Effect of Coconut (*Cocos nucifera*) Water on Elevated Leptin Levels in Rats fed High-fat and High-fructose Diets

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ABSTRACT

Background: Coconut water is the liquid endosperm of coconut (*Cocos nucifera*, Arecaceae) fruit rich in important nutrients such as vitamins, phytohormones, amino acids and phytochemicals. Tender coconut water, the fluid present in young green coconuts and mature coconut water, the clear liquid present in the older brown coconut have been shown to significantly reduce hyperlipidaemia in cholesterol fed rats. Leptin is a 16 KDa peptide hormone that is the product of *ob* gene in adipose tissues. Leptin level in circulation is directly related to adipose tissue mass.

Objective: The effect of tender and mature coconut water on the leptin level of high-fat and high-fructose diet fed rats was investigated in this study.

Materials and Methods: Fifty-five rats were separated into 11 experimental groups of which 4 groups each had high-fat and high-fructose feeding respectively with and without coconut water treatment. The rats were fed for 8 weeks followed by 4 weeks of treatment with tender coconut water, mature coconut water and atorvastatin as reference standard.

Results: Leptin level was significantly reduced ($p<0.01$) by tender and mature coconut water in a manner that was comparable to atorvastatin in both high-fat and high-fructose diet fed rats. Leptin lowering in the high-fat fed rats was similar in both mature and tender coconut water, but the lowering effect was more in the mature coconut water (MCW) than in the tender coconut water (TCW) in the high-fructose fed rats.

Conclusion: Tender and mature coconut water have a lowering effect on leptin level in circulation.

Keywords: Tender coconut water, mature coconut water, hyperlipidaemia, *ob* gene, adipose tissue, atorvastatin.

INTRODUCTION

Coconut is a tropical fruit that belongs to the palm family Arecaceae. It is botanically known as *Cocos nucifera*, with "nucifera" meaning "nut-bearing". It has two edible parts: coconut water and coconut meat, that constitute the endosperm tissue. Endosperm tissues undergo one of three main modes of development which are the nuclear, cellular and helobial modes and the development of coconut endosperm belongs to the nuclear mode (1-3). Coconut water is the fresh, clear fluid inside a coconut, the fruit of the well-known coconut palm tree. Coconut water has long been a prevalent fluid in the tropical countries and is native to continents like South America, Asia and Africa where it can be taken fresh as a natural fruit drink or processed and made available in can or bottle (2, 4). It is fat-free, cholesterol-free, and low in calories but high in nutrients and electrolytes (3). Coconut water is very rich in electrolytes. It has inorganic ions that are essential major and trace elements present in substantial quantities that give profound health benefits (4). Coconut water has close plasma concentration of potassium, calcium, sodium and magnesium; these ions can replenish the electrolytes of the human body and thus give coconut the property of serving as a rehydration drink (5).

Many studies have been carried out on coconut water to test its therapeutic potentials. Coconut water was reported to reduce hyperlipidaemia in cholesterol fed rats. This reduction in lipidaemia was found to be associated with increased activities of lipoprotein lipase in heart and adipose tissue, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (the enzyme that catalyses the rate limiting step in cholesterol biosynthesis) and plasma lecithin-cholesterol acyltransferase (LCAT) (6). Disorder of lipid metabolism has been associated with the consumption of high fat diet and high fructose diet (8, 9). Studies have shown that fructose might play a role in the onset of metabolic disorders and excess weight gain. In a rat model, high fructose diet induced hepatic insulin resistance, increased intrahepatocellular lipids and stimulated hepatic *de novo* lipogenesis after a few days of administration (10). When sustained over a longer period of time, high fructose diet induced hepatic steatosis and insulin resistance with an accompanying accumulation of intramyocellular lipids.

Lipid metabolism is regulated by many hormones and leptin is the most recently discovered and currently well studied. Leptin acts as a regulator of lipid reserves through changes in food intake, energy expenditure and fuel selection, with an emphasis on its direct effects on cellular lipid metabolism (11). Leptin levels in the body have been linked to increase or decrease in appetite which accordingly affects food intake and accounts for a healthy diet status or leads to obesity. Leptin has the primary physiological role of communicating to the central nervous system (CNS) the abundance of available energy stores and to restrain food intake while inducing energy expenditure.

Since diseases caused by lipid metabolism disorders are endemic in many countries of the world, the need to deliver an effective therapy is high and urgent. Science has responded by producing drugs (statins such as lovastatin and atorvastatin) that can help treat and manage these disorders. There are however, undesirable and inevitable side effects associated with the use of these drugs. To deal with the need to provide an effective treatment for lipid disorders while minimizing the side effects, natural plant products such as coconut water have been tested and found to be very effective (6).

This study was carried out to analyse the effect of coconut water on leptin levels in circulation. The results obtained will furnish valuable data on leptin levels in relation to lipid parameters and dieting (high-fat and high-fructose). It will also help to better understand how coconut water affects lipid levels at the hormonal level.

MATERIALS AND METHODS

Chemicals, Reagents and Drugs

The chemicals and reagents used in this study were of analytical grade and were purchased from Sigma Aldrich Chemical Company, Taufkirchen, Germany. Rat leptin kit purchased from B-Bridge International Inc., Santa Clara, CA, USA, was used for the leptin assay carried out in this study.

Atorvastatin 40 mg (Lipitor, Pfizer, New

York, USA) was supplied by Bernados Pharmacy located at Idi-Araba, Surulere metropolis of Lagos, Nigeria.

Experimental Rats

Male Sprague Dawley rats weighing 140-150 g used in this study were obtained from the Animal House of the College of Medicine, University of Lagos, Idi-Araba Campus, Lagos, Nigeria. The animals were housed individually in polypropylene cages and maintained at a temperature of 22±1 ^{o}C with alternate exposure to light and dark for 12 h. Animal study was carried out in line with the Helsinki declaration of 1964.

Animal Feed

The feeds used for this study were prepared locally at the Animal House of the College of Medicine, University of Lagos, Idi-Araba Campus, Lagos, Nigeria. Normal rat chow was composed of crude protein (21%), ether extract (5%), crude fibre (5%), ash (8%), calcium (1%), phosphorus (0.6%) and nitrogen free extract (52%); Fat enriched chow was composed of normal feed (78.8%), cholesterol pure (1%), cholic acid (0.2 %), lard (10%) and egg yolk powder (10%); Fructose enriched chow was composed of normal feed (75%) and pure fructose (25%).

Coconut Water

Fresh coconuts (*Cocos nucifera*) at tender stage (6 months maturity) and mature stage (10 months maturity) were harvested at the Coconut Plantation at Ebute, Badagry, Lagos State, Nigeria and authenticated at the University of Lagos Herbarium with voucher number LUH 6447. The coconuts (tender and mature) were dehusked, broken carefully and their liquid endosperm was collected into separate labelled bottles. The samples were refrigerated at 4 ^{o}C prior to use.

***In-vivo* Studies**

Animal Grouping

A total of 55 male Sprague Dawley rats were used. The rats were placed in 11 groups of 5 animals each and were treated as outlined below:

Group 1: Fed with normal rat chow and water only for the entire 12 weeks of experiment. Group 2: Fed with normal rat chow for 8 weeks and administered normal rat chow with tender coconut water for 4 weeks.

Group 3: Fed with normal rat chow for 8 weeks and administered normal rat chow with mature coconut water for 4 weeks.

Group 4: Fed with high fat chow for 8 weeks and administered normal rat chow with tender coconut water for 4 weeks.

Group 5: Fed with high fat chow for 8 weeks and administered normal rat chow with mature coconut water for 4 weeks.

Group 6: Fed with high fat chow for 8 weeks and administered normal rat chow with atorvastatin for 4 weeks.

Group 7: Fed with high fat chow for 8 weeks and administered normal rat chow only for 4 weeks.

Group 8: Fed with high fructose chow for 8 weeks and administered normal rat chow with tender coconut water for 4 weeks.

Group 9: Fed with high fructose chow for 8 weeks and administered normal rat chow with mature coconut water for 4 weeks.

Group 10: Fed with high fructose chow for 8 weeks and administered normal rat chow with atorvastatin for 4 weeks.

Group 11: Fed with high fructose chow for 8 weeks and administered normal rat chow only for 4 weeks.

Administration of Coconut Water and Atorvastatin

Coconut water and the aqueous solution of atorvastatin were administered orally. Two ml of tender and mature coconut water was administered to each rat on a daily basis throughout the four weeks of intervention. One ml of atorvastatin solution (0.5 mg/kg) was administered to each rat on a daily basis throughout the four weeks of intervention. 0.1 mg/ml atorvastatin was prepared by dissolving a caplet of atorvastatin (40 mg atorvastatin) in 400 ml distilled water.

Blood Collection

Blood collection was done by ocular puncture. Four ml of blood was collected into plain sample bottles to be used for electrolytes analysis and leptin assay. Serum was obtained as the liquid fraction of whole blood after the blood samples were allowed to clot in the sample bottles and centrifuged. The blood samples were refrigerated at 4 ^{o}C prior to use.

Electrolyte Analysis

Sodium and potassium levels were

determined by flame photometry (13) and chloride was determined by mercuric nitrate titrimetric method (14).

Leptin Analysis

This was done using the protocol described in B-Bridge International Inc. (Santa Clara, CA, USA) rat leptin ELISA kit manual. The kit reagents were allowed to equilibrate to room temperature (25 °C) prior to the start of sample preparation. Serum samples were diluted (20-fold) with Diluent Buffer and vortexed well to mix. 100 µl of diluted standards, quality control and serum samples in a microplate were incubated at room temperature for 1 h with shaking at 300 rpm on an orbital microplate shaker. The microplate wells were washed with Wash Solution (350 µl per well), followed by the adding of 100 µl of the diluted biotin-labeled secondary antibody solution into each well. Incubation was repeated at room temperature for 1 h while shaking at 300 rpm on an orbital microplate shaker. The wells were washed and 100 µl of streptavidin-HRP conjugate solution was added to each well. The plate was incubated at room temperature for 30 min and shaken. Again, the wells were washed after which 100 µl of substrate solution was added into the wells while avoiding exposure of the microtiter plate to direct sunlight by covering the plate with aluminium foil. The plate was incubated for 10 min at room temperature without shaking for 20 min at reaction temperature below 20 °C. There was a colour development which was stopped by adding 100 µl of stop solution. Absorbance reading of the plate was then done at 450 nm. Dual wavelength mode incorporating 620-650 nm filter was used to measure the reference absorbance. The absorbance reading was done within 5 min following the addition of stop solution.

Statistical Analysis

All data were presented as mean ± standard error of mean (SEM). One-way Analysis of Variance (ANOVA) followed by Bonferroni's Multiple Comparison Test was done to determine statistical significance of the data set using GraphPad Prism 5 (GraphPad Prism Software, Inc., San Diego, CA, USA). Differences were considered statistically significant at $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, meaning significant, very significant and extremely significant, respectively.

RESULTS

Electrolyte Composition

In the high-fat feeding groups, Na^+ was high in the high-fat untreated and atorvastatin treated groups and low in the high-fat tender coconut water treated group; K^+ was high in the normal diet untreated and high-fat + tender coconut water groups but low in the normal control; and Cl^- was high in the high-fat untreated group and the high-fat + mature coconut water treated group, but low in the high-fat + tender coconut water treated group (Figure 1).

The high-fructose feeding groups had high Na^+ levels in all the groups except the control and the normal diet tender coconut water treated groups; it was lowest in the normal diet + tender coconut water group. There was high K^+ in the untreated test group and low levels in the normal control and high-fructose + tender coconut water groups. Cl^- level was highest in the high-fructose + mature coconut water group and lowest in the high-fructose + tender coconut water group (Figure 2).

Leptin Assay

The leptin assay of Sprague Dawley rats fed high-fat diet for 8 weeks followed by administration of tender coconut water, mature coconut water and atorvastatin in respective groups for 4 weeks showed that leptin level was highest in the untreated test group with a value of 170±7.8 pg/ml and lowest in the control group fed normal diet and mature coconut water with a value of 73.78±1.51 pg/ml (Figure 3). The leptin levels of high fat + tender coconut water and high fat + matured coconut water groups varied significantly ($^{*}p<0.05$) when compared against the high fat + normal experimental control and normal control groups. The levels of high fat + atorvastatin group varied very significantly ($^{**}p<0.01$) when compared against the high fat + normal experimental control group but varied insignificantly ($p>0.05$) with the normal + normal control group.

In the rat groups fed high-fructose diet for 8 weeks followed by 4 weeks administration of either tender coconut water, mature coconut water, or atorvastatin, the untreated test group showed the highest level of leptin with a value of 157.40±2.28 pg/ml, while the lowest leptin remained in the normal diet group treated with mature coconut water having the value 73.78 ±1.51 pg/ml, similar to the high-fat feeding group

(Figure 4). When compared against the high fructose + normal experimental control group, leptin level of high fructose + tender coconut water group varied significantly ($^{*}p<0.05$), high fructose + matured coconut water group varied very significantly ($^{**}p<0.01$) and the high fructose + atorvastatin group varied extremely significantly ($^{***}p<0.001$). However, on comparison against the normal control group, high fructose + tender coconut water group varied significantly ($^{*}p<0.05$) while the high fructose + matured coconut water and high fructose + atorvastatin groups varied insignificantly ($p>0.05$).

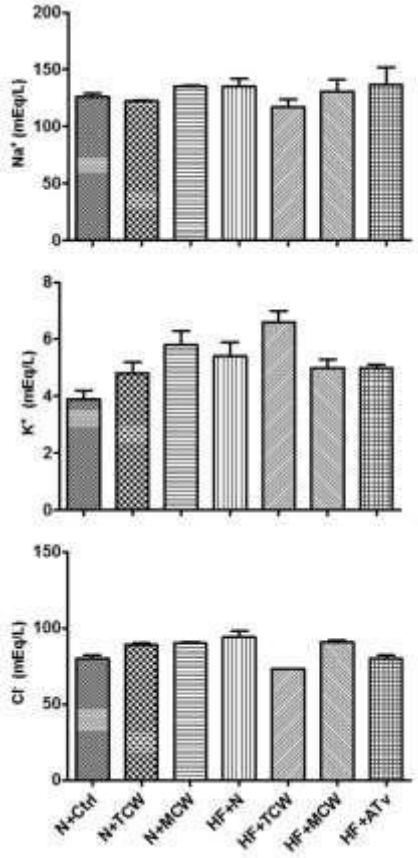

Fig. 1: Electrolytes level in the high-fat diet groups.
N = Normal diet; TCW = Tender coconut water; MCW = Mature coconut water; ATV = Atorvastatin; HF = High fat.
Sodium ion was highest in the high-fat + atorvastatin and lowest in the high-fat + TCW; potassium ion was highest in the high-fat + TCW and lowest in the control; chloride ion was highest in the high-fat + normal diet and lowest in the high-fat + TCW.

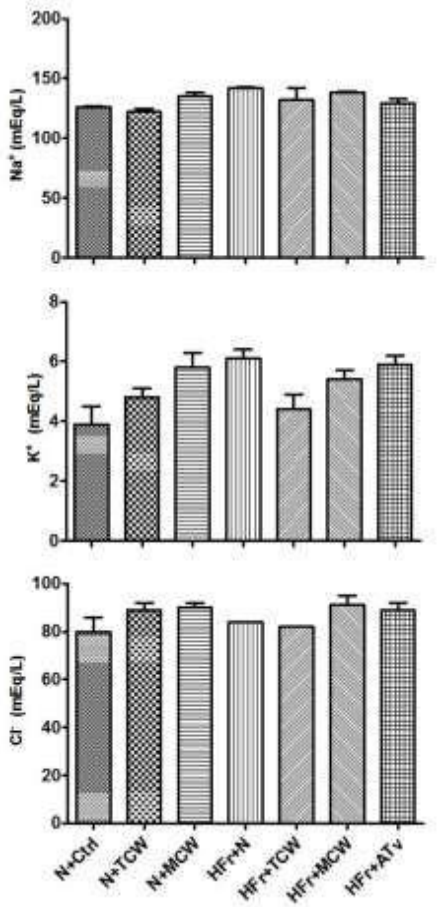

Fig. 2: Electrolytes level in the high-fructose diet groups.
N = Normal diet; TCW = Tender coconut water; MCW = Mature coconut water; ATV = Atorvastatin; HFr = high fructose.
Sodium ion was highest in the high-fructose + normal diet and lowest in the normal diet + TCW; potassium ion was highest in the high-fructose + normal diet and lowest in the normal control; chloride ion was highest in the high-fructose + MCW and lowest in the high-fructose + TCW.

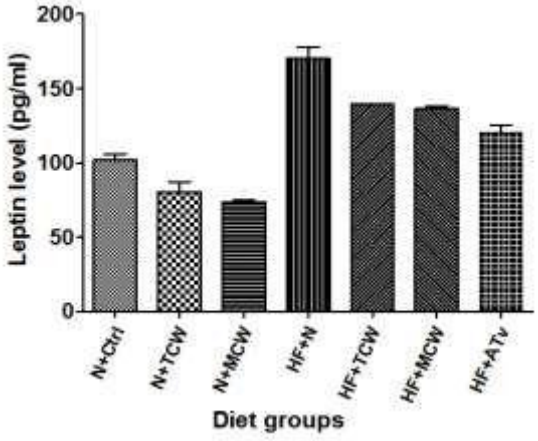

Fig. 3: Leptin levels of high-fat diet fed groups.
N = Normal diet; TCW = Tender coconut water;

MCW = Mature coconut water; ATV = Atorvastatin; HF = High fat.

Leptin level was highest in the experimental group fed high-fat diet for 8 weeks + normal diet for 4 weeks without treatment with tender or mature coconut water. It was lowest in the experimental group fed normal diet for 8 weeks followed by administration of MCW and normal diet for 4 weeks. The leptin levels of HF+TCW and HF+MCW groups varied significantly ($^{*}p<0.05$) when compared against the HF+N experimental control and N+Ctrl normal control groups. The levels of HF+ATV group varied very significantly ($^{**}p<0.01$) when compared against the HF+N experimental control group but varied insignificantly ($p>0.05$) with the N+Ctrl normal control group.

Fig. 4: Leptin levels of high-fructose diet fed groups.

N = Normal diet; TCW = Tender coconut water; MCW = Mature coconut water; ATV = Atorvastatin; HFr = high fructose.

Leptin level was highest in the experimental group fed high-fructose diet for 8 weeks + normal diet for 4 weeks without treatment with tender or mature coconut water. It was lowest in the experimental group fed normal diet for 8 weeks followed by administration of MCW and normal diet for 4 weeks. When compared against the HFr+N experimental control group, leptin level of HFr+TCW group varied significantly ($^{*}p<0.05$), HFr+MCW group varied very significantly ($^{**}p<0.01$) and the HFr+ATV group varied extremely significantly ($^{***}p<0.001$). However, on comparison against the N+Ctrl group, HFr+TCW group varied significantly ($^{*}p<0.05$) while the HFr+MCW and HFr+ATV groups varied insignificantly ($p>0.05$).

DISCUSSION

The analysis of plasma electrolytes of rats fed high-fat and high-fructose diet showed variation in the amounts of potassium, sodium and chloride ions. These ions had highest levels in the control and high-fat/high-fructose untreated experimental groups of this study. Reports from mineral analysis of coconut water have shown coconut water to be rich in potassium (3, 4). It would thus be expected that the experimental groups treated with either tender or mature coconut water should show the highest levels of this ion. There was a moderate conformity with this expectation in the high-fat diet group and a deviation from conformity in the high-fructose diet group. Coconut water as a wholesome fluid has many biologically important constituents that work synergistically to give it the potential for treating health conditions (4). Improvement of the ion composition of plasma might be one of the ways coconut water delivers its important health benefits.

The leptin assay of rat blood samples from the two main feeding groups, high-fat diet and high-fructose diet, showed very significant variation in means across their respective 4 experimental groups (high-fat/high-fructose untreated, high-fat/high-fructose + TCW, high-fat/high-fructose + MCW, high-fat/high-fructose + atorvastatin). The p-value for variability in leptin levels across the 4 experimental groups of the two major feeding groups was 0.0016 for the high-fat feeding group and 0.0029 for the high-fructose feeding group. This study demonstrated that both high-fat diet and high-fructose diet increase leptin secretion in the plasma. The potential of tender and mature coconut water treatment to reduce leptin levels in high-fat diet and high-fructose diet fed rats was investigated. Feeding of rats with normal diet accompanied by treatment with either tender coconut water or mature coconut water did not show any significant reduction of leptin levels in rat blood plasma when compared to the control group fed only normal diet without coconut water administration. High-fat diet alone increased leptin level in rat plasma extremely significantly when compared with the control group. This particular finding is in agreement with the report by Ji *et al*. (16) that high-fat diet increases the circulating levels of leptin in the blood. Treatment of the high-fat group with tender or mature coconut water significantly reduced plasma leptin level when compared with the untreated experimental group given the same diet. There was however, no significant difference between leptin level decrease by tender coconut water and mature coconut water which suggests that both types of coconut water elicit similar effects on leptin secretion. Leptin levels of the high-fat tender and mature coconut water treated experimental groups, compared to the control group, were significantly lower. This latter finding together with the former obtained from this study suggest that although tender and mature coconut water effectively inhibit leptin secretion, this inhibition is not sufficient to return leptin

level to the baseline level of the control group.

In the high-fat atorvastatin treated experimental group, leptin level decrease was very statistically significant when compared to the untreated high-fat diet experimental group. This shows that atorvastatin is relatively more effective at lowering circulating leptin levels than either tender or mature coconut water.

High-fructose diet feeding of rats also increased circulating leptin levels in ways similar to high fat diet feeding. However, treatment with coconut water affected leptin levels in ways that quite contrasted with the pattern showed by high-fat diet feeding. Leptin levels of rats fed normal diet and administered tender coconut water did not significantly differ from that in the control group (fed normal diet only). However, the leptin level of normal diet mature coconut water treated group significantly increased when compared with the control. High-fructose diet untreated experimental group showed marked significant increase in leptin level when compared with the control group. This latter finding corresponds with the report of Kim-Anne *et al.* (10) that high fructose feeding led to a continuous rise in fasting plasma leptin concentrations. Also, experiments by Cammisotto *et al.* (17) performed on isolated adipocytes showed that fructose, glycolytic substrates and metabolites increased leptin secretion. Treatment of the high-fructose group with tender and mature coconut water significantly caused a reduction in leptin level compared to the untreated experimental group. Leptin reduction was however more significant in the mature coconut water treated experimental group compared to the tender coconut water treated experimental group suggesting difference in their elicited effects on leptin secretion. No significant difference exists in leptin levels between high-fructose mature coconut water treated experimental group and the control group, but the difference between high-fructose tender coconut water treated experimental group and the control is significant. The insignificant difference in the former suggests that mature coconut water effectively reduces leptin level to the baseline level of the control group, whereas, the significant difference in the latter suggests that, although tender coconut water attenuates leptin secretion in high-fructose diet fed rats, it does not reverse the increase or return the leptin level to the baseline level.

Leptin level of high-fructose atorvastatin treated experimental group was reduced extremely significantly when compared to the untreated group. High-fructose diet atorvastatin treated experimental group also showed reduction in leptin level that was significant when compared to high-fructose mature coconut water treated experimental group and very significant when compared to the high-fructose tender coconut water treated experimental group. This shows that atorvastatin is the more effective of the three treatment types, followed by mature coconut water. Atorvastatin is one of a group of drugs known collectively as statins. These drugs have adverse effects associated with their usage. They cause mild memory loss, cognitive impairment, dementia and some myopathies. Concerns about these side effects make mature coconut water a more preferred treatment for dyslipidaemia (18, 19). Leptin is synthesized and secreted primarily from adipocytes and it acts centrally to regulate appetite and energy homeostasis (16). Increased adiposity has been linked to increase in circulating leptin levels (15, 16). High fat and high fructose feeding lead to positive energy balance and the stimulation of lipogenesis which leads to increase in adiposity. Tender and mature coconut water have been reported to reduce dyslipidaemia in fat-cholesterol fed rats (7). Also, the lipid lowering effect of tender and mature coconut water was found to be similar to lovastatin (an alternative to atorvastatin used in this study). Leptin is more effectively reduced by atorvastatin administration than mature and tender coconut water according to this study. However, the undesirable side effects associated with atorvastatin or other statin drug administration makes mature coconut water a more preferable and safe alternative.

CONCLUSION

Coconut water as a source of important nutrients and hormones has a lowering effect on leptin level in circulation. Tender and mature coconut water elicit similar lowering effect on leptin secretion in high-fat diet feeding but in high-fructose diet feeding, mature coconut water is more effective than tender coconut water.

CONFLICT OF INTEREST

The authors of this work declare that there is no conflict of interest.

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