67)	1.34 ± 0.88		86.30 ± 23.66	25 (37.3)
	36–45 (n=56)	1.05 ± 0.68		83.65 ± 22.36	28 (50.0)
	46–55 (n=41)	0.99 ± 0.44		86.29 ± 19.36	21 (51.2)
	56+ (n=50)	0.86 ± 0.41		78.60 ± 26.28	29 (58.0)
Riboflavin (mg/d)	Overall	1.51 ±1.53	0.9[d.]	80.09 ± 25.83	130 (50.0)
	19–25 (n=46)	2.07 ± 1.84		86.23 ± 21.07	17 (37.0)
	26–35 (n=67)	1.95 ± 1.78		81.58 ± 27.77	28 (41.8)
	36–45 (n=56)	1.35 ± 1.58		76.29 ± 26.38	34 (60.7)
	46–55 (n=41)	1.12 ± 0.97		79.49 ± 23.93	22 (53.7)
	56+ (n=50)	0.92 ± 0.55		77.18 ± 27.70	29 (58.0)
Niacin (mg/d)	Overall	18.93 ± 30.10	11[d.]	88.46 ± 22.01	89 (34.2)
	19–25 (n=46)	19.91 ± 14.32		92.45 ± 16.23	14 (30.4)
	26–35 (n=67)	18.94 ± 12.75		86.73 ± 25.18	22 (32.8)
	36–45 (n=56)	24.32 ± 61.04		88.94 ± 20.84	18 (32.1)
	46–55 (n=41)	17.67 ± 8.72		94.34 ± 12.86	11 (26.8)

Table 4. Contd.

	56+ (n=50)	13.02 ± 8.23		81.78 ± 27.38	24 (48.0)
Vitamin B6 (mg/d)	Overall	1.74 ± 1.23	1.1[d.]	86.54 ± 23.39	97 (37.3)
	19–25 (n=46)	2.03 ± 1.17		93.12 ± 16.12	11 (23.9)
	26–35 (n=67)	1.77 ± 1.15		85.63 ± 25.85	22 (32.8)
	36–45 (n=56)	1.97 ± 1.69		89.17 ± 19.12	20 (35.7)
	46–55 (n=41)	1.72 ± 0.92		89.16 ± 19.86	14 (34.1)
	56+ (n=50)	1.18 ± 0.79		76.62 ± 29.40	30 (60.0)
Folate (µg/d)	Overall	540.89 ± 484.06	320[d.]	82.37 ± 26.70	106 (40.8)
	19–25 (n=46)	553.74 ± 469.15		84.60 ± 22.97	18 (39.1)
	26–35 (n=67)	482.40 ± 431.61		81.39 ± 27.33	29 (43.3)
	36–45 (n=56)	713.98 ± 587.12		85.43 ± 24.13	18 (32.1)
	46–55 (n=41)	594.79 ±503.29		85.96 ± 23.56	17 (41.5)
	56+ (n=50)	369.41 ± 346.80		75.30 ± 33.05	24 (48.0)
Vitamin B12 (µg/d)	Overall	7.66 ± 18.53	2.0[d.]	74.91 ± 35.34	110 (42.3)
	19–25 (n=46)	11.02 ± 23.77		87.10 ± 24.58	12 (26.1)
	26–35 (n=67)	7.54 ± 13.20		76.46 ± 35.71	25 (37.3)
	36–45 (n=56)	10.90 ± 28.99		75.48 ± 35.08	22 (39.3)
	46–55 (n=41)	3.91 ± 3.86		77.59 ± 31.86	20 (48.8)
	56+ (n=50)	4.16 ± 7.68		58.80 ± 41.32	31 (62.0)
Vitamin C (mg/d)	Overall	58.02 ± 90.38	60[d.]	46.77 ± 39.68	191 (73.5)
	19–25 (n=46)	78.87 ± 93.00		67.14 ± 36.79	26 (56.5)
	26–35 (n=67)	91.40 ± 19.22		55.18 ± 42.44	39 (58.2)
	36–45 (n=56)	35.13 ± 48.47		41.56 ± 36.49	48 (85.7)
	46–55 (n=41)	34.81 ± 51.43		39.41 ± 34.20	36 (87.8)
	56+ (n=50)	38.77 ± 89.07		28.62 ± 36.15	42 (84.0)
Mean adequacy ratio	Overall	NA	NA	74.25 ± 19.96	NA
	19–25 (n=46)			81.34 ± 14.54	
	26–35 (n=67)			76.31 ± 23.23	
	36–45 (n=56)			73.21 ± 17.07	
	46–55 (n=41)			74.15 ± 16.36	
	56+ (n=50)			66.18 ± 22.77	

[a.] EER value for active women 30 years of age, of average height 1.45m and BMI of 24.99 kg/m^2, Adapted from IOM, 2005. [b.] Acceptable macronutrient distribution range (AMDR), adapted from IOM, 2005. [c.] AI value adapted from IOM, 2005. [d.] EAR values adapted from IOM, 2005. [§.] NAR values reported are capped at 100 to avoid reporting over intake.

legumes, nuts and seeds, fruits and fruit products, vegetables contributed less to calcium intake. However, these estimates of calcium intakes represent intakes from food only. They underestimate the true calcium intakes for several reasons, one of the most important being underreporting of food intakes. Numerous studies suggest that individuals do not report their full nutrient intake (Schoeller, 1990; Forbes, 1993). Secondly, we did not account for calcium intake from fortified foods and supplements. Because of this, calcium intakes estimated in our study are likely to be underestimates of the true dietary calcium intakes of the population, but they do give estimates of what is derived from the natural foods.

Relation between nutrient Intake and dietary diversity

Measures of DDS and FVS are often validated against

Table 5. A Linear regression analysis between Nutrient Adequacy Ratios for respective micronutrients with dietary diversity score, food variety score and individual food group diversity (n=260). Values presented are un-standardized coefficients with the standard error of the coefficient (SE β) in parentheses.

NAR of nutrients	β–values, (SE β)										
	DDS	FVS	Flesh	Dairy	Vegetable	Cereal	Legume	Vitamin A rich vegetable	Fruit	Fat/Oil	R2
Calcium (mg/d)	2.79 (1.10)*	–2.98 (4.58)	5.69 (4.69)	2.34 (5.22)	3.08 (4.91)	2.62 (4.41)	2.61 (5.50)	2.13 (4.68)	4.13 (4.72)	2.78 (5.03)	0.43
Iron (mg/d)	13.04 (1.15)***	–1.00 (4.74)	0.77 (4.85)	1.41 (5.40)	–0.15 (5.08)	2.39 (4.56)	–4.17 (5.70)	–2.54 (4.85)	0.03 (4.88)	7.82 (5.21)	0.88
Zinc (mg/d)	13.39 (1.16)***	2.21 (4.79)	–1.07 (4.91)	–2.23 (5.46)	–3.94 (5.14)	–1.03 (4.61)	–9.66 (5.76)	–7.56 (4.90)	–2.02 (4.94)	4.67 (5.26)	0.88
Vitamin A (RE) µg/d	12.02 (1.26)***	–2.99 (5.20)	3.95 (5.32)	2.25 (5.93)	2.04(5.57)	3.74 (5.00)	–1.50 (6.25)	–0.60 (5.31)	3.20 (5.36)	5.03 (5.71)	0.81
Thiamin (mg/d)	12.92 (1.13)***	–2.02 (4.67)	3.75 (4.79)	1.92 (5.33)	0.89(5.01)	2.79 (4.50)	–4.28 (5.62)	–2.05 (4.78)	1.69 (4.82)	6.94 (5.13)	0.88
Riboflavin (mg/d)	11.51 (1.16)***	–2.18 (4.81)	4.23 (4.92)	1.36 (5.48)	1.08(5.15)	3.29 (4.63)	–4.68 (5.78)	–1.43 (4.92)	2.50 (4.95)	7.84 (5.28)	0.86
Niacin (mg/d)	13.66 (1.14)***	–1.06 (4.71)	1.81 (4.83)	1.01 (5.37)	0.12(5.05)	2.14 (4.54)	–5.61 (5.67)	–3.62 (4.82)	0.52 (4.86)	6.72 (5.18)	0.88
Vitamin B6 (mg/d)	13.13 (1.25)***	1.41 (5.18)	0.45 (5.31)	–1.94 (5.91)	–2.92 (5.55)	–1.04 (4.99)	–9.24 (6.23)	–7.15 (5.30)	–0.63 (5.34)	5.57 (5.69)	0.85
Folate (µg/d)	12.19 (1.33)***	5.68 (5.49)	–3.55 (5.62)	–5.56 (6.26)	–6.61 (5.88)	–4.82 (5.28)	–13.81 (6.60)*	–12.75 (5.61)*	–5.85 (5.66)	0.69 (6.03)	0.82
Vitamin B12 (µg/d)	11.91 (1.51)***	–2.59 (6.23)	1.54 (6.38)	5.32 (7.10)	0.53(6.68)	2.07 (5.60)	0.36 (7.49)	–2.14 (6.37)	3.78 (6.42)	6.98 (6.84)	0.75
Vitamin C (mg/d)	9.88 (1.41)***	–14.15 (5.82)*	13.40 (5.96)*	15.00 (6.64)*	14.86 (6.24)*	13.65 (5.60)*	13.55 (6.70)	12.02 (5.95)*	15.09 (5.60)*	8.04 (6.39)	0.60
MAR	11.39 (1.02)***	–1.79 (4.21)	2.83 (4.31)	1.95 (4.80)	0.82(4.52)	2.32 (4.06)	–3.31 (5.06)	–2.28 (4.31)	2.05 (4.34)	5.77 (4.63)	0.87

*** p< 0.001; ** p< 0.01; * p< 0.05. Eggs were excluded from the model due to lack of variety in the food group.

nutrient adequacy ratio (NAR), Mean nutrient adequacy ratio (MAR), probability of adequacy (PA), mean probability of adequate nutrient intake (MPA), or anthropometric measurements, which indicate nutritional status and socio-economic characteristics (Rani et al., 2010).

Few studies within South Africa (Steyn et al., 2006c; Oldewage-Theron and Kruger, 2008a; Labadarios, 2011) have been conducted to relate dietary diversity and nutrient intake and adequacy. The current study documents a significant association between nutrient adequacy (measured as NAR and MAR) with only DDS as a measure of dietary diversity but not with food variety score (FVS) or individual food groups. Dietary diversity score has been reported to provide more relevant information than Food variety score (Swindale and Bilinsky, 2006). All micronutrients were positively significantly associated with the dietary diversity score (defined as the number of food groups consumed) rather than the food variety score (numbers of individual foods within the food groups) (Table 5). The measures of variability (r^2) in the predictors with respective micronutrient ratios were high (ranging from 0.43 to 0.88), indicating strong associations. In this context, increasing the number of food groups has a greater impact on nutrient adequacy than increasing the number of individual foods in the diet. These findings are in agreement with other authors, (Ogle et al., 2001; Onyango et al., 1998; Tarini et al., 1999). Similar trends were observed within age-groups (Table 6). Across all age groups, there was a strong significantly positive relation between the nutrient adequacy ratios and DDS, consistent for all. The reverse was true for FVS. This finding supports the priority message of South African dietary guidelines “enjoy a variety of foods”. Many kinds and combinations of food lead to a balanced diet, for, it is not only what the nutrients do once the body gets them but also what they do to each other that makes the difference in our health and wellbeing; most of which do their work when teamed with other nutrients (De Guzman and Diamaano, 1982).

Associations between the nutrient adequacy of respective micronutrients with individual food groups were not statistically significant except for vitamin C which was significant and positively associated with seven food groups ($p<0.05$), and likewise positively but not significantly associated with fat and legume groups. The findings on the association of dietary diversity and adequacy of intake confirms the consistent pattern of a positive association between diversity measures and nutrient adequacy previously documented in

Table 6. A Linear regression analysis between nutrient adequacy ratios for respective micronutrients with only dietary diversity score and food variety score by age-group.

NAR	Standardized Beta coefficient B, (sig)		
	Age–group (years)	DDS	FVS
Calcium (mg/d)	19–25 (n=46)	0.708 (0.002)**	0.073 (0.742)
	26–35 (n=67)	0.186 (0.329)	0.590 (0.003)**
	36–45 (n=56)	0.594 (0.012)*	–0.006 (0.980)
	46–55 (n=41)	0.717 (0.034)*	–0.229 (0.487)
	56+ (n=50)	0.245 (0.392)	0.325 (0.257)
Iron (mg/d)	19–25 (n=46)	1.200 (0.000)***	–0.283 (0.010)*
	26–35 (n=67)	1.178 (0.000)***	–0.268 (0.008)**
	36–45 (n=56)	1.157 (0.000)***	–0.259 (0.011)*
	46–55 (n=41)	1.329 (0.000)***	–0.480 (0.003)**
	56+ (n=50)	1.019 (0.000)***	–0.107 (0.418)
Zinc (mg/d)	19–25 (n=46)	1.216 (0.000)***	–0.303 (0.007)**
	26–35 (n=67)	1.206 (0.000)***	–0.310 (0.004)**
	36–45 (n=56)	1.262 (0.000)***	–0.371 (0.000)***
	46–55 (n=41)	1.399 (0.000)***	–0.554 (0.000)***
	56+ (n=50)	0.988 (0.000)***	–0.091 (0.530)
Vitamin A (RE) µg/d	19–25 (n=46)	1.203 (0.000)***	–0.293 (0.012)*
	26–35 (n=67)	1.077 (0.000)***	–0.183 (0.128)
	36–45 (n=56)	1.083 (0.000)***	–0.243 (0.077)*
	46–55 (n=41)	1.231 (0.000)***	–0.416 (0.032)*
	56+ (n=50)	1.017 (0.000)***	–0.160 (0.337)
Thiamin (mg/d)	19–25 (n=46)	1.207 (0.000)***	–0.283 (0.005)**
	26–35 (n=67)	1.165 (0.000)***	–0.256 (0.013)*
	36–45 (n=56)	1.152 (0.000)***	–0.254 (0.012)*
	46–55 (n=41)	1.402 (0.000)***	–0.569 (0.001)**
	56+ (n=50)	1.000 (0.000)***	–0.102 (0.476)
Riboflavin (mg/d)	19–25 (n=46)	1.129 (0.000)***	–0.199 (0.063)
	26–35 (n=67)	1.068 (0.000)***	–0.166 (0.153)
	36–45 (n=56)	1.084 (0.000)***	–0.197 (0.087)
	46–55 (n=41)	1.346 (0.000)***	–0.507 (0.002)**
	56+ (n=50)	0.979 (0.000)***	–0.563 (0.576)
Niacin (mg/d)	19–25 (n=46)	1.219 (0.000)***	–0.303 (0.005)**
	26–35 (n=67)	1.177 (0.000)***	–0.271 (0.010)*
	36–45 (n=56)	1.207 (0.000)***	–0.303 (0.001)**
	46–55 (n=41)	1.346 (0.000)***	–0.481 (0.001)**
	56+ (n=50)	1.042 (0.000)***	–0.152 (0.295)
Vitamin B6 (mg/d)	19–25 (n=46)	1.196 (0.000)***	–0.295 (0.018)*
	26–35 (n=67)	1.199 (0.000)***	–0.317 (0.007)**
	36–45 (n=56)	1.302 (0.000)***	–0.450 (0.000)***

Table 6. Contd.

	46–55 (n=41)	1.404 (0.000)***	–0.576 (0.001)**
	56+ (n=50)	0.966 (0.000)***	–0.086 (0.585)
Folate (µg/d)	19–25 (n=46)	1.239 (0.000)***	–0.381 (0.011)*
	26–35 (n=67)	1.270 (0.000)***	–0.425 (0.001)**
	36–45 (n=56)	1.266 (0.000)***	–0.424 (0.000)***
	46–55 (n=41)	1.470 (0.000)***	–0.678 (0.000)***
	56+ (n=50)	0.933 (0.000)***	–0.072 (0.671)
Vitamin B12 (µg/d)	19–25 (n=46)	1.136 (0.000)***	–0.220 (0.064)
	26–35 (n=67)	0.977 (0.000)***	–0.115 (0.423)
	36–45 (n=56)	1.142 (0.000)***	–0.343 (0.022)*
	46–55 (n=41)	1.523 (0.000)***	–0.782 (0.000)***
	56+ (n=50)	0.756 (0.001)***	0.024 (0.910)
Vitamin C (mg/d)	19–25 (n=46)	0.933 (0.000)***	–0.012 (0.932)
	26–35 (n=67)	0.537 (0.001)**	0.322 (0.047)*
	36–45 (n=56)	0.680 (0.000)***	0.096 (0.597)
	46–55 (n=41)	0.957 (0.001)**	–0.306 (0.274)
	56+ (n=50)	0.785 (0.007)**	–0.245 (0.385)

***. Significant at p< 0.001; **. significant at p< 0.01; *. significant at p< 0.05.

developed countries (Ruel, 2002). The results are surprisingly consistent, considering the wide differences between studies in definitions of foods, food groups, reference periods, dietary assessment methods, scoring systems, cut-off points used as well as age of study participants.

Calcium intake was positively associated with individual food groups, but not with total number of foods consumed. Iron intake on the other hand was positively associated with intake of flesh, dairy, cereal, fruits, and fat. Zinc intake was only seen to be positively associated with the total number of foods consumed and intake of fats and oils. Vitamin A, thiamin, riboflavin, Niacin, vitamin B12 intake and MAR were positively correlated with flesh, dairy, vegetable, cereal, fruit and fat intake. However, vitamin B6 and folate intake were only positively correlated with the total varieties consumed (FVS), flesh and fat consumption. Among the nutrients, calcium had the lowest r^2 value followed by vitamin C. Consequently, these were the nutrients, whose nutrient adequacy ratios were below 50.Given the importance of adequate intake of these two nutrients in the diet, and concern about low intakes among women, (although not possible to calculate a true nutrient adequacy for calcium), we believe that any composite nutrient adequacy score must include these two nutrients in the analysis so as to promote their intake. Torheim et al. (2003) also found that FVS was a poorer predictor of NAR and MAR than DDS.

Traditionally, African diets are not high in fat, but surprisingly, fat and oil consumption in this study group was high, and yet the same diets were inadequate in some important micronutrients. This suggests that through nutrition education, guidance given to consumers should focus on helping them to identify desirable level of variety in a day and avoid high consumption of fats.

Variability between age groups

Table 7 presents a comparison between estimated between-group variability inmicronutrient intake. Results indicated that group 5 (56 years and above) had lowest intake for all micronutrients, while group 1 (19–25 years) had the highest intake levels for most of the nutrients. Pair-wise comparisons between age groups (based on estimated marginal means) showed no significant difference in intake of calcium, iron, vitamin A, niacin and vitamin B12 between all age groups (p>0.05). However, a significant difference was observed in intake of zinc between group 3 (36–45 years) and group 5 (56 years and above), with a mean difference of 12.86. Low intake of zinc has been reported among elderly populations in South Africa (Oldewage-Theron et al., 2008b). Intake of thiamin, riboflavin, vitamin B6 and vitamin C, were also significantly higher among the younger groups of women (groups 1, 2 and 3) compared to the older age groups (4 and 5). Table 8 gives an illustration of these differences

Table 7. Micronutrient intake by age group. Data are presented as means, with standard deviations in parentheses.

Age category (years)	Mean (SD)										
	Ca	Fe	Zn	Vitamin A	Thiamin	Riboflavin	Niacin	Vitamin B6	Folate	Vitamin B12	Vitamin C
(19 – 25), n=46	477.41 (368.83)	13.47 (8.46)	18.93 (16.87)	1254.61 (2487.02)	1.47 (0.96)	2.07 (1.84)	19.91 (14.32)	2.03 (1.17)	553.74 (469.15)	11.02 (23.77)	78.87 (93.00)
(26 – 35), n=67	469.44 (398.52)	12.58 (8.43)	16.16 (15.73)	1198.75 (1860.55)	1.34 (0.88)	1.95 (1.78)	18.94 (12.75)	1.77 (1.15)	482.40 (431.62)	7.54 (13.20)	91.40 (119.22)
(36 – 45) n=56	354.36 (292.22)	10.62 (6.88)	23.85 (24.71)	1125.21 (2869.70)	1.05 (0.68)	1.35 (1.58)	24.32 (61.04)	1.97 (1.69)	713.98 (587.12)	10.90 (29.00)	35.13 (48.47)
(46 – 55) n=41	296.13 (228.04)	12.01 (7.94)	18.92 (19.51)	508.69 (466.99)	0.99 (0.44)	1.12 (0.97)	17.67 (8.72)	1.72 (0.92)	594.79 (503.29)	3.91 (3.86)	34.81 (51.43)
(56 and above), n=50	347.70 (326.40)	10.12 (5.40)	11.10 (12.61)	477.77 (633.81)	0.86 (0.41)	0.92 (0.55)	13.02 (8.23)	1.18 (0.79)	369.41 (346.80)	4.16 (7.68)	38.77 (89.07)

in a contrast analysis. Between groups 1 and 2, no significant variation in intake of all micronutrients was observed (all p values >0.05). Similarly, no significant variation was seen between groups 3 and 4. However, significant differences were observed between groups 2 and 3 in intake of zinc, thiamin, riboflavin, folate and vitamin C, which may suggest transition from young adulthood (18–35) through middle adulthood (35–50) and then to late adulthood (55/56 to death). Likewise, significant differences were observed in intake of zinc, vitamin B6 and folate between groups 4 and 5.

This current study had some limitations. Although three 24 hours recalls were administered, the reference period in dietary studies still remains subjective. Three recalls may be too short to measure habitual intake of some food groups. A seven-day record, suggested by other authors would probably give a better picture. Potentially, the greatest problem comes from the variability of food groups and food items used across studies. This makes it difficult to compare with wider studies of this nature. While FAO recommends use of nine food groups, Ruel (2002); Swindale and Bilinsky (2006) recommend use of 8 food groups which makes comparability difficult. Despite these limitations, this study gives a snapshot of food consumption patterns and dietary adequacy in these populations in South Africa. Lastly, accuracy in estimation of food intake is difficult except with very large sample sizes. Some of the large standard errors reported in our study reflect this fact. The results of our study can not therefore be generalized because the sample was limited to women in informal settlements. However, because such a magnitude of inadequate consumption of certain micronutrients was found in this population, it is possible that nutrient intake inadequacies among the general population will be even more severe.

Conclusion and recommendations

Dietary diversity was above average, there is a strong relationship between dietary diversity and micronutrient intake among women in informal settlements in Gauteng. Older women in the population (36-years and above) were found to be more at-risk of inadequate micronutrient intake, most notably, calcium; vitamins C and A. From these findings, decisions on the best allocation of resources can be made, which should include all women groups so as to promote good dietary habits and consequently good health among the women.

Table 8. Contrast between micronutrient intakes of the different age groups. Contrasts between groups are presented as mean difference, with standard error and 95% confidence interval.

Age categories	Micronutrient mean differences										
	Ca	**Fe**	**Zn**	**Vit A**	**Thiamin**	**Riboflavin**	**Niacin**	**Vit. B6**	**Folate**	**Vit. B12**	**Vit. C**
1 vs. 2											
Difference	7.97	0.89	2.77	55.86	0.13	0.12	0.97	0.26	71.34	3.48	–12.54
SE	64.13	1.44	3.51	376.93	0.14	0.28	5.77	0.23	90.68	3.53	16.75
P	*0.901*	*0.538*	*0.431*	*0.882*	*0.357*	*0.677*	*0.866*	*0.254*	*0.432*	*0.326*	*0.455*
95% CI	–118.32, 134.25	–1.95, 3.73	–4.14, 9.69	686.44, –798.16	–0.15, 0.40	–0.44, 0.67	–10.38, 12.33	–0.19, 0.72	–107.23, 249.91	–3.48, –10.43	–45.52, 20.45
2 vs. 3											
Difference	115.08	1.96	–7.70	73.54	0.29	0.60	–5.39	–0.20	231.58	–3.36	56.27
SE	60.64	1.36	3.32	356.42	0.13	0.27	5.45	0.22	85.74	3.34	15.84
P	*0.059*	*0.152*	*0.021**	*0.837*	*0.030**	*0.025**	*0.324*	*0.350*	*0.007**	*0.315*	*0.000****
95% CI	–4.33, 234.49	–0.73, 4.64	–14.24, –1.16	–628.36, 775.44	0.03, 0.55	0.08, 1.12	–16.12, 5.35	–0.63, 0.23	–400.43, –62.73	–9.93, 3.21	25.08, 87.46
3 vs. 4											
Difference	58.23	–1.39	4.94	616.52	0.07	0.23	6.66	0.25	119.20	6.99	–0.33
SE	68.84	1.55	3.77	404.61	0.15	0.30	6.19	0.25	97.34	3.79	17.98
P	*0.398*	*0.371*	*0.192*	*0.129*	*0.650*	*0.450*	*0.283*	*0.306*	0.222	*0.066*	*0.985*
95% CI	–77.33, 193.79	–4.44, 1.66	–2.49, 12.36	–180.30, 1413.33	–0.23, 0.36	–0.37, 0.82	–5.53, 18.85	–0.23, 0.74	–72.49, 310.88	–0.47, 14.45	–35.09, 35.73
4 vs. 5											
Difference	–51.57	1.89	7.93	30.92	013	0.20	4.64	0.53	225.380	–0.25	–3.96
SE	70.56	1.59	3.86	414.75	0.15	0.31	6.35	0.25	99.77	3.88	18.43
P	*0.466*	*0.234*	*0.041**	*0.941*	*0.409*	*0.521*	*0.465*	*0.037**	*0.025**	*0.949*	*0.830*
95% CI	–190.53, 87.38	–1.23, 5.02	0.32, 15.53	–785.85, 847.69	–0.18, 0.43	–0.41, 0.81	–7.85, 17.14	0.03, 1.03	28.90, 421.86	–7.90, 7.40	–40.26, 32.33

Hypothesized value=0. 1 = 19–25 years, 2= 26–35 years, 3= 36–45 years, 4= 46–55 years, 5= 56+.

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Full Length Research Paper

Antagonistic salubrious effects of Tahitian Noni in *Momordica charantia*-induced cytoarchitectural alterations in rat testes: Parallel light microscopic findings

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Drug interaction is a situation in which a substance affect the activity of another to either increased or decreased its outcome. It could also produce an entirely new effect. To determine if addition of Tahitian Noni dietary supplement can alleviate *Momordica charantia*-induced testicular alterations in Sprague-Dawley rats. Thirty adult male 16 - 20 weeks' old Sprague-Dawley rats randomized into 6 groups (n=5) were used. Group I (DW_{8wk}) the control: administered distilled water for 8 weeks. Group II ($MC_{50\ (8wk)}$): received 50 mg/100 g b.w. of *M. charantia* seed extracts for 8 weeks. Group III ($MC_{50\ (4wk)}$-$N_{5\ (4wk)}$): gavaged 50 mg/100 g b.w. of *M. charantia* seed extracts for 4 weeks thereafter 5 ml/kg of Noni for another 4 weeks. Group IV ($MC_{50\ (8wk)}$+$N_{5\ (8wk)}$): received 50 mg/100 g b.w. of *M. charantia* seed extracts and 5 ml/kg of Noni concurrently for 8 weeks. Group V ($N_{5\ (8wk)}$): received 5 ml/kg of Noni alone for 8 weeks. Group VI ($N_{5\ (4wk)}$-$MC_{50\ (4wk)}$): treated with 5 ml/kg of Noni for 4 weeks and 50 mg/100 g b.w. of *M. charantia* seed extracts for another 4 weeks. The animals were sacrificed on day 57. The testes harvested cleared off surrounding connective tissues and processed for histology. Comparing the slides from treated rats to control, histology showed depletion of spermatogenic cells lines in the seminiferous tubular lumen in $MC_{50\ (8wk)}$. This indicates a harmful effect of the extract. Those exposed to Noni, concurrently or otherwise ($N_{5(8wk)}$, $MC_{50(4wk)}$-$N_{5\ (4wk)}$ and $MC_{50\ (8wk)}$ +$N_{5\ (8wk)}$) tended towards the normal reference. Light microscopic evidence supports the ameliorative effect of Tahitian Noni dietary supplement on the testicular toxicity induced by high dose *M. charantia* extract.

Key words: *Momordica charantia*, Tahitian Noni sprague-dawley, histology, testes.

INTRODUCTION

The use of plant product has increased significantly among adults over the past 15 years, with products such as Echinacea for colds or ginger for nausea now in common use (Teen prescription and OTC drug abuse, 2008). Plant products have enjoyed unparallel patronage either as supplement alternatives or out rightly replacing mainstream medications. Amongst such plants is *Momordica charantia* (MC), which is now being widely publicized and utilized for its hypoglycemic effects. Its efficacy has continually received accolade and described in one study described to improved glucose tolerance to a degree similar to the oral hypoglycemic agent, tolbutamide (Alternative Medicine Review, 2007).

However, in some other cases healthy subjects have employed MC culinary usage simply for its bitter taste to season several menus. Herbal formations are known for being accidentally misused because they are often consumed without prescription (Teen prescription and OTC drug abuse, 2008). The lack of relevant knowledge about the exact quantity of the active ingredients in these substances makes 'frivolous abuse' inevitable. There is

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also the flawed conviction that medications from plant materials are usually or generally safe. This is not true as many abuses with resulting adverse effects have been well documented in literature (Vandana, 2005). Little is known of about the organo-toxicity effect of MC. A recent research work established a negative effect of MC seed extract on the testes of 'healthy' Sprague-Dawley (S-D) rats. It was observed to suppress sperm quotient in a dose dependent manner (Yama et al., 2011a). It also depressed the testicular weight/volume and extra-testicular testosterone concentrations (Yama et al., 2011a). In another study, a conclusive finding suggests the extract resulted in increased testicular oxidative stress amid inclusive compromise of fertility in the treated rats (Yama et al., 2011b).

Tahitian Noni dietary supplement is a liquid dietary supplement made up of 89% *Morinda citrifolia*. Noni, a common name for *M. citrifolia* is one of the most common plants used in herbal remedies (Bassey et al., 2011). Apart from literature citations of the use of the fruit juice in the remedy of a plethora of illnesses (alternative medicine) and in the management of drug addiction (Wang et al., 2002), we have also confirmed qualitatively its ameliorative prospect (Bassey et al., 2011).

Interactions is known to exist between drugs and foods (drug-food interactions), as well as drugs and herbs (drug-herb interactions) (Bushra et al., 2011). In this present study, we evaluated parallel seminiferous tubular (ST) variations in the interactive effect of Tahitian Noni on the testicular micro-anatomy of rats treated with high dose of MC extract.

MATERIALS AND METHODS

Tahitian Noni (*M. citrifolia*) dietary supplement

The regular bottled commercial form of Tahitian Noni dietary supplement produced by Morinda Inc., United States of America was obtained from a registered Tahitian Noni distributor. The producing company's bottle cap was observed intact before commencement of use.

Plant procurement and processing of seed extract

Fresh fruits of *M. charantia* (from plants grown in Southern part of Nigeria) were acquired in a local market at Mushin, Lagos State Nigeria. The same was identified and authenticated by Professor J. Olowokudejo, a taxonomist in the Botany Department of the University of Lagos, where the voucher specimen was deposited (ascension number FHI 108422) in the herbarium.

The processes leading to the constitution of the appropriate formulation of 230 g of MC in 1000 ml of methanol was done in the Pharmacognosy Department CMUL. These included drying the fruits to get seeds which were then weighed and Soxhlet extraction done using alcohol (absolute methanol) as solvent (Yama et al., 2010). The percentage yield of the seed extract obtained was 23.0% w/w; from the dried extract (alcohol evaporated), 50 mg/100 g body weight (b.w.) dose was prepared in distilled water immediately before use.

Animals

This study was conducted on thirty healthy adult male albino rats of Sprague-Dawley breed weighing between 105 - 200 g. The rats were allotted randomly into six groups: a control and five experimental groups. They were obtained from the Department of Biochemistry, College of medicine University of Lagos (CMUL). The rats were housed in well-ventilated metal cages under standard conditions (temperature: 28 - 31°C; light: approximately 12 h natural light alternating with 12 h darkness per day; humidity; 50 - 55%) in the Department of Anatomy, CMUL. They were allowed free access to standard laboratory food and water *ad libitum* throughout the experiment. The animals were kept for at least 2 weeks to acclimatize to the laboratory conditions before experimentation.

Experimental procedure, necropsy schedule and animal ethics

The investigation protocol was approved by the Departmental Ethics Committee CMUL. The test solutions (MC, Noni and distilled water) were administered between the 7 - 9 h daily to the treated and control animals with a metal canula by gastric gavage. The total experimental duration was 56 days to ensure the test solutions effects were observed for one complete spermatogenic cycle (Jegou et al., 2002). The animals were treated with distilled water, extract and Noni as follows:

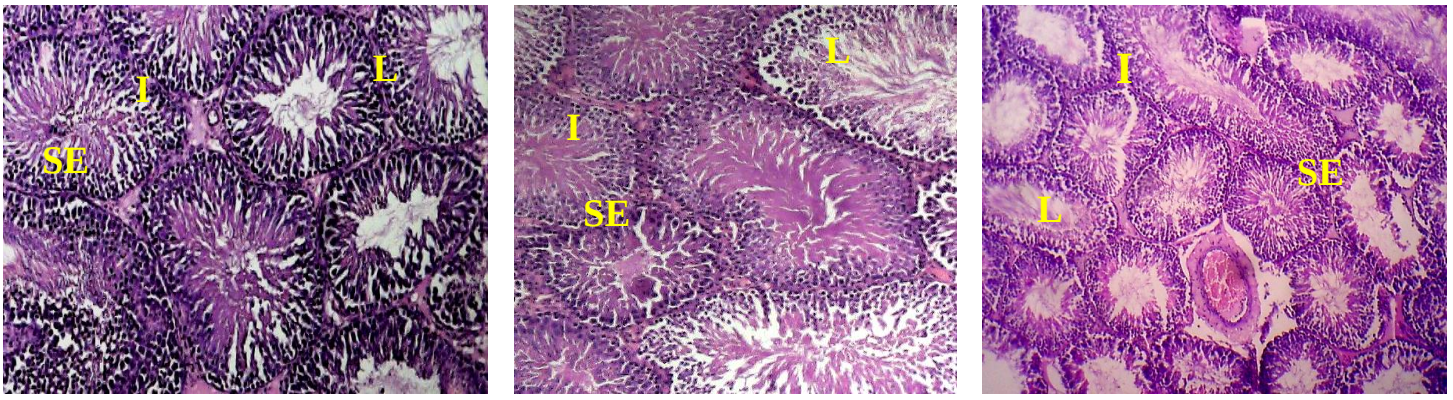
1. Group I (DW_{8wk}), control: Distilled water (4 - 5 ml) for 8 weeks.
2. Group II ($MC_{50(8wk)}$): MC seed extract (50 mg/100 g b.w.) for 8 weeks.
3. Group III ($MC_{50(4wk)}$-$N_{5(4wk)}$): MC seed extract (50 mg/100 g b.w.) for 4 weeks alternating with Noni (5 ml/kg) for another 4 weeks.
4. Group IV ($MC_{50(8wk)}$+$N_{5(8wk)}$): MC seed extract (50 mg/100 g b.w.) and Noni (5 ml/kg) concurrently for 8 weeks.
5. Group V ($N_{5(8wk)}$): Noni (5 ml/kg) alone for 8 weeks.
6. Group VI ($N_{5(4wk)}$-$MC_{50(4wk)}$): Noni (5 ml/kg) for 4 weeks thereafter MC seed extract (50 mg/100 g b.w.) for another 4 weeks.

On the 57th day, the animals were made unconscious by cerebral dislocation which allowed the surgical procedure involving a ventral laparotomy to be carried out. The testes were delivered *per* abdomen.

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

Tissue processing for histological studies

The harvested testes were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were processed by the method described previously (Animal tissue techniques, 2009) with slight modification. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 h, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicular to the long axis of the testes. The sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of



(a) DW_{8wk} **(b)** $N_{5(8wk)}$ **(c)** $MC_{50(4wk)}$-$N_{5(4wk)}$

Figure 1. Cross-section of the seminiferous tubules of rats treated with: (a) 4-5 ml distilled water: DW_{8wk}; (b) 5 ml/kg of Noni: $N_{5(8wk)}$.(c) 50 mg/100 g of *Momordica charantia* seed extract for 4 weeks and afterwards 5 ml/kg of Noni for 4 weeks: $MC_{50(4wk)}$-$N_{5(4wk)}$). Stains: Haematoxylin and Eosin. Mag. × 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium.

tissue, fixed on clean slides to which Mayer's egg albumin had been coated to cement the sections to the slides properly and later stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene the sections were oven-dried between 35 and 40°C.

RESULTS

Histological assessment the testes

Histological sections from groups DW_{8wk}, $N_{5(8wk)}$ and $MC_{50(4wk)}$-$N_{5(4wk)}$ were similar. The ST, contained germ cells at all levels of differentiation with maintenance of normal polarity of germ cells. The interstitium contained normal Leydig cells. However $N_{5(8wk)}$ showed more densely populated ST (Figure 1).

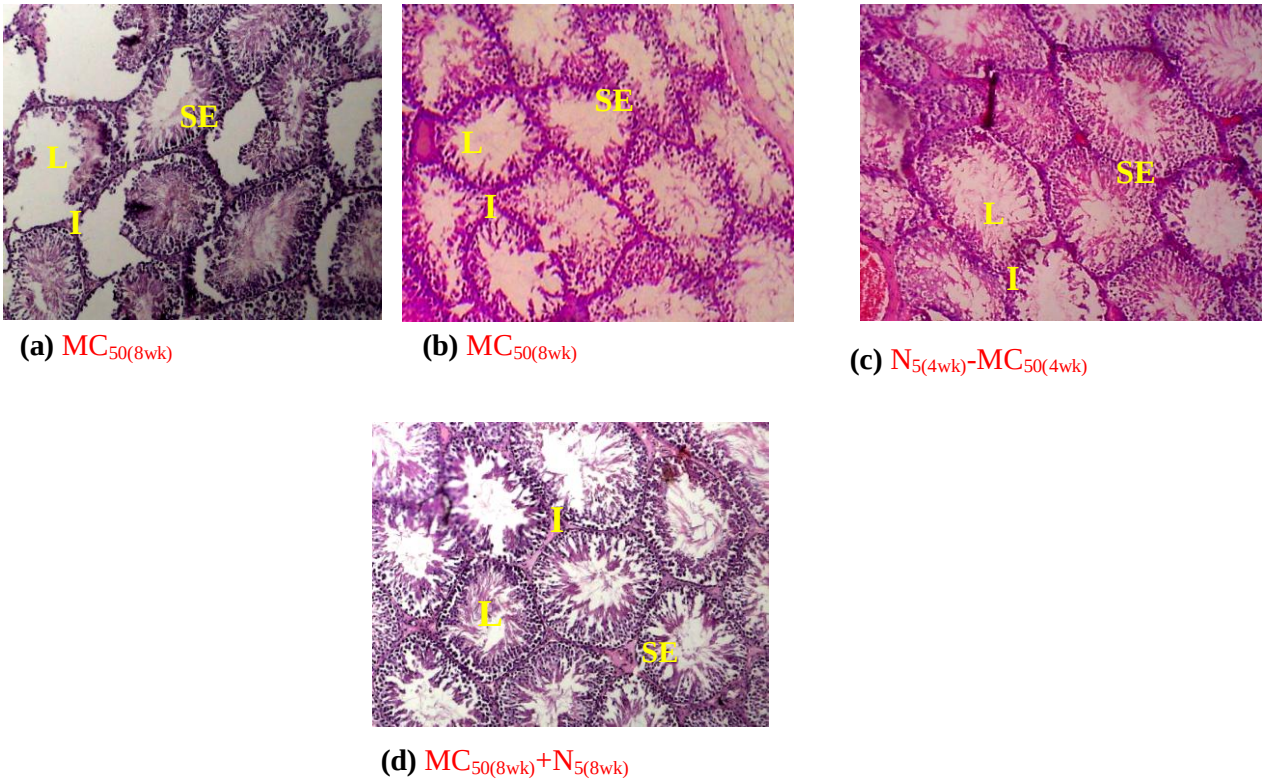
Sections from $MC_{50(8wk)}$ showed tubular hypocellularity, interstitium diminution and destruction of the spermatid layer with few to absent luminal spermatozoa (Figure 2). $N_{5(4wk)}$-$MC_{50(4wk)}$ slides were similar only degree of alteration was lesser.

Finally, histological sections from $MC_{50(8wk)}$+$N_{5(8wk)}$ rats revealed basal epithelium and the tubular lumen was fairly well populated with viable germ cells at various spermatogenic stages. The tubular cross sections showed profiles that were fairly regular, signifying some protective role.

DISCUSSION

Just as the recreational use of over-the-counter and prescription drugs are on the rise, there is also a growing trend of use of herbal drugs. One reason for this shift to herbal drugs is that users tend to think of herbal products as natural and safe. But many of these products can be dangerous and even deadly (Teen prescription and OTC drug abuse, 2008). Different herbal formulations act in different ways causing harmful/toxic effects to human physiology (Vandana, 2005). MC herb of ubiquitous importance and gaining popularity as alternative in the management of diabetes. It was recently shown to result in dose dependent alteration in the sperm production and androgen levels when administered for a duration equivalent to one spermatogenic cycle (Yama et al., 2011a). Failure of the pituitary gland to produce adequate amounts of gonadotrophins can lead to decreased sperm counts (Al-Daghistani and Abdel-Dayem, 2002) and might be the factor in the reduction of sperm counts. This correlates with features from our histological findings of rats fed high dose of MC. The extract showed it was capable of causing seminiferous testicular alteration. There was decreased tubular cellularity, as well as modification in the interstitium (Leydig cells); while in certain regions the epithelia showed focal degeneration with few to absent luminal spermatozoa. Conversely, rats treated pre-treated with MC before administering the dietary supplement recuperated because they had a comparable histoarchitecture as the normative control. Their germ cells well organised and stratified and all cells of the spermatogenic series represented. The tubular lumens observed to contain many viable germ cells.

The histological findings of the testes in the slides reviewed from the rats administered MC and Noni showed modulating effect and patterns similar to control. There were no marked disruptions in the basal epithelium and the tubular lumen was fairly well populated with viable germ cells at various spermatogenic stages. The tubular cross sections showed profiles that were fairly regular, signifying some protective role. The testes from rats inspected for a probable prophylactic effect showed sections similar to those treated with MC although



(a) $MC_{50(8wk)}$ **(b)** $MC_{50(8wk)}$ **(c)** $N_{5(4wk)}$-$MC_{50(4wk)}$

(d) $MC_{50(8wk)}$+$N_{5(8wk)}$

Figure 2. Cross-section of the seminiferous tubules of rats treated with: (a) and (b) 50 mg/100 g of *Momordica charantia* seed extract for 8 weeks: $MC_{50(8wk)}$ (c) 5 ml/kg of Noni for 4 weeks afterwards 50 mg/100 g *Momordica charantia* seed extract for another 4 weeks: $N_{5(4wk)}$-$MC_{50(4wk)}$; (d) concurrently with 50 mg/100 g *Momordica charantia* seed extract and 5 ml/kg Noni for 8 weeks: $MC_{50(8wk)}$+$N_{5(8wk)}$). Stains: Haematoxylin & Eosin. Mag. x 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium.

there were moderately populated areas.

The exact mechanism by which Tahitian Noni brought about the effects is unknown the following pathways are speculative. It is known that Tahitian Noni acts as a powerful immune modulator (Bassey et al., 2011; Heinicke, 1985); hence it helps in keeping the endocrine system in a well balanced condition and so may help in cases of sterility. In a case of altered spermatogenic cell lines, Noni may have helped to improve this condition. Studies have shown that MC possesses antioxidant property (Technical data on *M. charantia*, 2002; Dhanasekar and Sorimuthu, 2005) which is expected to produce a protective effect on the testes. Researchers have shown that depending on the dose a substance may act either as a pro-oxidant or antioxidant (Tan et al., 2000). The MC extract acted as a pro-oxidant due to the prolonged daily exposure (4–8 weeks) and at a high concentration 50 mg/100 g (Yama et al., 2011b) the body could not adapt or overcome to its effect, generating free radicals. Thus if the observed histological alteration produced by MC is due to a high increase in free radicals' damage, Noni may have helped to improve this condition by its 'supreme' anti-oxidant properties. Also Noni is able to affect so many systems through its ability to promote new cellular growth and to repair damaged cells (Bassey et al., 2011; Heinicke, 1985).

Conclusion

Although more work needs to be done in identifying biochemical and cellular events such as apoptosis, necrosis and cellular proliferation to corroborate further the testicular histoarchitectural findings. This study has presented histological evidences which gives clue to the primary cells affected during the interaction of Tahitian Noni and MC. The dietary supplement may possibly be useful in antagonizing the effects of high dose of the extract by preventing or reversing testicular toxicity.

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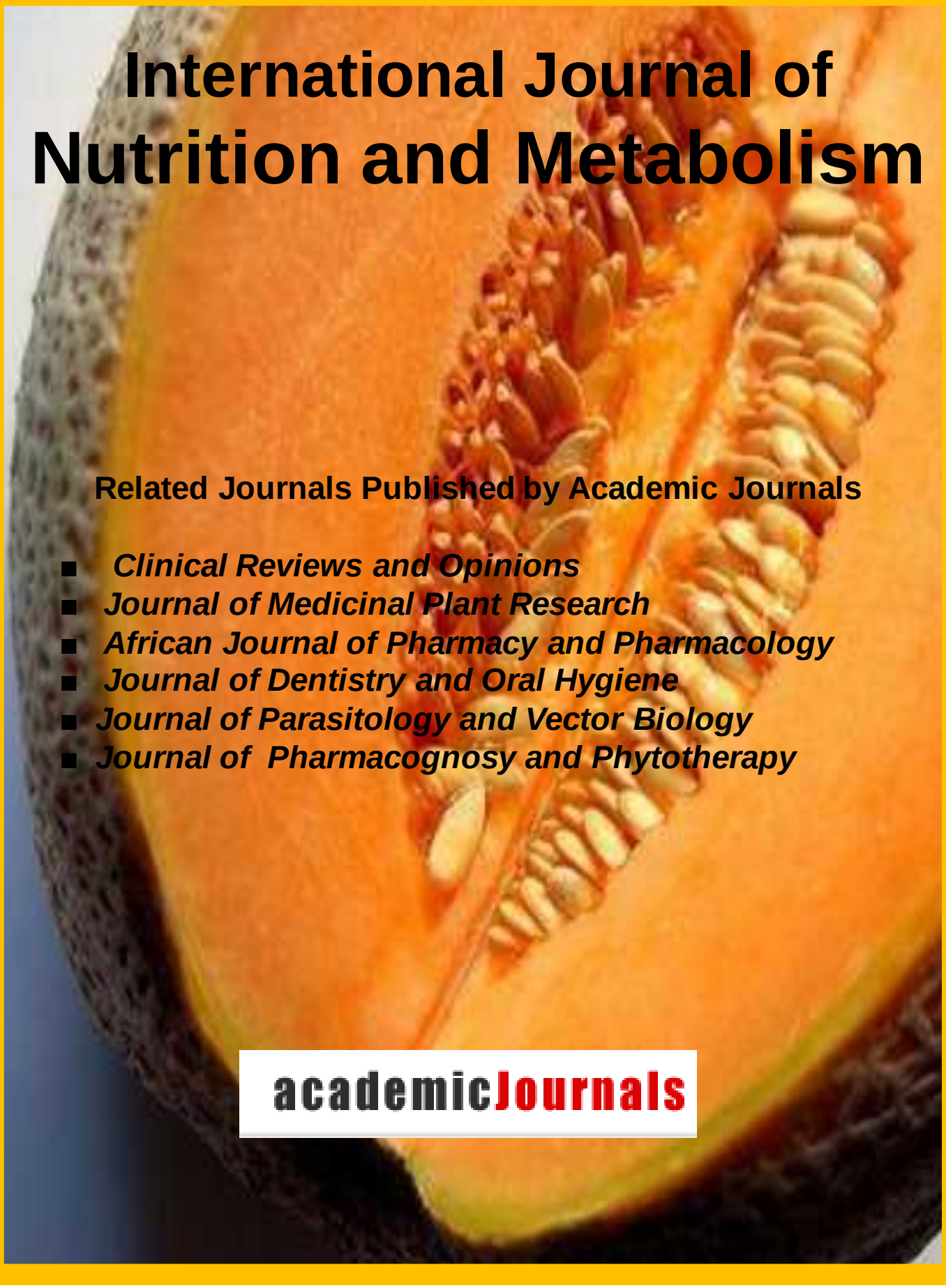

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