LASU Journal of Medical Sciences
Official Publication of the Faculty of Basic Medical Sciences and Faculty of Basic Clinical Sciences
Lagos State University College of Medicine, Ikeja
www.lasucom.edu.org.
E-mail: lasujms@lasucom.edu.ng

Research Article

Cellgevity Supplementation Improves Blood Flow Velocity and Haemodynamic Indices in Internal Carotid, Middle Cerebral and Anterior Cerebral Arteries in Healthy Volunteers

Ogungbemi S. I.[1], Abidoye A. O.[2], Balogun O. J.[3], Tijani O. K.[1], Imonigie L. O.[1], Oluwaji E. A.[1], Anigbogu C. N.[1]
Department of Human Anatomy and Cell Biology, Delta State University, Abraka

[*]***Author for Correspondence:***
Ogungbemi S. I.
08087764885 <sogungbemi@unilag.edu.ng>

Keywords:
cellgevity, glutathione, cerebral blood flow indices, healthy volunteers

ABSTRACT

Objectives: Changes in intracranial artery blood flow velocity (BFV) and indices and plasma GSH level in healthy volunteers (HV) were investigated after chronic cellgevity (CGV) supplementation.

Method: Thirty-four (34) age- and sex-matched adult healthy volunteers were studied for changes in the time average mean of maximum velocity (TAMMV) of blood flow, Lindegaard (LR), resistivity (RI), pulsatility (PI) and systolic-diastolic (S-DR) indices in the internal carotid (ICA), middle cerebral (MCA) and anterior cerebral arteries (ACA) using transcranial doppler; and plasma GSH level after chronic oral CGV supplementation (2320 mg/d) in the HV .

Results: Before supplementation, TAMMV and LR were higher in left MCA than in right MCA ($p<0.001$), whereas TAMMV was higher in right ACA than in left ACA ($p<0.05$). TAMMV was lower in right ACA, much lower in left ACA and left ICA than in left MCA ($p<0.05$, $p<0.001$). After supplementation in the HV, there were reductions in TAMMV in right and left MCA, left ICA and ACA and in right LR ($p<0.05$, $p<0.001$). TAMMV reduced in right and left ACA more than in ICA and MCA and in left and right MCA more than in left and right ICA but (least in right ACA) increased in left ACA ($p<0.001$) after supplementation. RI, S-DR increased after supplementation ($p<0.001$, $p<0.01$). Increase in plasma GSH level correlated negatively with changes in TAMMV and LR.

Conclusion: Chronic CGV supplementation improved cerebral artery TAMMV, RI, LR and S-DR in HV by boosting plasma GSH level.

INTRODUCTION

Cerebral blood flow (CBF) is supplied by left and right internal carotid arteries (ICA) and basilar artery – from the left and right vertebral arteries.[1] The CBF is directly dependent on cardiac output (CO): an increase in CO results to an increase in CBF. The left and right ICA divide into left and right middle and anterior cerebral arteries with left and right anterior communicating arteries.[1]The CBF ensures continuous perfusion of the brain tissues with blood in order to supply oxygen, glucose, micronutrients and electrolytes.[1] The normal CBF represents about 14% of the CO.[2] A direct relationship has been demonstrated and reported among CBF, cerebral perfusion pressure, area, diameter and length of cerebral vessels, dynamic viscosity of blood, mean arterial pressure and cerebrovascular resistance.[1] The brain consumes high amount of glucose and oxygen to produce and utilize large number of adenosine triphosphate (ATP) molecules.[3] Regulation of CBF depends on these metabolic function demands of the brain mentioned above in order to maintain efficient supply of oxygen and glucose.[4] The high activity of mitochondria in the brain has been linked to the generation of reactive oxygen and nitrogen species (RONS). The sources of the RONS are the electron transport chain, cyclooxygenases, lipoxygenases, cytochrome P450 reductase, xanthine oxidase, nitric oxide metabolites.[4] There have been reports associating generation of cerebral RONS and RONS/antioxidant-balance with cerebral vasculature and CBF dynamics.[5] The RONS that are produced in the brain subsequent to its high metabolic activity are superoxide, peroxide, nitric oxide metabolites, peroxynitrite, nitrogen oxides, hypocholorous radical, hydroxy radical.[6] The powerful antioxidants in cerebral oxidative defense system include: glutathione (GSH), glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, minerals, vitamins, antioxidant free fatty acid.[4] GSH has been described as a master antioxidant on which other antioxidants depend. GSH is utilized by glutathione peroxidase as the final neutralizer of all oxidants.[7,8] GSH functions in all cells to maintain and restore the wholesome life processes, including calories, respiratory, water, vitamins, growth, pH, ion, redox, hormone metabolisms. This GSH is a tripeptide found in all mammalian cells with unique functions like elimination of RONS, removal of endogenously produced toxicants and trace metal homeostasis.[4] Studies have provided evidence that brain

cells' activities are wholly dependent on systemic GSH levels. The effectiveness brain blood flow dynamics may increase with increasing level of GSH.[9] Riboceine chemically known as D-Ribose-L-Cysteine is a GSH precursor.[10] The GSH is synthesized from L-glycine, L-glutamate and L-cysteine with L-cysteine being the rate limiting amino acid. Several dietary supplements: pro-drugs of L-cysteine have been reported to increase the level of GSH in the body. Riboceine has been shown to be significantly more effective than other GSH enhancers.[7] Cellgevity (CGV) is a dietary supplement that contains riboceine which delivers ribose and cysteine into the cell and replenishes GSH level.[7] The study investigated the changes in blood flow velocity, Lindegaard and systolic-diastolic ratios, resistivity and pulsatility indices (in the internal carotid, middle cerebral and anterior cerebral arteries) and plasma GSH level in healthy volunteers before and after eight-week, oral, 2320 mg/d CGV supplementation.

METHODOLOGY

The Healthy Volunteers

A written ethical approval was granted (CMUL/HREC/06/17/199) for this study by the Medical Research Grant and Experimentation Committee of the College of Medicine of the University of Lagos, Lagos, Nigeria. Thirty-Four (34) healthy adult volunteers (age- and sex-matched) were studied. Each volunteer gave informed consent and was served with subject information and consent, subject data and subject record forms. They were non-smokers, non-hypertensive (arterial blood pressure ≤ 140/90 mm Hg), non-alcoholic, not on medication, having no sickness or blood transfusion six (6) months prior to the course of the study. The volunteers were students of College of Medicine of the University of Lagos, Lagos, Nigeria. The studied parameters were measured before and after CGV supplementation in the Human Research Laboratory (Department of Physiology, College of Medicine of the University of Lagos) and Transcranial Doppler Laboratory (Sickle Cell Foundation, Surulere, Lagos, Nigeria). The volunteers were then followed up daily and/or weekly via social media channels. After the measurements, each volunteer was administered orally 2320 mg/d CGV capsules (Max International, USA) for eight (8) weeks. At the end of the 8 weeks, all the measurements were made again for each volunteer in order to study the effects of CGV on those measured parameters. Results are presented as mean ± standard error of mean (SEM). Statistical analyses were made using GraphPad Prism 5 and Microsoft Excel 2007 softwares. Significance level was accepted at $p < 0.05$ for each analysis.

Measurement of Biophysical Parameters

The age (in years), height (in cm) and weight (in Kg) using Avery Stadiometer (England) of each volunteer were measured. Body mass index (BMI) and body surface area (BSA) of the volunteers were calculated. The body temperature (in ^{o}C) was measured on the forehead using non-contact infrared thermometer (DT-8806C, Body Infrared Thermometer, China) at room temperature (25^{o}C).

Measurement of Blood Pressure and Heart Rate Parameters

The volunteers lay supine on a couch for twenty (20) min to come to physiological baseline. Arterial blood pressure (BP) and heart rate (HR) were then measured in this position. The arterial systolic (SBP) and diastolic (DBP) blood pressures were measured on the right brachial artery using Omron Automated Sphygmomanometer (Healthcare Co Ltd, Kyoto, Japan). Pulse pressure (PP) and mean arterial pressure (MAP) were then calculated from SBP and DBP. The HR was calculated from the R-R interval on the ECG tracing. The rate-pressure product (RPP) was calculated as the product of SBP and HR.[11,12]

Determination of Blood Flow Velocity and Indices in Internal Carotid Artery

The transcranial Doppler ultrasound measurement was done by the assistance of a trained Technologist. The volunteers were lay on bed during the test. The transcranial probe was placed over the volunteers' temporal bone. The probe insonated sound into the internal carotid artery (ICA), middle cerebral artery (MCA) and anterior cerebral artery (ACA) and blood tissue and at the same time received echoed sound from ICA, MCA and ACA as the case may be and blood tissue as a transducer for the ICA, MCA and ACA blood flow parameters.[13]

Determination of Plasma Glutathione Level

The blood samples collected in heparinized bottles were centrifuged. Glutathione (GSH) levels were measured from plasma samples.[14] 0.2 mL of 0.4 M Phosphate buffer at pH 7.0; 0.1 mL of 10 mM Sodium azide; 0.2 mL GSH and 0.1 mL of 0.2 mM H_2O_2 were combined to form a reaction mixture with 0.4 mL of 10% Trichloroacetate (TCA) and centrifuged at 2000 rpm for 10 min. Then, 1.0 mL supernatant was treated with 0.5 mL Ellman's reagent in 100 mL of 1.0% Sodium nitrate ($NaNO_3$) and 1.0 mL of Phosphate buffer in 2 M at pH 8.0. The absorbance was then read at 412 nm in a spectrophotometer and GSH level was determined (U/mg protein).

Data Analyses

Body Mass Index (BMI) was calculated as: BMI = Body W ÷ H^2 (Kg/m^2). Where W = weight (Kg) and H = height (m).[15] Body Surface Area was calculated as: BSA (m^2) = $0.001315 \times H^{1.214}$ (cm) × $W^{0.262}$ (Kg). Where W = weight; H = height; 0.001315 = constant for Negroes BSA.[16] Pulse Pressure (PP) was calculated as: PP = SBP – DBP (mm Hg).

Mean Arterial Pressure (MAP) was calculated as: MAP = (SBP + 2DBP) ÷ 3 (mm Hg)

Where SBP = systolic blood pressure (mmHg); DBP = diastolic blood pressure (mm Hg).[15] Heart Rate (HR) (b/min) was calculated as: HR = 25 × 60 ÷ R-R Interval (b/min).[11,15] Mean Flow Velocity (MFV: cm/s) = MFV = time average mean of maximum velocity (TAMMV). Gosling's pulsatility index (PI) = (peak systolic velocity – end diastolic velocity) ÷ TAMMV. Pourcelot resistivity index (RI) = (peak systolic velocity, PSV – end diastolic velocity) ÷ PSV. Lindegaard ratio (LR) = $TAMMV_{MCA} \div TAMMV_{ICA}$.[13]

RESULTS

Physical Parameters of the Volunteers before and after Cellgevity Supplementation

There was little or no change in the physical

parameters of the volunteers after CGV supplementation (Table 1). The age and height of the volunteers were 23.0 ± 2.9 y and 175.0 ± 2.2 cm before and after CGV supplementation. There were also little or no changes in the body weight (74.3 ± 5.8 Kg vs 76.1 ± 6.3 Kg), body mass index (24.2 ± 1.8 Kg/m^2 vs 24.9 ± 2.1 Kg/m^2), body surface area(2.13 ± 0.01 m^2 vs 2.23 ± 0.02 m^2) and body temperature (36.8 ± 0.10°C vs 36.54 ± 0.10°C).

Effect of Cellgevity Supplementation on Arterial Blood Pressure and Heart Rate Parameters in the Volunteers

There were slight reductions in systolic blood pressure (SBP: 125.0 ± 4.7 mm Hg vs 118.0 ± 3.9 mm Hg), diastolic blood pressure (DBP: 80.0 ± 3.4mm Hg vs 73.0 ± 4.2 mm Hg), and mean arterial pressure (MAP: 95.0 ± 3.7mm Hg vs 88.0 ± 3.2 mm Hg) in the healthy volunteers following CGV supplementation (Table 2). However, there was little or no change in pulse pressure (PP: 45.0 ± 1.3 mm Hg vs 45.0 ± 1.5 mm Hg) in the volunteers. After CGV supplementation, heart rate (HR: 78.6 ± 2.5b/min vs 66.8 ±1.9 b/min, $p < 0.05$) and rate-pressure product (RPP: 9825.0 ± 150.4 AU vs 7882.4 ±129.1 AU, $p < 0.001$) reduced in the volunteers (Table 2).

Table 1: Showing the Biophysical Parameters of the Volunteers before and after Cellgevity Supplementation

Parameters	Before Supplementation	After Supplementation
Age (y)	23.0 ± 2.9	23.0 ± 2.9$^{\#}$
Height (cm)	175.0 ± 2.2	175.0 ± 2.2$^{\#}$
Weight (Kg)	74.3 ± 5.8	76.1 ± 6.3$^{\#}$
Body Mass Index (Kg/m^2)	24.2 ± 1.8	24.9 ± 2.1$^{\#}$
Body Surface Area (m^2)	2.13 ± 0.01	2.23 ± 0.02$^{\#}$
Body Temperature (°C)	36.8 ± 0.103	6.6 ± 0.10$^{\#}$

= not significant

Table 2: Showing the Arterial Blood Pressure Parameters in the Volunteers before and after Cellgevity Supplementation

Parameters	Before Supplementation	After Supplementation
SBP (mm Hg	125.0 ± 4.7	118.0 ± 3.9$^{\#}$
DBP (mm Hg)	80.0 ± 3.4	73.0 ± 4.2$^{\#}$
PP (mm Hg)	45.0 ± 1.3	45.0 ± 1.5$^{\#}$
MAP (mm Hg)	95.0 ± 3.7	88.0 ± 2.4$^{\#}$
HR (b/min)	78.6 ± 2.5	66.8 ± 1.9*
RPP (AU)	9825.0 ± 150.4	7882.4 ± 129.1***

SBP = systolic blood pressure; DBP = Diastolic blood pressure; PP = pulse pressure
MAP = mean arterial pressure; HR = heart rate; RPP = rate-pressure product
# = not significant; * = $p < 0.05$ and *** = $p < 0.001$ = significant

Effect of Cellgevity Supplementation on Time Average Mean of Maximum Velocity and Lindegaard Ratio in the Cerebral Artery in the Volunteers

Before CGV supplementation in the healthy volunteers, time average mean of maximum velocity (TAMMV) was high in the left middle cerebral artery (MCA) compared to that of right MCA (149.4 ± 5.1cms^{-1} vs 126.0 ± 4.8 cms^{-1}, $p < 0.001$). There was little or no difference between the TAMMV in the left internal carotid artery (ICA) compared to that of the right ICA (115.0 ± 5.6 cms^{-1} vs 118.8 ± 3.3 cms^{-1}) before supplementation (Table 3). However, TAMMV was high in the right anterior cerebral artery (ACA) compared to left ACA (67.8 ± 3.4 cms^{-1} vs 56.9 ± 2.6 cms^{-1}, $p < 0.05$) before supplementation (Table 3). Similarly, the Lindegaard Ratio (LR) was high in the right cerebral vessels compared to LR of the left cerebral vessels (3.2 ± 0.10 vs 1.27 ± 0.08, $p < 0.001$) (Table 3). After CGV supplementation in the healthy volunteers, TAMMV reduced in the right MCA (from 126.0 ± 4.8 cms^{-1} to 115.0 ± 3.1 cms^{-1}, $p < 0.001$), left MCA (from 149.4 ± 5.1 cms^{-1} to 105.3 ± 5.3 cms^{-1}, $p < 0.001$), left ICA (from 118.8 ± 3.3 cms^{-1} to 104.9 ± 3.1 cms^{-1}, $p < 0.001$).on the other hand, TAMMV in the left ACA increased from 56.9 ± 2.6 cms^{-1} to 65.3 ± 2.5 cms^{-1} ($p < 0.001$) (Table 3). There was little or no reduction in TAMMV in the right ICA (115.0 ± 5.6 cms^{-1} vs 110.3 ± 3.4 cms^{-1}) and right ACA (67.8 ± 3.4 vs 64.5 ± 7.8 cms^{-1}) after supplementation. LR reduced from 3.21 ± 0.10 to 1.06 ± 0.07 ($p < 0.001$) in the right cerebral arteries, whereas LR in the left cerebral arteries reduced slightly from 1.27 ± 0.08 to 1.01 ± 0.07 in the healthy volunteers after CGV supplementation (Table 3).

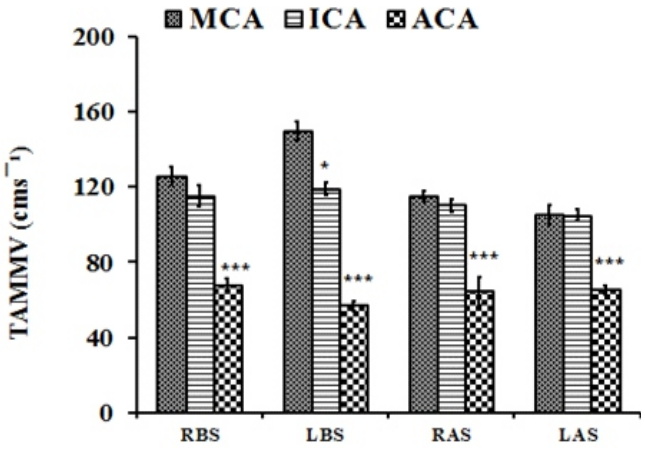

Figure 1: Showing time average mean of maximum velocity in the cerebral arteries in the volunteers before and after cellgevity supplementation
MCA = middle cerebral artery; ICA = internal carotid artery; ACA = anterior cerebral artery
RBS = Right before supplementation; LBS = Left before supplementation; RAS = Right after supplementation; LAS = Left after supplementation; * = $p < 0.05$ and *** = $p < 0.001$ = significant

Table 3: Showing Time Average Mean of Maximum Velocity and Lindegaard Ratio in the Cerebral Arteries in the Subjects before and after Cellgevity Supplementation

Vessels/Parameters	Before Supplementation R-TAMMV (cms^{-1})	After Supplementation L-TAMMV (cms^{-1})	After Supplementation R-TAMMV (cm^{-1})	After Supplementation L-TAMMV (cms^{-1})
MCA	126.0 ± 4.8	149.4 ± 5.1***	115.0 ± 3.1***	105.3 ± 5.3***
ICA	115.0 ± 5.6	118.8 ± 3.3#	110.3 ± 3.4#	104.9 ± 3.1***
ACA	67.8 ± 3.4	56.9 ± 2.6*	64.5 ± 7.8#	65.3 ± 2.5***
LR	3.21 ± 0.10	1.27 ± 0.08***	1.06 ± 0.07***	1.01 ± 0.07#

R = right; L = left; TAMMV = time average mean of maximum velocity;
MCA = middle cerebral artery; ICA = internal carotid artery; ACA = anterior cerebral artery
LR = Lindegaard ratio; # = not significant; * = $p < 0.05$ and *** = $p < 0.001$ = significant

Before supplementation, TAMMV was lower in the right ACA than in the right ICA and MCA ($p < 0.001$) while in the left ACA, TAMMV was lower than in the left ICA and MCA ($p < 0.001$). TAMMV was also lower in the left ICA than in the left MCA ($p < 0.05$) (Figure 1).

After supplementation, the TAMMV in the right and left ACA were lower than the TAMMV in the right and left MCA and ICA ($p < 0.001$). There were little or no differences between the TAMMV in the right and left MCA and ICA (Figure 1).

More so after CGV supplementation in the healthy subjects: the reduction in TAMMV was greatest in MCA followed by that in ICA and least in ACA (Figure 2); the reduction in TAMMV in the left MCA and left ICA were more than the reduction in the right MCA and right ICA ($p < 0.001$) (Figure 2); Figure 2 also shows that while there was reduction in TAMMV in the right ACA, there was increase in TAMMV in the left ACA.

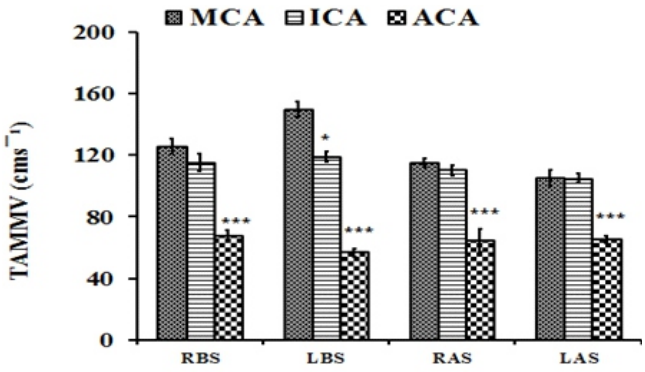

Figure 1: Showing time average mean of maximum velocity in the cerebral arteries in the volunteers before and after cellgevity supplementation
MCA = middle cerebral artery; ICA = internal carotid artery; ACA = anterior cerebral artery
RBS = Right before supplementation; LBS = Left before supplementation; RAS = Right after supplementation; LAS = Left after supplementation; * = $p < 0.05$ and *** = $p < 0.001$ = significant

Effect of Cellgevity Supplementation on Resistivity Index, Pulsatility Index and Systolic-Diastolic Ratio in the Volunteers

Results show increase in resistivity index from 0.59 ± 0.04 to 0.71 ± 0.04 ($p < 0.001$) and systolic-diastolic ratio from 2.61 ± 0.21 to 3.21 ± 0.15 ($p < 0.001$) and a slight increase in pulsatility index from 0.74 ± 0.06 to 0.86 ± 0.02 in the volunteers following CGV supplementation (Table 4).

Plasma Glutathione Level in the Volunteers before and after Cellgevity Supplementation

Plasma glutathione level increased by 118.5± 3.7mM/L (from 197.3± 12.9 mM/L to 315.8± 16.6 mM/L) in healthy volunteers following CGV supplementation (Figure 3).

After CGV supplementation, change in plasma glutathione (GSH) level correlates negatively with reductions in heart rate (ΔHR: $r = -0.52$, $p < 0.05$), rate-pressure product (ΔRPP: $r = -0.65$, $p < 0.01$), time average mean maximum velocity (ΔTAMMV) of right and left middle cerebral arteries (MCA: $r = -0.79$, $p < 0.001$ and $r = -0.5$, $p < 0.05$ respectively), right internal carotid artery (ICA: $r = -0.51$, $p < 0.05$) and Lindegaard ratio (LR: $r = -0.73$, $p < 0.001$) in the healthy volunteers.

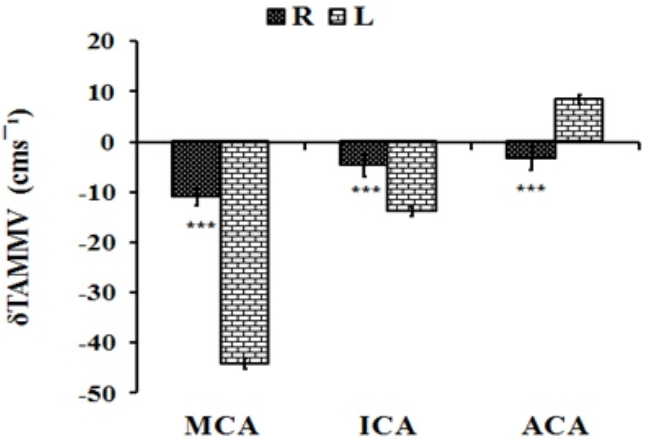

Figure 2: Showing change in time average mean of maximum velocity in the cerebral arteries in the volunteers before and after cellgevity supplementation
R = right; L = left; MCA = middle cerebral artery; ICA = internal carotid artery
ACA = anterior cerebral artery; * = $p < 0.05$ and *** = $p < 0.001$ = significant

Table 4: Showing the Indices of Cerebral Blood Flow in Volunteers before and after Cellgevity Supplementation

Parameters	Before Supplementation	After Supplementation
Resistivity Index	0.59 ± 0.04	0.71 ± 0.04***
Pulsatility Index	0.74 ± 0.06	0.86 ± 0.02#
Systolic-Diastolic Ratio	2.61 ± 0.21	3.21 ± 0.15**

= not significant; ** = p < 0.01; *** = p < 0.001 = significant

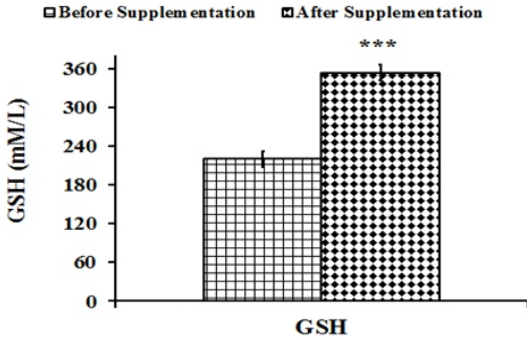

Figure 3: Showing plasma glutathione (GSH) level before and after supplementation in the healthy volunteers.

DISCUSSION

Effect of Cellgevity Supplementation on the Biophysical Parameters of the Healthy Volunteers.

The physical feature data of the volunteers: age, height, weight, BSA, BMI and body temperature before and after supplementation suggest they were in good health.[17] The slight decreases in SBP, DBP and MAP obtained in the volunteers after CGV supplementation indicate CGV possesses a blood pressure lowering effect on arterial vasculature in humans.[17] The increase in plasma GSH following CGV supplementation co-existed with reductions obtained in SBP, DBP and MAP in the volunteers. GSH has been linked to vascular smooth muscle contraction and relaxation with vasoprotective substances at the endothelium, vascular smooth muscle adventitia and perivascular level via reduction in α-adrenal receptor activity and of plasma nitric oxide (NO) level.[18,19,20] The reductions in HR and SBP obtained produced the reduction in RPP in the volunteers following CGV supplementation. The reduction in HR and RPP are suggesting reduction in myocardial work or stress and myocardial oxygen consumption in the volunteers following supplementation.[21,22] The negative correlation between the increase in plasma GSH and reduction in HR and RPP shows the possible association between plasma GSH and reduced HR and RPP in the volunteers. The results show that increase in plasma GSH following supplementation might been responsible for the reductions in SBP and HR and the subsequent decrease RPP in the volunteers. Increase in GSH level has been linked with decrease in α-adrenal receptor activity and increase in NO level and the subsequent decrease in arterial blood pressure.[18,19] Similarly, Ogungbemi *et al*., (2013) have linked reductions in arterial blood pressure, HR and RPP to increase in plasma NO level.[22]

Effect of Cellgevity Supplementation on the Blood Flow Time Average Mean of Maximum Velocity and Indices in the Cerebral Arteries

Before supplementation, the higher time average mean of maximum velocity (TAMMV) in the left middle cerebral artery (MCA) (than that obtained in the right MCA), in the right anterior cerebral artery (ACA) (than that obtained in the left ACA), and the increase in the right Lindegaard ratio (LR) than that obtained in the left suggest that haemodynamic baseline, features and activities have different physiological baselines in the right and left cerebral vessels in the volunteers. In addition, the results show a consistent higher TAMMV in MCA greater than that obtained in internal carotid artery (ICA) which in turn was greater than that obtained in the ACA in both left and right cerebral vessels before supplementation in the volunteers.

The reductions in TAMMV obtained in the right and left ICA, MCA and ACA as well as the reduction in the LR (both being within the physiological range) in the volunteers suggest overall improvement in cerebral blood flow after CGV supplementation.[23] These reductions in TAMMV in the cerebral arteries indicate a reduction in intracranial pressure with improvement in cerebral blood flow and vasodilation.[24] The reduction in cerebral blood flow velocity in volunteers in this study is corroborated with decrease in arterial blood pressure and peripheral resistance, and increase in cardiac output obtained after supplementation. Several authors have associated decrease in cerebral blood flow velocity (within physiological range) with improvement in cerebral blood flow and vasodilatation and prevention of stenosis, vasospasm, hyperdynamic flow and ischaemic stroke.[25]The reduction in LR obtained in the volunteers after CGV supplementation is an absolute indicator of increased cerebral blood flow and vasodilation in them.[1,13] The study shows that reduction in LR is compatible or corroborated with the reductions in TAMMV, arterial blood pressure and peripheral resistance in the volunteers after supplementation. The similarity in the TAMMV reductions (in the volunteers after supplementation) in left and right MCA and ICA which were greater than those in the left and right ACA as well as the consistency of the reductions in the MCA which was greater than that of ICA, which in turn was greater than that of ACA (in the volunteers after supplementation) imply a maintained cerebral blood flow auto-regulation and haemodynamic relationship and among cerebral arteries in addition to the improvement in cerebral blood flow after supplementation. The increases in RI, PI, systolic-diastolic ratio following supplementation in the volunteers show improvement or modification of cerebral blood flow pulse and resistance indices within physiological range. This modification may be a physiological compensation for the reduction in TAMMV and LR.

The findings in this study imply possible improvement cerebral blood flow, cerebral blood flow velocity, and cerebral vessel vasodilatation, which may prevent hyperdynamic flow, vasospasm, stenosis, occlusion or ischaemic stroke in the cerebral vessels of healthy individual on CGV supplementation.[25] The negative correlation between the increase in plasma concentration of glutathione

and the changes in TAMMV, LR, RI, PI, of the ICA, MCA and ACA are indications that these findings are associated with the increase in plasma glutathione level in the volunteers after CGV supplementation. Previous studies have linked the level of plasma glutathione with improvement in carotid artery blood flow, reduction in carotid artery intima-media thickness, vasodilatation, via a direct antioxidant activity and elevation of plasma nitric oxide level.[18,26,27]

Effect of Cellgevity Supplementation on the Plasma Glutathione Level in the Volunteers

The increase in plasma glutathione (GSH) levels in the volunteers after CGV supplementation shows that CGV supplementation might have increased plasma GSH levels in them. CGV has been reported to produce GSH in humans and animals, because it contains D-ribose and L-cysteine.[27] Riboceine might have delivered D-ribose-L-cysteine into the body cell and boosted plasma GSH level following supplementation in the volunteers.[7] The negative correlations between increase in the plasma GSH and reductions in other measured parameters show that increase in plasma GSH is a possible mechanism by which these findings are obtained in the studied healthy volunteers after CGV supplementation.

CONCLUSION

Chronic CGV supplementation improved cerebral artery blood flow velocity, resistivity index, Lindegaard and systolic-diastolic ratio by boosting plasma GSH level in healthy volunteers. The study therefore established that chronic, oral CGV supplementation attenuated time average mean of maximum velocity and modified haemodynamic indices of cerebral blood flow via elevation of plasma GSH in healthy volunteers.

REFERENCES

1. Tameem A. and H. Krovvidi. Cerebral Physiology. The British Journal of Anaesthesia, 2013;13(4):113-118
2. Scheinberg P. and Jayne H. W. Factors Influencing Cerebral Blood Flow and Metabolism: A Review. Circulation, 1952;5(2):225-234
3. Mergenthaler P, Lindauer U, Dienel GA and Meisel A Sugar for the Brain: The Role of Glucose in Physiological and Pathological Brain Function. Trends in Neurosciences, 2013;36(10):587-597.
4. Carvalho C and Moreira PI. Oxidative Stress: A Major Player in Cerebrovascular Alterations Associated to Neurodegenerative Events. Frontiers in Physiology, 2018;9:806.
5. Wevers NR and de Vries H. E. Morphogens and Blood-Brain Barrier Function in Health and Disease. Tissue Barriers, 2016;4(1):e1090524.
6. Goetz ME and Luch A. Reactive Species: A Cell Damaging Rout Assisting to Chemical Carcinogens. Cancer Letters, 2008;266:73–83.
7. Nagasawa HT. Inventor: Max International, LLC Membership Interest Assignment. Method to Enhance Delivery of Glutathione and ATP Levels in Cells. United States Patent Application US 14/838, 2015;274:p.27.
8. Boron W. F. and Boulpaep E. L. Free Radical Theory of Aging and Special Circulation: The Brain In: Medical Physiology; Updated 2nd Ed. Saunders Elsevier Philadelphia; 2017.1333-1340,3003-3006.
9. Damy T, Kirsch M, Khouzami L, Caramelle P, Le Corvoisier P, Roudot-Thoraval F. *et al*. Glutathione Deficiency in Cardiac Patients is Related to the Functional Status and Structural Cardiac Abnormalities. Public Library of Science One, 2009;4(3):e4871.
10. Kader T, Porteous CM, Williams MJ, Gieseg SP, McCormick S. P. Ribose-Cysteine Increases Glutathione-Based Antioxidant Status and Reduces LDL in Human Lipoprotein(A) Mice. Atherosclerosis, 2014;237:e725-e733.
11. Araoye MA. Heart Rate: In Basic Electrocardiography. 1st Ed Nathadex Publishers Ilorin, 2004.p14
12. Jaja SI and Ogungbemi SI. Electrocardiographic and Blood Pressure Changes in Apparently Healthy Male Subjects following Oral, Chronic, Low-Dose Vitamin C Supplementation and/or Change in Posture. Nig. Quart. Journ. Hosp. Med., 2008;18(2):96100.
13. Naqvi J, Yap KH, Ahmad G and Ghosh J. Transcranial Doppler Ultrasound: A Review of the Physical Principles and Major Applications in Critical Care. International Journal of Vascular Medicine, 2013;629378.
14. Ellman GL. Tissue Sulphydryl Groups. Archives of Biochemistry and Biophysics, 1959;82:70-77.
15. Sofola OA. Typical Electrocardiogram In: Cardiovascular System In: A Revision Text in Medical Physiology. 4th Ed, Miral Printing Press, Lagos; 2019.p27.
16. Nwoye LO. Body Surface Area of Africans: A Study Based on Direct Measurement of African Males. Human Biology, 1989;61(3):439-457.
17. Ogungbemi SI, Ale OK, Ejalonibu ML, Ojubanire OY, Tijani KO, Balogun OJ, Anigbogu CN and Olatunji-Bello II. Apha-Wave Voltage, Daytime Sleep and Plasma Glutathione Level Following D-Ribose-L-Cysteine Supplementation in Healthy Participants, 2019;2(2):59-68.
18. Tejedor C, Lorente JA, Delgado MA, Fernāndez-Segoviano P, De Paula M, Tobalina R *et al*. One-Gram Intravenous Glutathione Reversed Septic Induced Vasoconstriction, and Pressor Responses via Free Haemoglobin in Aneasthetised Septic Pigs by Interactions between NO and Reduced Sulfrydryl Group in Haemoglobin. Critical Care Medicine, 2002:493-500
19. Kevin PK. Glutathione lower blood pressure. California, State Science and Engineering PAS Book of Abstract, 2004:Ap2/04
20. Sena CM, Leandro A, Azul L, Seiça R and Perry G. Vascular Oxidative Stress: Impact and Therapeutic Approaches. Frontiers in Physiology, 2018;9:1668.
21. Uehara M and Sakane KK. Thermodynamics of the Heart: Application of Thermodynamics to Biological and Material Science; Tadashi (Ed), ISBN; 2011.p980-986.
22. Ogungbemi SI, Anigbogu CN, Kehinde MO and Jaja SI. L-arginine Increases Nitric Oxide and Attenuates Pressor and Heart Rate Responses to Change in

Posture in Sickle Cell Anemia Subjects. Nigerian Journal of Physiological Sciences, 2013;28(1):45-50.
23. Bathala L, Mehndiratta MM and Sharma VK. Transcranialdoppler: Technique and Common Findings (Part 1). Annals of Indian Academy of Neurology, 2013;16:174-179.
24. Xu H and Hepel M. "Molecular Beacon"-Based Fluorescent Assay for Selective Detection of Glutathione and Cysteine. Analytical Chemistry, 2011; 83(3):813-819.
25. Nicoletto HA and Burkman HA. "Transcranial Doppler Series Part III: Interpretation," American Journal of Electroneurodiagnostic Technology, 2009;49(3):244–259.
26. Schutte R, Schutte AE, Huisman HW, Van Reeyen JM, Malan NT, Peter S. African Men with Subdue all Atherosclerosis. American Journal of Hypertension, 2009;22(11):11541159.
27. Awodele O, Badru WA, Busari AA, Kale OE, Ajayi TB, Udeh RO *et al*. Toxicological Evaluation of Therapeutic and Supra-Therapeutic Doses of Cellgevity on Reproductive Function and Biochemical Indices in Wistar Rats. BioMed Central Pharmacology and Toxicology, 2018;19:68.