

## Stability of Vitamin A in Nigerian Retailed Biscuits

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### Abstract

Vitamin A deficiency is a major public health problem affecting poor populations in developing countries. Biscuits baked with Nigerian vitamin A fortified flour (30 IU/g) have been consumed by pre-school children. This study aims at determining vitamin A content and stability in retailed biscuits at point of consumption. Pre-tested, semi-structured, interviewer-administered questionnaire was used to collect biscuit consumption pattern of pre-school children (n=1600). Out of 18 brands of biscuits reported, eight cartons of 8 commonly consumed brands were purchased from major markets in Lagos. Vitamin A (retinol) stability was determined by storing biscuit samples for 30 days. Pre- and post-storage retinol analyses were carried out using high performance liquid chromatography. Vitamin A stability was calculated as percentage of initial vitamin A biscuit values. Crunchiness and packaging of biscuit samples were also assessed. Data were analysed using descriptive and T-test at  $p < 0.05$ . At pre-storage level, 62.5 % and 37.5 % samples had vitamin A and zero contents respectively. At post-storage, 25% had vitamin A content while 75% had zero content. Pre- and post-storage vitamin A content of samples was  $5.2 \pm 4.9$  IU/g and  $1.9 \pm 1.8$  IU/g. Mean vitamin A stability and loss in retailed biscuits at 2 months was 16.8% and 83.2% respectively. A significant difference was found in vitamin A content and stability of biscuits at pre- and post-storage levels. Biscuits lost crunchiness at post-storage level. Vitamin A content of retailed biscuit samples was below 30 IU/g resulting in very low stability. Use of fortified quality raw materials and compliance are essential.

**Keywords:** biscuits, flour, fortification, high performance liquid chromatography, pre-school children, retinol, vitamin A deficiency, vitamin A stability

### 1. Introduction

The World Health Organization estimates that at least a third of the world's population is affected by micronutrient malnutrition (Allen et al. 2006). Vitamin A is a fat-soluble vitamin which plays an important role in vision, bone growth, reproduction, and in the maintenance of healthy skin, hair, and mucous membranes (FAO/WHO, 2002). Vitamin A cannot be synthesised within the body and so can only be derived from dietary intake from animal and plant sources. Inadequate consumption of vitamin A sources results in vitamin A deficiency leading to adverse effects on growth, reproduction and resistance to infection. Xerophthalmia is a severe consequence of vitamin A deficiency (VAD) which might lead to an irreversible blindness especially in pre-school children, pregnant and lactating mothers. A common cause of vitamin A deficiency might be a shift in the local diet to imported and ready-to-eat foods (Englberger et al. 2005). The increasing use of highly refined foods, and foods prepared from highly purified ingredients, may contribute to dietary vitamin A inadequacies in certain population (Manan, 1994). It has been suggested that the high prevalence of VAD in Nigerian communities was due to low dietary intake of vitamin A, dominant dietary staples being cassava and other carbohydrate-rich foods that are virtually devoid of vitamin A and carotenoids (Tee, 1995).

Vitamin A deficiency has also been reported as essentially a result of poor-socio-economic environment (Oomen, 1976). Vitamin A deficiency (VAD) is a global public health nutritional problem. In Nigeria 29.5% pre-school children are vitamin A deficit (NFCNS 2001-2003, 2004; Maziya-Dixon, Akinyele, Sanusi, Oguntona, Nokoe, and

Harris, 2004; Sight and Life, 2013). Forty one percent of the childhood deaths are caused by measles while 4% are as a result of diarrhoeal disease (World Health Organization (WHO, 2014). These diseases are related to vitamin A deficiency (VAD). Flour fortification is one of the long-term sustainable nutritional strategies to eradicate micronutrient deficiencies in vulnerable populations in the world. Nigeria is the only country in Sub-Saharan Africa fortifying three staples with vitamin A: wheat (*Triticum aestivum*) and maize (*Zea mays*) flour (30 IU/g), refined sugar - white and brown (25 IU/g) and margarine (26 – 33 IU/g). Wheat flour is one of the major raw materials for baking biscuits also referred to as cookies in some countries.

Vitamin A is unstable to many environmental, physical and chemical factors such as moisture, heat, sunlight, metals (copper, iron, zinc), humidity, high temperature and pH. Many developed countries have reported good stability of vitamin A in flour and its products (Murphy, 1995; Piza and Nelson, 1998; Mansoor, 2007; USAID/DSM, 2007a, b). However, the standard technology for fortification of different foods have not been fully resolved with regard to nutrient levels, stability, physical property characteristics and consumer acceptance in terms of cooking properties and taste, among other factors (WHO/FAO/UNICEF/GAIN/MI/FFI, 2009). Stability of staple food additives under different storage and cooking conditions is a one of the problems encountered in vitamin A fortification (Mannar and Gallego, 2002). Vitamin A degrades with time (Wirakartakusumah and Hariyadi, 1998). Significant losses can occur on storage if the encapsulation and antioxidant protection system is poor (Flour Fortification Initiative, 2014). While fortification of flour with vitamin A has been initiated in a few countries, questions remain about the cost of adding vitamin A to flour, as well as the stability of the vitamin A in flour and flour products (Flour Fortification Initiative, 2008).

There have been some few reports on vitamin A stability in biscuits. According to Piza and Nilson, (1998), when wheat flour is fortified with vitamin A, the typical losses during the production of bread and biscuits are 30 % and 40 %. Vitamin A losses from premixes with different forms of added iron ranged from 3 % to 46 % in products such as baked loaves of bread, raw noodles (prepared from hard flour), and biscuits (prepared from soft flour). Vitamin A losses, however, were 20 % to 30 % in biscuits, irrespective of the type of iron fortificant (Solon, Sancex-Fermin, Wambangco, Solon, 1999). Naturally, biscuits contain no vitamin A. It is the fortified flour that is used as major raw material that is expected to introduce vitamin A into biscuits. The aim of this study therefore is to determine vitamin A content and stability in Nigerian retailed biscuits produced with fortified flour at point of consumption.

## **2. Materials and Methods**

### *2.1 Assessment of Consumption Pattern of Biscuits in the Diet of Pre-School Children*

The consumption pattern of biscuits by pre-school children was assessed using a pre-tested semi-quantitative food frequency questionnaire (FFQ) as done by Steyn and Labadarios (2002) and Blanton et al. (2006).

### *2.2 Identification of Commonly Consumed Brands of Biscuits by Pre-School-Aged Children*

#### **2.2.1 Inclusion Criteria**

Samples were selected for inclusion in the study based on percentage of the population reporting the food. Only samples consumed by at least 5% of the total population in each LGA were considered as commonly consumed as done by Fox, Reidy, Karwe and Ziegler (2006). Out of 18 brands of biscuits reported, 8 were commonly consumed by at least 5% of the children.

#### **2.2.2 Sample Sourcing**

Eight commonly consumed biscuit brands were sourced at point of consumption (wholesale outlets) according to methods of Nalubola, Nestel, Dexter and Alnwick (1998) and Yusufali, Sunley, de Hoop and Panagides (2012). The most current batch of selected commonly consumed biscuit samples in the market were purchased in cartons from major markets in the same study locations - Ejigbo, Mushin, Oshodi, Agege and Ikorodu markets in Lagos State. Samples were bought in cartons to be sure of their production dates. Production date helped to determine sample freshness. Sampling replicates were carried out according to consumption pattern as follows: Sample A: 2; Sample B: 3; Sample C: 1; Sample D: 1; Sample E: 3; Sample F: 3; Sample G: 1 and Sample H: 1. The following information was recorded for each sample: (i) Date of production written on cartons and packets in order to determine how long the sample has stayed or the post-production time. (ii) Date of sampling/analysis (iii). Brand name (iv). Batch number if given and (v). Laboratory sample code for identification of each sample.

#### **2.2.3 Selection of Samples**

As standard laboratory procedure, 30% of biscuit samples were randomly selected from each biscuit carton for analysis as done by Oyunga-Ogubi, Okwach, Waudu, Makokha, and Oiye (2009). Suitable sample interval was used to

select packets of biscuits from cartons. This procedure was followed for selecting samples from all the eight brands of biscuits.

#### 2.2.4 Sample Storage

Biscuit samples were stored for 30 days after initial analysis under room temperature similar to that done by F. Solon, M. Solon and Nano, (1998); Solon et al. (1999); Solon, Klemm, Sanchez, Darnton-Hill, Craft, Christian and West Jr., (2000); F. Solon, M. Solon, Nano, Limson, Mondoza, Sanchez and Wambangco (2008); Cort, Borenstein, Harley, Osadca and Scheiner (1975) and Cort, Borenstein, Harley, Osadca and Scheiner (1976).

#### 2.2.5 Vitamin A Content Analysis

Pre- and post-storage retinol analyses were carried out using high performance liquid chromatography (HPLC) according to Association of Official Analytical Chemists methods (AOAC, 2000) as described in Uchendu and Atinmo (2012); Uchendu, Atinmo and Oyewole (2012) and Uchendu and Atinmo (2016). The analysis was carried out in batches in three laboratories at different times of sample collection: Standard Organization of Nigeria (SON), Honeywell Flour Mills Plc and BATO Chemical Laboratories Limited. All samples were analysed for vitamin A content within 24 hours of collection in duplicates and mean values taken. Each sample was analysed on the day it clocked 30 days post-production.

##### 2.2.5.1 Preparation of Sample Matrices

- i). Random selected samples of biscuits were shredded and crushed into tiny pieces.
- ii). The samples were quickly ground to a fine powder just before the analysis started using Sonik grinder. This increased the surface area of the samples for the subsequent analysis.
- iii). Samples were homogenized very well. A random sample was taken by dividing the sample into four equal parts and samples were taken from each part of the quarter.
- iv). Appropriate weights of samples were quickly transferred into 250mls quick-fit/50mls centrifuge bottles, corked and labelled A, B, C, D, E, F, G, H, etc for easy identification ready for analysis.

##### 2.2.5.2 Vitamin A Extraction and Quantification Procedures

#### **Sample matrix: biscuits**

##### **Procedure**

1. About 5.0 g unknown sample (biscuits) was weighed into a 30 ml screw cap bottle.
  2. About 1.0 g of Ethylene diamine tetra acetic acid (EDTA) was weighed and added to each of the samples. EDTA is a chelating agent and was added to remove elementary iron in the flour. It is also an anti-oxidant which improves the stability of vitamin A even at high temperatures.
  3. 25 ml Ascorbic acid was added to each of the samples using pipette filler under fume extractor as antioxidant to avoid vitamin A oxidation from peroxides present in ethyl acetate, diethyl ether, and isopropyl ether used in vitamin A extraction.
  4. The samples were agitated with vortex mixer for two minutes.
  5. 10mls 50 % KOH\* was added to the samples. This was done three times.
  6. The samples were agitated with vortex mixer for two minutes.
  7. The samples were incubated in a water bath for 45 minutes at 65 °C to melt the retinol (MP = 62-64 °C) in the matrix.
  8. The samples were brought out and allowed to cool for about 10 minutes.
  9. 25 ml Hexane was added to the samples each. This extraction was done three times.
  10. The samples were agitated with vortex mixer for two minutes.
  11. The samples were allowed to sediment into layers for 5minutes.
- If emulsion was formed, the sample bottle was tapped or that side of the bottle was placed on the vortex mixer to check it off.
12. From the two layers formed, the upper layer which is assumed to be the vitamin A is pipetted into another 30 ml screw cap bottle already labelled using micro pipette.
  13. The vitamin A extracts were filtered into the 1.5 ml ember vial bottles using 0.45 µl disposable filter and syringe.

14. The 1.5ml ember vial bottles were put in the HPLC and the readings taken.  
 15. The machine was flushed for 1 hour after the analysis to avoid clogging the column.  
 16. The calculation of this HPLC is automated. It read the vitamin A contents of the samples in IU/Kg.

Quantification is with HPLC- UV detector

Mobile Phase: Hexane 99 %, Isopropanol 1 %

Flow rate- 1 ml/min

Response time – 1.0 sec

UV Detection at 325 nm

Premix was treated as sample.

17. Calculation of vitamin A content was similar to that of A2Z USAID, 2010 and Andarwulan et al. 2014.

$$C_{\text{vitamin As}} = \frac{(\text{RFA}) (\text{Ps}) (\text{Vml})}{(\text{Ws})} \quad (1)$$

where:

$C_{\text{vitamin As}}$  = Vitamin A concentration in sample, IU/Kg (as retinol)

RFA = Response /Correction factor for vitamin A

Ps = Total peak area of test sample (all trans- and 13-cis retinol)

Vml = Volume of hexane used for dilution/extraction (ml)

Ws = Sample weight (g)

#### 2.2.6 Calculation of Percentage Vitamin A Stability in Samples

Vitamin A stability was calculated as percentage of initial vitamin A value as follows:

$$\% \text{ vitamin A stability} = \frac{\text{Mean sample post-storage vitamin A content}}{\text{Pre-storage vitamin A content}} \times 100 \quad (2)$$

Pre-storage vitamin A content

#### 2.2.7 Calculation of Vitamin A Stability Losses in Samples

Vitamin A losses were computed in percentages to estimate baking losses as follows:

$$\text{Vitamin A stability losses (\%)} = 100 - \text{Vitamin A stability (\%)} \quad (3)$$

#### 2.2.8 Tastes for Biscuit Crunchiness

At purchase and post-storage, biscuit samples were tasted to check freshness and crunchiness (hardness and crispiness).

#### 2.2.9 Biscuit Packaging

The packaging of the biscuit samples were examined to know the type of packaging used.

#### 2.2.10 Statistical Analysis

Samples were grouped as 1 and 2 months using sample production dates. Data were arranged in tables and percentages. Data were analysed using descriptive and student T-test to test significance of sample vitamin A content and stability at  $p < 0.05$  significant level.

### 3. Results

Table 1 shows that out of 18 brands of biscuits reported, 8 were commonly consumed by at least 5% of the children.

Table 1. Brands/ Types of Biscuits Consumed by Pre-school-aged Children (N = 1599)

Brand of biscuits:	LGAs				
	Oshodi/Isolo	Agege	Mushin	Lagos Island	Ikorodu
None	62(19.4)	171(53.4)	205(64.0)	167(52.4)	170(53.1)
A	19(5.9)	2(0.6)	15(4.7)	4(1.3)	12(3.7)
B	43(13.5)	43(13.5)	10(3.1)	65(20.3)	10(3.1)
C	27(8.5)	4(1.3)	5(1.6)	2(0.6)	8(2.5)
D	32(10.0)	3(0.9)	4(1.3)	1(0.3)	5(1.6)
E	22(6.9)	9(2.8)	25(7.8)	16(5.0)	10(3.1)
F	38(11.9)	41(12.9)	10(3.1)	37(11.6)	12(3.8)
G	46(14.4)	3(0.9)	4(1.3)	1(0.3)	9(2.8)
H	3(0.9)	8(2.5)	2(0.6)	2(0.6)	48(15.0)
I	7(2.2)	1(0.3)	2(0.6)	4(1.3)	10(3.1)
J	1(0.3)	2(0.6)	0(0.0)	0(0.0)	0(0.0)
K	1(0.3)	2(0.6)	2(0.6)	2(0.6)	0(0.0)
L	2(0.6)	11(3.4)	0(0.0)	8(2.5)	0(0.0)
M	2(0.6)	2(0.6)	14(4.4)	1(0.3)	8(2.5)
N	2(0.6)	1(0.3)	5(1.6)	2(0.6)	0(0.0)
O	2(0.6)	3(0.9)	1(0.3)	3(0.9)	7(2.2)
P	1(0.3)	7(2.3)	6(1.9)	1(0.3)	5(1.6)
Q	3(0.9)	4(1.3)	0(0.0)	0(0.0)	0(0.0)
R	7(2.2)	3(0.9)	10(3.1)	3(0.9)	6(1.9)
	320(100.0)	320(100.0)	320(100.0)	319 (100.0)	320(100.0)

\*Numbers in brackets are in percentages

Table 2 shows the pre- and post-storage vitamin A content in retailed biscuit samples. There was a significant difference between vitamin A content obtained from retailed biscuit brands at pre- and post-storage (1 and 2 months) levels ( $p < .05$ ).

Table 2. Pre- and Post-storage Vitamin A Content in Retailed Biscuit Samples

Biscuit brands	Pre-storage vitamin A content (IU/Kg) (1 months)**	Post-storage vitamin A content(IU/Kg) (2 months)**	P-value
A	0.0 <sup>a</sup>	0.0 <sup>a</sup>	
B	1,847.7 <sup>a</sup>	0.0 <sup>b</sup>	0.000
C	6,797.6 <sup>a</sup>	0.0 <sup>b</sup>	0.000
D	12,873.0 <sup>a</sup>	3,136.0 <sup>b</sup>	0.001
E	0.0 <sup>a</sup>	0.0 <sup>a</sup>	
F	0.0 <sup>a</sup>	0.0 <sup>a</sup>	
G	3,361.5 <sup>a</sup>	0.0 <sup>b</sup>	0.000
H	943.8 <sup>a</sup>	563.6 <sup>b</sup>	0.020
<b>*Mean</b>	<b>5,164.7 ±4,851.7</b>	<b>739.9 ±1361.5</b>	

\*Mean excludes samples with zero vitamin A content at 1 month and 2 months

\*\*Means in same row followed by different letters are significantly different ( $p < 0.05$ ).

Table 3 shows the pre- and post-storage vitamin A stability in biscuit samples. Significant difference existed in vitamin A stability among biscuit brands ( $t = -7.100$ ,  $df = 4$ ,  $p = .002$ ).

Table 3. Pre- and Post-storage Vitamin A Stability in Biscuit Samples

Biscuit brands	Pre-storage vitamin A content (IU/Kg) (1 months)	Post-storage vitamin content(IU/Kg) (2 months)	Vitamin A stability % (2months)	P-value
A	0.0	0.0	0.0	.002
B	1,847.7	0.0	0.0	
C	6,797.6	0.0	0.0	
D	12,873.0	3,136.0	24.4	
E	0.0	0.0	0.0	
F	0.0	0.0	0.0	
G	3,361.5	0.0	0.0	
H	943.8	563.6	59.7	
<b>*Mean</b>	<b>5,164.7 ±4,851.7</b>	<b>739.9 ±1361.5</b>	<b>16.8 ±26.2</b>	

#### 4. Discussions

Vitamin A level in biscuits was not specified in the Standard Organization of Nigeria standard (NIS: 111: 1992). Vitamin A content standards for flour-based products should be provided to guide manufacturers. The pre-storage vitamin A content obtained in this study is similar to that obtained by Ogunmoyela et al. (2013) but higher than that reported by Anyika and Uwaegbute, (2005). Mean vitamin A stability was approximately 17%. Individually, one of the biscuit samples (Sample H) had vitamin A stability close to 60% similar to the stability of the premix food starch matrix used. Type of food matrix used for the vitamin A premix encapsulation might have influenced the vitamin A stability of the sample. Nigeria uses 250 CWS premix which is encapsulated with modified food starch matrix. It has been reported that food starch matrix has the least stability among other matrices used for vitamin A premix encapsulation. Vitamin A stability of various matrices used as vitamin A coatings were reported as mannitol (90 %), lactose (89 %), mannitol + sucrose (88 %), mannitol + dextrose (83 %), dextrose (81 %), sucrose (80 %), calcium sulphate (75 %), kaolin (75 %), aluminium hydroxide (73 %), mannitol + starch (70%), mannitol + aluminium hydroxide (60 %), and starch (59 %) after one month storage (Kee-Neng, DeKang & Banker, 1962). The stability of flour obtained in this study at one month post-production time (60.7 %) is similar to the stability of the modified starch matrix used (59 %). This might explain the low stability obtained in this study. More than three quarter of vitamin A stability losses in biscuits confirms the low stability of premix matrix. Mean sample vitamin A stability losses as  $83.2 \pm 26.2$  %.

Vitamin A stability loss is closely related to vitamin A stability of matrices. Vitamin A stability losses in biscuits were higher than that reported by Piza and Nilson, (1998) and Solon et al. (1999).

More than half (62.5 %) of the samples had vitamin A content at purchase even though not up to 30 IU/g standard level. The 37.5 % samples that had zero vitamin A contents indicate partial compliance. Incomplete compliance places the pre-school children that consume these brands of biscuits at more risk of VAD. At post-storage level, only 25% (samples D and H) still had vitamin A content while three quarter (75%) had no vitamin A content. This might be as a result of the quality of the premix. Comparing the vitamin A content of samples with the recommended flour fortification level (30,000 IU/kg), it is clear that vitamin A was greatly reduced in retailed biscuits or that there was low compliance. The samples that had zero vitamin A at purchase and after storage (38%) could be because unfortified flour or un-encapsulated vitamin A premix which is cheaper was used for baking them. Samples that had vitamin A at pre-storage and zero content after storage (62.5%) might be due to reasons such as baking ingredients, baking temperature, level of encapsulation of vitamin A premix used, and quality of packaging material and sealing. Interestingly, the two samples (D and H) that had vitamin A at post-storage level were manufactured by the same company. This company declared on the biscuit labels ingredients such as orange cream (sample H) and skimmed milk, vegetable fat, butter flavour for sample D. Sample D had the highest vitamin A content at pre- and post-storage levels (12,873.0 IU/Kg; 3,136.0 IU/Kg). Sample C (6,797.6 IU/Kg) declared enriched with carotenes. Sample G (3,361.5 IU/Kg) had ingredients such as whole wheat flour, sugar, vegetable oil and milk while sample B (1,847.7 IU/Kg) had sugar, vegetable oil and butter flavour as ingredients among others.

The high vitamin A content in some of the samples might have been contributed by the ingredients used which are all good sources of vitamin A. Nigeria is the only country in Sub-Saharan Africa fortifying four staples- flour (30,000 IU/Kg), sugar (25,000 IU/kg), vegetable oil (20,000 IU/kg) and butter/margarine (butter/margarine 26,000-33,000 IU/kg) with vitamin A. Milk is a very good source of vitamin A. Use of vitamin A fortified staples as biscuit raw materials will enhance the vitamin A content of biscuits. However, sample E had the same ingredients on its label as sample G except whole wheat flour but it had zero vitamin A and the samples were manufactured by the same company. This difference in vitamin A content might depend on label claim integrity and the vitamin A quality of ingredients used for each of the samples. Biscuits are susceptible to rancidity. Rancidity or high peroxide value in vegetable oil, butter and margarine used for baking flour products could reduce their vitamin A contents. Several studies have shown that vitamin A oxidizes faster and losses its activity in the presence of highly-oxidized oils with a high peroxide values and that high peroxide values in oil prior to fortification could be a potential barrier to ensuring the stability of retinyl palmitate (Andarwulan, Gitapratwi, Laillou, Fitriani, Hariyadi, Moench-Pfanner & Martianto, 2014).

All the samples had similar polyethylene aluminum laminated packaging but some laminates were better than others. At post-storage analysis, some of the biscuits were already soft. They have lost their characteristics hardness and crunchiness. This might be as a result of moisture and air pick up from the environment. Presence of moisture and air in biscuits will oxidize the vitamin A and this might also explain the low vitamin A content of the samples. The packaging for biscuits should be able to provide rancidity, moisture and air barrier to avoid vitamin A oxidation. The barrier properties of packages are a combination of the basic moisture-proofness of the

materials used and the effectiveness of the seals. Some of the seals were rough and could have pinholes especially sample E. Pinholes enable moisture and air to penetrate the biscuits. None hermetical sealing of the biscuit packets might have exposed them to moisture and air leading to the oxidation of vitamin A. A standard should be set for moisture-proofness of biscuit packages. Biscuits are exposed to air, heat and sunlight all day long in front of retailers' shops. Isomerization of retinoid compounds is often by exposure to light with or without the addition of any catalyst (Manan, 1994). Aluminium coating can provide a complete barrier to light, moisture, grease and air but it depends on the thickness. The aluminium coating used for biscuit samples had light thickness and this might be due to the expensiveness of aluminium considering that the biscuits samples are sold at lower prices (₦5, ₦10, ₦20, ₦30).

It has also been reported that gluten protects vitamin A from degradation from water activity than starch (Arya and Thakur, 1990). This might be one of the reasons vitamin A stability was best in sample D made with whole wheat flour. It is recommended that biscuit fortification premix should have a higher standard to cushion the effect of low gluten content of soft flour and higher temperature (350 °C) used in baking biscuits.

## 5. Conclusions

This study showed that vitamin A stability in retailed biscuit was low. The low vitamin A stability might have been as a result of none or low vitamin A fortification compliance, quality of ingredients and packaging used. It was discovered that use of vitamin A fortified ingredients such as sugar, vegetable oil, milk, margarine/butter and whole wheat flour in baking might have improved the quantity of vitamin A in some of the biscuit samples. Use of fortified quality raw materials and compliance are essential.

## Conflict of Interest

The authors declare no conflict of interest.

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