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Effect of Natural Cocoa Powder Supplementation on Oxidative Stress in Healthy Ghanaians

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Authors' contributions

This work was carried out in collaboration between all authors. Author ESAA designed the study, wrote the protocol, performed the statistical analysis, managed the literature searches and author GAA wrote the first draft of the manuscript, supervised the bench work and managed the analyses of the study. Author DAA supervised the thesis. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Natural cocoa powder (NCP) has been recognized to possess significant amounts of flavonoids and methylxanthines with healthful benefits. Little or no work has been done on adult Ghanaians involving supplementation with NCP. We hypothesized that chronic consumption of NCP would improve oxidative stress biomarkers and endothelial integrity.
Aims: The study aimed at evaluating the long term effect of NCP supplementation on selected oxidative stress biomarkers in healthy adult Ghanaians.
Study Design: This was a longitudinal intervention study design.
Place and Duration of Study: Chemical Pathology Unit Laboratory,School of Allied Health Sciences (SAHS), Korle-Bu,Virology and Physiology Department Laboratories, University of Ghana Medical School (UGMS) and the Central Laboratory of the Korle-Bu Teaching Hospital, Accra,

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between August 2011 and June 2013.
Methodology: Seventeen subjects were randomly selected. NCP was consumed as a beverage twice daily before meals for 12 weeks. Pre- and post- supplementation blood draw, anthropometry and analysis of advanced glycated end-products (AGEs), vascular cell adhesion molecule1 (VCAM-1), tumour necrosis factor alpha (TNF-α), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and hemeoxygenase 1 (HO-1) were determined.
Results: Mean age was 30.8 ± 10.2 years. Weight and BMI, were significantly decreased by 1.67 kg and 0.05 kg/m^2 (P= 0.009 and 0.006, respectively). Percentage skeletal muscle and resting metabolism showed significant increases of 2.46% and 55 kcal (P= 0.031 and 0.028, respectively). GSH-Px significantly increased by 0.23 ng/L (P= 0.001) while MDA showed a significant decrease of 0.05 ng/L (P = 0.033).
Conclusion: NCP caused a significant reduction in BMI and increase in antioxidant levels. NCP reduced oxidative stress and a cardiovascular risk factor such as obesity.

Keywords: Natural cocoa powder; oxidative stress; supplementation; healthy adults; humans.

ABBREVIATIONS

BMI; body mass index; BMR; basal metabolic rate; CVD; cardiovascular disease; LDL; low-density lipoprotein; NCP; Natural cocoa powder; NO; nitric oxide; Nrf2; Nuclear factor (erythroid-derived 2)-like 2; RONS; reactive oxygen and nitrogen species; TBARS; thiobarbituric acid reducing substances.

1. INTRODUCTION

Ghana produces 26% of the world's cocoa '*Theobroma cacao'* bean which is the gold standard but there is still paucity of scientific documentation of its effects in health and disease states of humans. Flavanoids are beneficial compounds found in many foods including Natural Cocoa Powder (NCP), fruits, vegetables, teas and wines [1]. Cocoa flavanoids are said to possess activities like increased muscle recovery and energy metabolism [2]. In addition to these, cocoa is said to have vasodilatory and hypotensive effects [3-5], anti-malarial [6], antioxidant [1,7,8], aphrodisiac [9,10] and anti-carcinogenic properties. Cocoa also showed its hypo-lipidemic ability to decrease rat LDL-cholesterol and triglycerides [11], weight control and/or anti-obesity effects in humans [9], cardiopulmonary functions [12-14], and decreased LDL lipid peroxidation [8].Oxidative stress affects the inflammatory process and atherogenesis as well as the mutagenic power of the human genome. Thus, the protective effect of cocoa polyphenols may have important consequences for oncogene expression and cancer pathogenesis [15]. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) regulates enzymes involved in antioxidant functions or detoxification (e.g. thioredoxin reductase-1, glutathione peroxidases, heme-oxygenase-1, glutathione-*S*-transferases) via antioxidant- or stress- response elements [16].

The available epidemiological evidence is most consistent for its protective effect against oxidative stress [17]. These beneficial effects are supported by a few human intervention studies using polyphenol-rich foods that have shown consistent effects on a number of intermediate markers for CVD [4]. Literature review does not show any work done in healthy adults Ghanaians involving the long term supplementation of NCP, hence the aim of the study.

2. MATERIALS AND METHODS

2.1 Subjects

The Ethical and Protocol Review Committee of the University of Ghana Medical School (UGMS), Korle-Bu gave approval with reference number MS.Et/M.8-P.4.2/2011-1012. Written informed consent was sought form all the participants. The study was an interventional longitudinal study with humans using natural "unsweetened" cocoa powder labeled as a food supplement (Golden tree Royale®). The open label masking was employed. The supplementation period was for three months. Subjects were age and weight matched and apparently healthy black Africans from the diverse ethnic groups in Ghana recruited from the School of Allied Health Sciences (SAHS), College of Health Sciences, University of Ghana, Korle-Bu.

The inclusion criteria were subjects who had not been hospitalized or seen medical personnel with

disease conditions for at least 2 months prior to the start of the study. Subjects underwent the wash-out pre-selection stage and were advised to minimize their consumption of flavonoid-rich diets and cocoa-related products throughout the study period to reduce bias in the treatment groups and as well as the outcome assessment. The subjects' age ranged from 21 to 55 years with a mean of 30.8 ± 10.2 years indicative of the productive age bracket of the Ghanaian population. Non-alcoholics, non-smokers, non-allergists to cocoa, non-pregnant, non-menopausal subjects with BMI between 18.5 and 24.99 kg/m^2 were selected [18]. The participants maintained their energy intake during the study period. Food intake was evaluated during the study through food frequency recall and menu lists.

2.2 Anthropometric Measurements

Anthropometric and biochemical assays were performed on week 0 day 1. This served as the pre-supplementation control (pre-) group and on the completion day of the supplementation week 12 day 85, this was referred to as the post-supplementation (post-) group. Thus, each subject in the test group was his or her own respective control at week 0 day 1. Height and weight were measured from 08:00 to 10:00 h after a 12 h fast. Participants stood barefooted, knees and heels together, such that the Frankfurt plane was parallel to the floor. Height was measured using a Seca stadiometer (Hamburg, Germany) to the nearest 0.1 cm, and weight was obtained using Seca 770 scale (Hamburg, Germany) to the nearest 0.1 kg, with participants wearing light clothing and no shoes. BMI was calculated using the standard equation (kilograms per meters squared). Anthropometric measurements were taken twice and mean values were used in all analyses. Percentage body fat, visceral fat, percentage skeletal muscle, BMI (kg/m^2) and basal metabolic rate (BMR) were estimated by TBF-501 Tanita Corp bio-electrical impedance fat analyzer (Seattle, USA). These were measured to the nearest whole number. The systolic, diastolic blood pressures and pulse rates were measured using a Lumiscope Co, Inc., digitalized auto-inflating blood pressure monitor (East Brunswick, New Jersey). Each subject in a comfortably seated position after 15 minutes in warm room with the cuff placed at the left upper arm, 2 – 3cm above the antecubital vein as described by Grassi et al. [5].

2.3 Biochemical Assays

Blood sampling was done twice. The pre- and post- fasting blood samples were collected into gel separator tubes and centrifuged at 3000 rpm for 5 minutes at room temperature. This was then aliquoted into labelled Eppendorf tubes and stored in a - 20℃ freezer until subsequent analyses using Mybiosource® Inc. ELISA kits (California, USA) according to the manufacturer's instructions. The following markers were used: malondialdehyde (MDA), reduced glutathione peroxidase (GSH-Px), hemeoxygenase (HO-1), advanced glycated end-products (AGEs), vascular cellular adhesion molecule (VCAM-1), tumour necrosis factor alpha (TNF-α).

2.4 Cocoa Supplementation

Major dietary components in NCP were as follows: total fat 3g per 100g, 450 mg flavonoids, 612 kcal /2568 KJ per 100g. Two heaped tablespoonfuls (each weighing 15g) were reconstituted into 200 ml of warm water. Subjects drank NCP beverage twice daily for 12 weeks because it was intended to be a chronic study.The first serving was taken at 7.00 am in the morning (breakfast) and the second at 7.00 pm in the evening (dinner). Subjects were instructed to maintain their usual physical activity and diet except to refrain from flavonoid-rich foods and beverages, alcoholic beverages, vitamin supplements, certain medications before and during the study. The twice daily supplementation was adopted to mimic local tropical behaviour of consuming hot beverages.

2.5 Statistical Analysis

Paired t- and Wilcoxon tests were used to compare differences between the Pre- and Post-supplementation means. The parametric data was tested using the paired t test. The Wilcoxon signed-rank test was used for data not satisfying assumptions of normality. The Pearson's chi-square test for independence was used to discover if there is a relationship between two variables. Analyses were performed using SPSS (version 20, IBM SPSS software for Windows 2010). Statistical significance was accepted at $P< 0.05$. Data was shown in tabular form as mean ± SD.

3. RESULTS AND DISCUSSION

Seventeen (17) subjects successfully completed the study, out of the initial 54 that were recruited.

Twelve (12) of them were males and five (5) females, ranging from 21 to 55 years with a mean age of 30.8±10.2 years, mean height of 170.9±8.7 cm, mean weight of 65.19±10.29 kg (post), mean BMI 22.96±3.53 kg/m^2 (post), mean pulse rate of 71.29±5.40 bpm (post), mean systolic blood pressure 121.11±9.11 mmHg (post) and mean diastolic blood pressure of 78.24±8.40 mmHg (post) (Table 1). Weight loss and BMI changes were significant (*P*=0.009 and 0.006, respectively). Furthermore, resting metabolism and skeletal muscle changes were significantly higher at the end of the 12 week supplementation (*P* = 0.028 and 0.031, respectively) (Table 1). Endothelial integrity marker VCAM-1 reduced, although not significant. However, lipid peroxidation biomarker MDA was significantly lower at the end of supplementation (*P* = 0.033) which was complemented by a significantly higher enzyme antioxidant (GSH-Px) level (*P* = 0.001) (Table 2).

Oxidative stress is a dynamic condition amplified by a continuous vicious circle of metabolic stress, tissue damage and cell death. This culminates into increased free radical production and compromised defence systems that exacerbate oxidative damage [13,19]. The 12 weeks of NCP supplementation significantly increased the antioxidant GSH-Px status (*P* = 0.001), resting metabolism (*P* = 0.028) and percentage skeletal muscle (*P* = 0.031). In addition to this, NCP decreased overall weight (*P* = 0.009), BMI (*P*= 0.006), pro-oxidant MDA levels (*P* = 0.033). To the best of our knowledge, this is the first study to examine the long term effects of NCP supplementation on oxidative stress parameters in healthy free-living Ghanaian subjects. The overall weight loss that translated into decreased BMI maybe attributed to the increased skeletal muscle bulk and the resting metabolism. The increase in the resting metabolic rate may be due to the increased circulating levels of glucose used for energy. Polyphenols have been found to attenuate postprandial glycemic responses and fasting hyperglycemia while improving acute insulin secretion and insulin sensitivity [7,20,21].

The improvement in antioxidant status observed after NCP consumption is most likely related to elevated concentrations of plasma phenolic hydroxyl group(s), which is the basis of their antioxidant activity in vitro and in vivo resulting in reduced accumulation of reactive oxygen and nitrogen species (RONS). This suggests that cocoa could be used to increase the antioxidant glutathione (GSH) levels within the RBCs.

Table 1. Mean Pre- and Post-supplementation levels of the anthropometric parameters

Parameter	Pre-	Post-	Change	*P*-value
Weight (kg)	66.86±10.45	65.19±10.29	1.67±0.14	0.009*
BMI (kg/m^2)	23.01±3.80	22.96±3.53	0.05±0.01	0.006*
SBP (mmHg)	124.53±11.25	121.12±9.11	3.41±2.01	0.162
DBP (mmHg)	80.12±9.62	78.24±8.40	1.88±1.10	0.271
Pulse (bpm)	70.18±8.80	71.29±5.40	1.11±0.51	0.550
Resting metabolism (kcal)	1475±270	1530±206	55.0±5.2	0.028*
Skeletal muscle (%)	35.18±10.26	37.64±8.89	2.46±1.24	0.031*
Visceral fat (%)	4.41±2.85	4.47±3.66	0.06±0.01	0.745
Body fat (%)	23.27±12.59	23.00±12.79	0.27±0.03	0.407

*Wilcoxon's sign rank test *Significant at 5%SBP – Systolic blood pressure, DBP – Diastolic blood pressure, BMI – Basal metabolic rate*

Table 2. Mean Pre- and Post-supplementation levels of the selected oxidative stress markers (MDA, GSH-Px, VCAM-1, ACEs, HO-1, TNF-α)

Parameter	Pre-	Post-	Change	*P*-value
MDA (ng/L)	0.69±0.10	0.64±0.10	0.05±0.01	0.033*
GSH-Px (ng/L)	1.73±0.18	1.96±0.10	0.23±0.01	0.001*
VCAM-1 (mg/L)	0.53±0.13	0.44±0.18	0.09±0.03	0.068
AGEs (ug/L)	0.26±0.07	0.26±0.12	0.00±0.00	0.538
HO-1 (mg/L)	1.74±0.35	1.85±0.22	0.11±0.02	0.554
TNF-α (ng/L)	0.31±0.02	0.46±0.06	0.15±0.12	0.981

*Wilcoxon's sign rank test*Significant at 5%AGEs; advanced glycated end-products, GSH-Px; glutathione peroxidase, HO-1; hemeoxygenase 1, MDA; malondialdehyde, TNF-α; tumour necrosis factor alpha, VCAM-1; vascular cell adhesion molecule1*

Cocoa polyphenols are physiologically processed as xenobiotics which activate signalling pathways that result in increased expression of cytoprotective genes [16]. These results are in agreement with work done by Spadafranca et al. [15] showing that cocoa improved DNA resistance to oxidative stress in healthy subjects even though this effect was transient, probably due to flavonoid kinetics [22]. Recently, the European Food Safety Authority (EFSA) disapproved the use of thiobarbituric acid reducing substances (TBARS) and the *ex vivo* oxidation lag time of LDL as biomarkers for lipid peroxidation [23] hence, the use of MDA in this study. The results of this study on lipid peroxidation and antioxidant status are consistent with studies done on cocoa in various forms such as chocolates and cocoa powders in several different populations around the world [22,24,25]. In view of the possible study limitations, it cannot be ruled out that the positive changes in the major oxidative stress markers induced by NCP were not due to the increased NO availability. Nevertheless, the decrease in systolic and diastolic blood pressures observed after the supplementation though not statistically significant supports this hypothesis. The data obtained are consistent with the fact that increased antioxidant status and decreased lipid peroxidation represents a potential benefit derived from cocoa [25]. The contribution of other substances such as methionine and choline present in NCP, to the positive effects on the selected oxidative stress markers cannot be completely excluded. Therefore, flavanoids are the most likely biomolecules responsible for the above-mentioned effects [26].

No changes in the selected markers assessing the cell membrane stability (VCAM-1), advanced glycated end-products (AGEs), RBC hemolysis (HO-1) and tissue damage (TNF-α) were observed. Further phytotherapeutic studies could be undertaken on active cocoa polyphenols components to ascertain the effects on a molecular not organismic basis.

4. CONCLUSION

In conclusion, the present study indicates that NCP has a beneficial physiological effect on antioxidant status, obesity and energy expenditure rate in subjects after the supplementation. These results suggest that cocoa powder might be of therapeutic value in patients with increased risk of cardiovascular diseases such as hypertension and atherosclerosis, perhaps through mechanisms of antioxidant elevation and obesity reduction. Furthermore, the unique feature of the naturally unsweetened cocoa powder has posed major advantage over the enhanced products like chocolate in providing individuals who are prohibited from sugar-related additives intake with the feasibility and flexibility to enjoy the benefits of cocoa, without the concern of the side effects induced by sweeteners and additives.

CONSENT

Written informed consent was obtained from each participant.

ETHICAL APPROVAL

Ethical clearance was obtained from the Ethics and Protocol Review Committee of the University of Ghana Medical School (UGMS), Korle-bu.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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