The Effects of Acute Energy Drink Consumption on the Cardiovascular System of University Students

by

Ayanda Nompumelelo Nyalela

Submitted in accordance with the requirements

for the degree of

Master of Science in Life Sciences

in the

College of Agriculture and Environmental Sciences

Department of Life and Consumer Sciences

at the

University of South Africa

Supervisor: Dr C Myburgh

Co-supervisor: Dr EA Symington

January 2023

Dedication

This dissertation is dedicated to my brother, Sibusiso Nyalela, for his love, wisdom, warrior-spirit and endless support, and to my partner, Sive Nodada. Thank you for your love, companionship and for being my inspiration and beacon of light.

I'd also like to dedicate this dissertation to my late parents, Elsie and Mbulelo Nyalela. Thank you for life itself and teaching me to walk in God's path. I am eternally grateful, for you're the ones who taught me how to fly.

Declaration

UNISA | college of agriculture and environmental sciences

Student number: 54233550

I Ayanda Nompumelelo Nyalela, hereby declare that the dissertation/thesis, with the title: The Effects of Acute Energy Drink Consumption on the Cardiovascular System of University Students which I hereby submit for the degree of Master's in Life Sciences at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

I declare that the dissertation /thesis does not contain any written work presented by other persons whether written, pictures, graphs or data or any other information without acknowledging the source.

I declare that where words from a written source have been used the words have been paraphrased and referenced and where exact words from a source have been used the words have been placed inside quotation marks and referenced.

I declare that I have not copied and pasted any information from the Internet, without specifically acknowledging the source and have inserted appropriate references to these sources in the reference section of the dissertation or thesis.

I declare that during my study I adhered to the Research Ethics Policy of the University of South Africa, received ethics approval for the duration of my study prior to the commencement of data gathering, and have not acted outside the approval conditions.

I declare that the content of my dissertation/thesis has been submitted through an electronic plagiarism detection program before the final submission for examination.

Student signature:  Date: 19 January 2023

Acknowledgements

I would like to express my sincerest gratitude for the following members who significantly contributed their expertise, patience, and tireless efforts, resulting in the completion of this research project:

- To Mr. Hosana Mkoyi for his availability, assistance, and expertise in laboratory techniques
- As well as to all the participants who sacrificed their time to contribute to the study.

I wish to give a special word of thanks to my research supervisor, Dr Caitlynd Myburgh. Thank you, Dr Myburgh, for your guidance, patience and being an inspiration. It is an honour to have been nurtured academically from your teachings in the various disciplines of human physiology, which have become a significant foundation to my body of scientific and clinical knowledge, to supervising me for my master's degree. Thank you for being a role model, an adviser, and for your endless motivation and abundant support that has no boundaries.

I also wish to give a special word of thanks to my co-supervisor, Dr Elize Symington. Thank you, Dr Symington for all the knowledge, advice, and expertise you have shared with me for the full duration of my studies under your supervision. Not only did I gain your support in the synthesis of my dissertation, but you have played a key-role in the broadening of my knowledge and became an inspiration for my newly found passion for the integration of medical sciences and nutrition. Thank you for nurturing my personal, academic, and scientific development.

It has been nothing short of a blessing to have been supervised by the both of you. Thank you again, for your support and aiding my professional development as a physiologist and researcher.

Lastly, I would like to thank my extended family, friends, and loving partner for being my solace, support and source of power and motivation which carried me through to the completion of this research project. My gratitude is infinite.

Abstract

Introduction: Among the changes in dietary behaviour in South Africa is the increased consumption of energy drinks (EDs), shown to affect the cardiovascular system (CVS) due to high caffeine and bioactive compound concentrations. Cardiovascular disease (CVD) is a concern, especially in black cohorts with a reported high prevalence of hypertension, as increased consumption highlights the adverse effects of EDs on the CVS. **Aim:** This study's aim was to determine the effects of acute ED exposure on the CVS of generally healthy, black, male university students. **Methodology:** A randomised, controlled, cross-over study was conducted on 26 male students aged between 18-29 years. A *Monster*® carbonated ED (intervention) and a diluted fruit juice concentrate with carbonated water (placebo) were administered to participants in a single-blinded manner on two non-consecutive days. Heart rate (HR; bpm) and blood pressure (BP; mmHg) were measured twice on the left arm of participants using an automated BP monitor. Measurements were recorded at 0-; 10-; 30-; 60-; 90-; and 120-minutes. Questionnaires were used to obtain demographical data and assess ED and caffeine usage. **Results:** Self-reported consumption frequency for caffeinated beverages was predominantly ≥14 servings/week (50%). The reported purpose for caffeine consumption was attributed to mainly academic purposes (61.5%). The preferred caffeinated beverage was EDs (77%). For ED consumption relative to baseline, systolic and diastolic BP were higher from 30-minutes (p<0,001 and p=0,015, respectively), HR was reduced at 10-minutes (p=0,015), while pulse pressure and MAP were increased from 30-minutes (p=0,043 and p<0,001). Following placebo consumption, blood pressure remained stable relative to baseline with BP reaching maximum values at 120-minutes. Although overall HR dropped below baseline in both groups over the study period, HR increased much later in the placebo group compared to the ED group, reaching its lowest point at 90-minutes, which in contrast, was the peak time point for ED consumption. For ED consumption relative to the placebo, BP was higher at all time points from baseline (p<0,001), while HR showed a significant increase at 90-minutes (p<0,001). Pulse pressure and MAP were higher for ED consumption, with significance observed at 90-minutes (p<0,001) for pulse pressure, and at all time points for MAP. The study found that drink type had a significant effect on BP (p<0,001) and a partial significance on HR (p=0,052). The interaction between drink type and time points had a significant effect on systolic BP

(*p*<0,001) and MAP (*p*<0,001) and a partial significance on diastolic BP (*p*=0,057). **Conclusion:** EDs significantly increased BP over a period of 2-hours relative to the placebo. These observations can be attributed to the sympathomimetic actions of the bioactive contents of EDs, especially as a result of the synergy between caffeine and taurine. Heart rate decreased slower in the ED group versus the placebo group which presented more stability. These changes can be attributed to caffeine's stimulatory effects as well as its half-life in an adult body.

Keywords:
Energy drink; cardiovascular physiology; cardiovascular disease; hypertension; heart rate; blood pressure; black males; university students

Opsomming

Inleiding: Die verhoogde inname van energiedrankies (ED's) maak deel uit van Suid-Afrikaners se dieet. Daar is bevind dat ED's vanweë hulle hoë kaffeïeninhoud en bioaktiewe verbinding konsentrasies die kardiovaskulêre stelsel (KVS) affekteer. Kardiovaskulêre siektes is veral onder swart mense rede tot kommer gesien die nadele wat die verhoogde inname van ED's vir die KVS inhou. **Oogmerk:** Die oogmerk van hierdie studie was om vas te stel watter uitwerking 'n akute inname van ED's op die KVS van gesonde swart manlike universiteitstudente het. **Metodologie:** Altesame 26 manlike studente tussen 18 en 29 jaar het aan hierdie ewekansige, beheerde oorkruisstudie deelgeneem. 'n *Monster*®-ED (ingryping) en 'n vrugtesapkonsentraat verdun met sodawater (plasebo) is op twee nieopeenvolgende dae volgens die enkelblindmetode aan deelnemers toegedien. Hulle hartklop (HK; bpm) en bloeddruk (BD; mmHg) is twee keer met 'n geoutomatiseerde sfigmomanometer aan die linkerarm gemeet. Meterlesings is na 0; 10; 30; 60; 90 en 120 minute aangeteken. Deelnemers se demografiese data en inname van ED's en kaffeïen is met behulp van vraelyste bepaal. **Bevindings:** Die self-gerapporteerde innamefrekwensie van kaffeïendrankies was oorwegend ≥14 porsies/week (50%). Volgens die deelnemers het 'n hoë kaffeïeninname hulle beter laat studeer (61,5%). ED's was hulle gunstelingkaffeïendrankie (77%). Vir die inname van ED met betrekking tot die aanvangsmeting was die sistoliese en diastoliese BD ná 30 minute hoër (p<0,001 en p=0,015 onderskeidelik), HK ná 10 minute stadiger (p=0,015), terwyl polsdruk en gemiddelde arteriële druk (GAD) ná 30 minute hoër (p=0,043 en p<0,001). Vir ED-inname met betrekking tot die plasebo was BD op alle metingstye hoër as die aanvangsmeting (p<0,001) en was HK ná 90 minute beduidend hoër (p<0,001). Polsdruk en GAD was hoër ná ED-inname. Beduidendheid is veral vir polsdruk ná 90 minute (p<0,001) en vir GAD op alle metingstye waargeneem. Die studie het bevind dat die soort drankie 'n beduidende effek op BD (p<0,001) en 'n gedeeltelike beduidende effek op HK gehad het (p=0,052). Die wisselwerking tussen die soort drankie en die metingstye het 'n beduidende effek op sistoliese BD (p<0,001) en GAD (p<0,001), maar 'n gedeeltelike beduidendheid op diastoliese BD (p=0,057) gehad. **Gevolgtrekking:** ED's het BD met betrekking tot die plasebo oor 'n tydperk van twee uur beduidend laat styg. Ofskoon HK gedaal het, was waardes vir ED hoër met betrekking tot die plasebo. Hierdie waarnemings kan toegeskryf word aan die

vasostimulant en simpatomimetiese werking van die bioaktiewe inhoud van ED’s as gevolg van veral die sinergie tussen kaffeïen en tourien.

Sleutelbegrippe:
Energiedrankie; kardiovaskulêre fisiologie; kardiovaskulêre siekte; hipertensie; hartklop; bloeddruk; swart mans; universiteitstudente

Isicatshulwa

Intshayelelo: Phakathi kweenguqu ezenzekileyo eMzantsi Afrika kwindlela yokutya kukunyuka kokusetyenziswa kweziselo ezinika amandla (iziselo ezinika Amandla,EDs), eziboniswa zichaphazela inkqubo yemithambo yentliziyo (CVS) ngenxa ye-khafini (caffeine) eninzi kunye nomxube oyingqumbululu wezinto ezisebenzayo (bioactive). Isifo semithambo yentliziyo (CVD) yinkxalabo, ngakumbi kumaqela abantu abamnyama, njengoko ukwanda kokusetyenziswa kuveza imiphumo emibi yeziselo ezinika amandla (ED) kwimithambo yentliziyo (CVS). **Injongo**: Injongo yolu phononongo yayikukuveza imiphumo yeziselo ezinika amandla eziyingozi kwinkqubo yemithambo yentliziyo (CVS) kubafundi abangamadoda abantsundu baseyunivesithi abasempilweni ngokubanzi.Indlela. **yokwenza**: Uphononongo olulawulwe ngokungakhethiyo olunqamlezileyo lwenziwe kubathathi-nxaxheba abaneminyaka ephakathi kwe-18 kunye ne-29. I-Monster® isiselo esinika Amandla esinekharbon (carbonated ED) (ungenelelo) kunye nengqumbululu yencindi engxengiweyo kunye namanzi anekharbon (i-placebo) inikezelwe kubathathi-nxaxheba ngendlela eyodwa kwiintsuku ezimbini ezingalandelelaniyo. Isantya sokubetha kwentliziyo (HR; bpm) kunye noxinzelelo lwegazi (BP; mmHg) zilinganiswe kabini kwingalo yasekhohlo yabathathi-nxaxheba kusetyenziswa isixhobo esizihambelayo i-sphygmomanometer. Imilinganiselo yarekhodwa kwi-0; 10; 30; 60; 90 kunye 120 yemizuzu. Imibuzo yayisetyenziselwa ukufumana idatha yabantu bendawo ethile ngokwamanani neemeko zabo kunye nokuhlola i-ED (iziselo ezinika Amandla) kunye nokusetyenziswa kwe-khafini (caffeine). **Iziphumo**: Izihlandlo zokusetyenziswa njalo kweziselo ezinekharbon ubukhulu becala zazine ≥14 ngokokuphaka / ngeveki (50%). Ukusetyenziswa kwekhafini kwabalelwa kwiinjongo zemfundo (61.5%). Esona siselo sikhethwayo esinekhafini yayiyisiselo esinika amandla i-EDs (77%). Ukusetyenziswa kwesiselo esinika amandla (ED) ngokuthelekswa nesiseko, uxinzelelo lwegazi xa intliziyo iqala ukumpompa igazi (systolic BP) kunye noxinzelelo lwegazi xa intliziyo iyeka ukumpompa igazi (diastolic BP) zaziphezulu ukusuka kwimizuzu engama-30 ($p<0,001$ kunye ne-$p=0,015$, ngokulandelanayo), i-santya sokubetha kwentliziyo (HR) yehla kwimizuzu eli-10 ($p=0,015$), ngelixa uxinzelelo lokubetha kwentliziyo kunye ne-MAP zonyuka ukusuka kwimizuzu engama-30 ($p=0,043$ kunye ne $p<0,001$).

Ukusetyenziswa kwe-ED ngokuthelekiswa nento efaniswa neyeza kodwa elingelilo esetyenziselwa ekukholiseni nje (placebo), unxinzelelo lwegazi (BP) lwaluphezulu kuzo zonke indawo ukususka kwisiseko ($p<0,001$), ngelixa isantya sokubetha kwentliziyo (HR) kubonise ukunyuka okukhulu kwimizuzu engama-90 ($p<0,001$). Uxinzelelo lokubetha kwentliziyo (Pulse) kunye ne-MAP zaziphezulu ekusebenziseni iziselo ezinika amandla (ED), zabonakala kakhulu kwimizuzu engama-90 ($p<0,001$) yoxinzelelo lokubetha kwentliziyo(pulse), kwaye kuzo zonke iindawo ze-MAP.
Uphononongo lufumanise ukuba uhlobo lwesiselo lunento oluyenzayo ebonakalayo kuxinzelelo lwegazi (BP) ($p<0,001$) kwaye lwabonisa okuyinxalenye kwisantya sokubetha kwentliziyo (HR) ($p=0,052$). Ukusebenzisana phakathi kohlobo lwesiselo kunye neendawo zexesha kunefuthe olubonakalayo kuxinzelelo lwegazi xa intliziyo liqala ukumpompa igazi (systolic) ($p<0,001$) kunye ne-MAP ($p<0,001$), kunye nokuyinxalenye kuxinzelelo lwegazi xa iyekile ukumpompa igazi (diastolic) ($p=0,057$).
Isiphelo: I-EDs (Iziselo ezinika Amandla) zinyuse kakhulu unxinzelelo lwegazi (BP) kwixesha elimalunga neyure ezi-2 kuthelekiswa nento efaniswa neyeza kodwa elingelilo esetyenziselwa ekukholiseni nje (placebo). Nangona isantya sokubetha kwentlinziyo (HR) sisehla, amaxabiso ayephezulu kwiziselo ezinika amandla (ED) ngokumalunga nento efaniswa neyeza kodwa elingelilo esetyenziselwa ekukholiseni nje (placebo). Olu qwalaselo lunokuthi lubalelwe kwizenzo zesivuseleli i-vaso kunye nechiza ukuvelisa iziphumo zokusebenza kophawu lwenkqubo yemithambo-luvo enovelwano ngokukhuthaza ukuvuselela imizwa yovelwano. yomthamo we-bioactive kwiziselo ezinika Amandla (EDs), ngakumbi ngenxa yokuhambelana phakathi kwe-khafini (caffeine) kunye nezinto ezimuncu ezisetyenziselwa ukunika amandla kwiziselo i-taurine.

Amagama angundoqo:
Energy drink; Isiselo esinika amandla; i-cardiovascular physiology; ukusebenza kwemithambo yentliziyo; isifo semithambo yentliziyo; uxinzelelo lwegazi; uxinzelelo lwegazi oluphezulu kunesiqhelo; umlinganiselo wokubetha kwentliziyo; uxinzelelo lwegazi; amadoda amnyama; abafundi baseyunivesithi

Table of Contents

List of Tables and Figures

List of Figures

List of Tables

Acronyms and Abbreviations

ADP	Adenosine diphosphate
AIDS	Acquired immunodeficiency syndrome
ATP	Adenosine triphosphate
AR	Adenosine-receptor
BP	Blood pressure
CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
CI	Confidence interval
CNS	Central nervous system
CV	Cardiovascular
CVD	Cardiovascular disease
CVS	Cardiovascular system
ECG	Electrocardiogram/electrocardiograph
ED(s)	Energy drink(s)
GPA	Grade point average
HIV	Human immunodeficiency virus
HR (bpm)	Heart rate (beats per minute)
HVD	Heart valve disease(s)
NCD	Non-communicable disease(s)
PhD	Doctorate in philosophy
PNS	Peripheral nervous system
RNA	Ribonucleic acid
SNS	Sympathetic nervous system
SSA	Sub-Saharan Africa

TB	Tuberculosis
UK	United Kingdom
USA	United States of America
VO_2 max	Maximum oxygen respiration volume
WHO	World Health Organisation

CHAPTER 1
INTRODUCTION

1. Background

In South Africa, a shift in lifestyle and dietary behaviours, due to rapid economic developments and urbanisation, has been linked to the increase in noncommunicable diseases (NCDs) (Nnyepi et al., 2015). As defined by the World Health Organization (WHO), NCDs are a class of chronic non-infectious medical conditions or diseases caused by the combination of behavioural, environmental, physiological, and genetic factors (World Health Organization, 2021). This transition observed in nutrition involves the abandonment of unrefined traditional diets for highly refined foods – mostly concentrated in energy; saturated and trans fats; salts; and simple sugars (Nnyepi et al., 2015). One of the more alarming changes seen worldwide however, includes the increased consumption of energy drinks (EDs) which are high in sugar, caffeine, and other bioactive constituents (Nowak and Jasionowski, 2015). In this dissertation, the term “energy drinks” describes cold beverages distributed and consumed in the form of a typically carbonated liquid which contains bioactive compounds, a high caffeine concentration, and claims energy-boosting properties or increased cognitive and physical performance (Plamondon, 2013).

Functional foods, loosely known as health-promoting foods (Corbo et al., 2014) , are those described by the European Commission as food products that have beneficial physiological effects on one or multiple functions of the human body beyond basic nutrition (Ozen et al., 2012), thus, improving the general physical health and/or decreasing the risk for potential diseases (Ozen et al., 2012). Amongst various sub-divisions of functional foods accessible to the market, Orru et al. (2018), states that beverages are most popular due to meeting the demands of consumers based on the product’s shape, size, storage, and potential to provide the desired nutrients and bioactive compounds (Orrù et al., 2018). Although EDs, together with sports drinks, are found under this sub-division of functional beverages (Corbo et al., 2014), there are emerging studies which highlight possible physiological adverse effects of ED consumption, attributed to the ingestion of high caffeine concentrations. With that being said, there is a rise in concern for increased ED consumption particularly in the younger population group, considering that they are the predominant target market (Reid et al., 2015).

1.1.1 The Brief Evolution of Sports and Energy Drinks

Prior to the existence of EDs, sports drinks were first introduced in the year 1927 to assist with the body meeting the demands of prolonged physical activities (Higgins et al., 2010). Sports drinks, also known as isotonic drinks or fluid-replacement drinks (Guo, 2009), are generally regarded as beverages created to provide fast and efficient replacements of carbohydrates, fluids, and electrolytes to working muscles (Guo, 2009). Traditionally used by athletes, primarily for electrolyte supplementation (Laquale, 2007), sports drinks play a significant role in rehydration; enhancing physical performance and endurance; and aid in the healing or prevention of certain medical conditions (Orrù et al., 2018). Popular brands of such drinks include *Powerade, Energade, Lucozade, Gatorade,* and *Vitamin Water*, which are usually found sharing the same shelves as EDs in local and major chain stores across South Africa, as depicted in Figure 1 below.

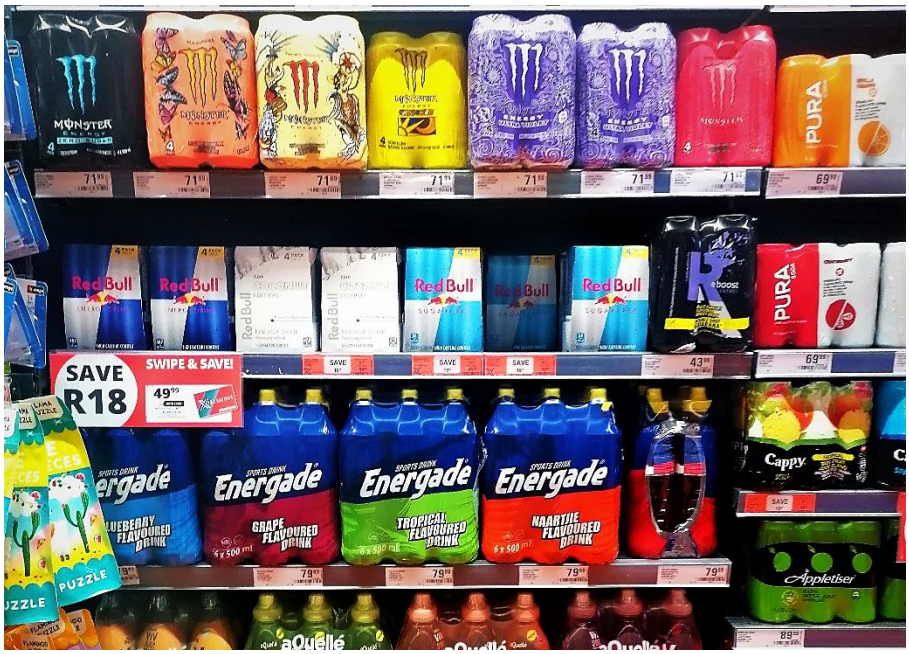


Figure 1 Energy drinks and sports drinks displayed on the shelf of a local chain store in South Africa

Many individuals often confuse sports drinks with EDs. However, sports drinks, as described in Table 1 below, are traditionally non-carbonated and are free of caffeine or any other bioactive stimulants found in EDs such as taurine, guarana seed, or yerba mate extract (Plamondon, 2013). Furthermore, sports drinks contain less sugar and a greater concentration of mineral salts in relation to that of EDs. Sports drinks are known to come in three different forms: *isotonic* – when salt and sugar concentrations

of the beverage match that of the blood glucose and sodium concentrations in the body; *hypertonic* – when salt and sugar concentrations of the beverage are greater than that of the blood; and *hypotonic* – when salt and sugar concentrations of the beverage are lower than that of the blood concentrations (Orrù et al., 2018; Urdampilleta et al., 2015). Since hydration can be a limiting factor to physical and athletic activities (Urdampilleta et al., 2015), the variety of tonicity in sports drinks is essential and plays a key role in enhancing performance, especially during ultra-resistance exercises, due to its effects in the amounts of fluid and nutrients that enter the bloodstream, as well as the absorption rate of nutrients in working muscles (Urdampilleta et al., 2015). Subsequently, added benefits of electrolytes in sports drinks, such as sodium, potassium, magnesium, and calcium have been reported to contribute to the reduction of hyponatraemia; promote the reabsorption of water; and play a role in the recovery of muscle tissue, following performance (Diel and Khanferyan, 2018).

Table 1 Ingredient list of various sports drinks (Adapted from nutritional labels of beverage bottles)

Name of Drink	***Gatorade (591ml)***	***Energade (500ml)***	***Powerade (500ml)***
Calories (kcal)	140	155	100
Sodium, mg	270	160	260
Potassium, mg	72	25	31
Total carbohydrate, g	36	29	23
Sugar, g	34	28	18
Total Fat, g	-	-	-
Caffeine	-	-	-
Other	-	-	Vitamins B6, B12, B3 and magnesium
Ingredients	Water, sucrose syrup, glucose-fructose syrup, citric acid, natural grape flavour with other natural flavours, salt, sodium citrate, monopotassium phosphate, red 40, blue 1	Filtered water, high fructose corn syrup, sucrose, glucose, citric acid, taurine, glycerol, salt, sodium citrate, monopotassium phosphate, panax ginseng, gum arabic, natural flavours, ascorbic acid, caffeine, niacin, turmeric, ester gum, sucralose, vitamin B2, vitamin B6, vitamin B12	Water, high fructose corn syrup, maltodextrin (glucose polymers), citric acid, acacia, potassium citrate. salt, potassium phosphate, natural flavours, glycerol ester of wood rosin, brominated vegetable oil

Dehydration, occurring when water loss is greater than its intake, can result in short-term physiological adverse effects such as epigastric cramps, constipation, nausea, and vomiting (Barnes and Devries, 1999). In addition, not only does dehydration disrupt the fluid and electrolyte balance in myocytes but can significantly affect the electroactivity of both the central nervous system (CNS) and the cardiovascular system (CVS), as well as alter body-temperature control (Diel and Khanferyan, 2018), ultimately compromising physical performance. To substantiate the efficiency of sports drinks, Barnes and DeVries (1999) conducted an experiment to determine the effects of sports drinks versus water hydration prior to physical exercise. With the objective to prove whether sports drink consumption had any influence on running performance

(assessing changes in HR (bpm); time of run; respiratory exchange ratio; expiratory volume; as well as maximal oxygen respiration volume [$VO2_{max}$]), they found that sports drink consumption prior to physical exercise (middle-distance running) improved the $VO2_{max}$, when compared to that of water. Hence, the supplementation of water and nutrients, which significantly contributes to the accumulation of glycogen in active muscles (Diel and Khanferyan, 2018), increased physical performance.

In advancing years, however, EDs became popular and in the year 1962, *Lipovitan D*, also known as *Libogen* or *Livita*, was first introduced as a high-energy beverage to the Asian market by the Japanese pharmaceutical company, Taisho (Antes, 2018). *Lipovitan D* was initially created with the objective to assist employees in working efficiently for extended hours, by increasing alertness and providing a short-term boost of energy (Antes, 2018). Following its inception, the success of the beverage inspired the development of advanced EDs which consisted of increased sugar concentrations and added bioactive compounds, thus resulting in larger volumes per serving for the newly developed beverages. For instance, when comparing the ingredient list for a single serving of *Lipovitan* D to that of Red Bull® (Table 2) – being the most successful ED brand, founded in the year 1984 by Dietrich Mateschitz (Pangakar and Agarwal, 2013), one can identify the differences in dietary composition and volume between the two beverages.

Table 2 Ingredient list of a single serving of Lipovitan D versus that of Red Bull® (adopted from nutritional lists of beverages)

Ingredients	Lipovitan-D (100ml)	Red Bull® (250ml)
Water	Present	Present (carbonated)
Calories, cal		112
Fat (saturated), g	<0.1	0.1
Sugar (sucrose), g	18	27
Total Sodium, g	-	* 0.105
Potassium, g	-	0.003
Taurine (0.4%), g	1	1
Magnesium carbonate, g	-	0.008
Inositol, g	0.05	Present
Caramel, g	-	Present
Caffeine, g	0.05	0.08
Niacin, g	-	0.02
Vitamins: • Pantothenic acid, g	0.005	0.005
• Vitamin B6, g	0.005	0.005
• Vitamin B1, g		0.005
• Vitamin B2 (riboflavin), g	0.005	0.005
• Vitamin B3		0.005
• Vitamin B12, g	0.005	0.005
Energy, kJ	356	487
Protein, g	0.8	1
Total Carbohydrates, g	20	27
Glucuronolactone, g	0.4	0.6
Citric acid, g	0.5	Present
Flavourings	Present	Present (Artificial)
Colouring	(tartrazine)	Present
Salt, g	* 0.3	-

***Both beverages contain variants of sodium, with Lipovitan D containing sodium chloride (salt) and sodium benzoate as a preservative, and Red Bull consisting of sodium bicarbonate listed as "sodium" on the nutrition list**

Following the introduction of *Red Bull*® to the US market in the year 1997 (Akinmolusun et al., 2012), there has been significant global proliferation of ED sales. Although both sports drinks and EDs were created with similar intention, EDs are marketed as acute energy boosters which guarantee sustenance, improve physical performance, cognitive concentration, and endurance (Plamondon, 2013). Energy drinks achieve this through their ability to suppress lethargy by inducing high concentrations of sugar and caffeine to increase energy levels and stimulate the CNS (Marczinski and Fillmore, 2014), as opposed to supplementing for loss in water, vitamins, and electrolytes, like sports drinks. Aside from the inadequate supplementation of vital minerals and vitamins in EDs, it has also been reported that EDs also promote dehydration (Higgins et al., 2010). Even though these beverages contribute very little in overall nutritional value, they are consumed due to their high-energy effects.

1.1.2 Rising concerns of ED consumption on health

Even with the claimed physical benefits of acute consumption, research has shown that EDs may result in adverse physiological effects, primarily due to caffeinism, defined as a state of intoxication induced by consuming excessive amounts of caffeine (Greden, 1974; Gunja and Brown, 2012; Turnbull et al., 2017). Caffeinism has been observed from caffeine plasma concentrations of 15mg/L or more (Cappelletti et al., 2018), and this can be achieved through the minimum ingestion of approximately 1000-1500mg of caffeine, per day (Cappelletti et al., 2018). These amounts, put in the context of daily consumption, can be equated to consuming between 13-20 cups of *Nescafe*® coffee or 12-18 small cans (250ml) of Red Bull® (Sereshti and Samadi, 2014). In this dissertation, adverse effects are defined as physiological and/or psychological impairments or pathologies which reduce the ability of an organism to optimally perform all necessary functions that ensure survival (Turnbull et al., 2017).

Caffeinism, defined above, is known to cause various adverse physiological events. These events are documented as those affecting multiple organ systems, namely the hepatic, renal, gastrointestinal, and predominantly, the neurological and cardiovascular (CV) systems (Stacey et al., 2017). Common medical conditions induced by caffeinism which have been reported by patients include heart palpitations;

hypertension; diuresis; the over-stimulation of the CNS resulting in jitters and hyperactivity; vomiting and regurgitation; nausea; hypokalaemia; metabolic acidosis; and convulsions (Breda et al., 2014). Additionally, psychological impairments have also been observed from caffeine intoxication and are identified as those in concordance with anxiety and stress neuroses (Greden, 1974; Jee et al., 2020), thus producing symptoms such as muscular twitches, tremors, irritability, nervousness, insomnia, as well as tachypnoea (Greden, 1974). Focusing on the CVS, acute caffeine consumption may directly affect myocardial stimulation by increasing cardiac output, tachycardia, and ectopic beats (Lee et al., 2009). Cardiovascular conditions can be triggered by a daily caffeine intake of more than 400mg, which has been associated with an acutely elevated BP and an increased HR (Nowak and Jasionowski, 2015). Consequently, adverse effects in the CVS can result from consuming as little as two 500ml cans of EDs per day, a quantity far lesser than the reported volumes that trigger caffeinism. With acknowledgement that the effects of caffeine differ in individuals according to whether consumption is acute or chronic; level of intake; as well as tolerance development, in very rare cases, caffeinism has been documented to result in death due to direct cardiac causes, with ventricular fibrillation being the most prominent (Cappelletti et al., 2018).

Similar to global trends, EDs distributed in South Africa are marketed on their presumed ability to improve mental and physical performance (Stacey et al., 2017). Although nutritional information provided on ED labels show contents such as concentrations of caffeine, B vitamins and herbal extracts, consumers are often unaware of the compounding stimulatory effects of the herbal extracts (such as guarana seed extract and yerba mate) when combined with caffeine (Nowak et al., 2018). This synergy has been shown to raise the caffeine concentration from 80mg/can to an alarming approximation of 300mg/can (Nowak et al., 2018). However, even with documented findings of adverse physiological effects, ED consumption has continuously experienced global growth over the last two decades (Reid et al., 2015). At present, little to no regulation of their distribution and consumption has been identified, especially in South Africa (Stacey et al., 2017).

1.2 Significance and Motivation

As mentioned previously, some reports across the globe have documented an increased use of EDs (Kurtz et al., 2013; Reid et al., 2017). Regarding consumption patterns amongst population groups, results from a cross-sectional survey conducted in tertiary institutions located in Trinidad and Tobago confirmed that males were more likely to have used EDs than females, with 76% of males initially using EDs before the age of 20 years (Reid et al., 2015). Additionally, the study also reported that males were twice as likely, in comparison to their female counterparts, to consume EDs for more than seven days a month. Similar findings in a Polish student cohort indicated that the male population (74.6%) used EDs more than the female population (60.8%), with 37% of males consuming EDs daily compared to 18% of females (Nowak and Jasionowski, 2015). Focusing on the different ethnic groups, from the Trinidad and Tobago study, Reid et al., (2015) determined that the largest ethnic group to consume EDs amongst university students was the black group (35.9%), in comparison to East Indian (32.9%); mixed (28.4%) and other races (2.1%). A study in the United States of America reported similar findings regarding ethnicity (Boehm et al., 2021).

Stacey et al. (2017), identified similar ED consumption trends in South Africa through the assessment of participants above the age of 15 years (n=25 005), from the annual national *All Media and Products Survey (AMPS) 2013* (Stacey et al., 2017). The survey looked at the number of EDs consumed (one serving=500ml can) in the previous seven days, with the research question being trichotomized into three variables: prevalence of any ED consumption (1-2 EDs in previous week); prevalence of moderate ED consumption (3-6 EDs in previous week); and prevalence of heavy ED consumption (≥ 7 EDs in previous week) (Stacey et al., 2017). The research data took into consideration the following categories across the sample group: socio-economic status; ethnicity; gender; and age. Looking at the ED consumption prevalence amongst the different age groups, the study concluded that young respondents (15-19 years of age) had the highest mean intake per week (0,597 servings/week), followed by respondents aged between 20-24 years (0,564 servings/week) (Stacey et al., 2017). Subsequently, they determined that consumption of EDs in South Africa was much higher in younger males of a high socio-economic group (mean consumption: 0,591 serving/week) relative to females (mean consumption: 0,445 serving/week). Assessing consumption patterns across the different ethnic groups, the

research determined that black respondents (reference category) had a significantly higher prevalence for both moderate and heavy consumption compared to other racial groups (Stacey et al., 2017).

When considering the prevalence of hypertension amongst the black ethnic group, these statistics are worrisome. In South Africa, cardiovascular diseases (CVDs) are the second leading cause of death following HIV/AIDS – being responsible for one in every six deaths (Ntuli et al., 2015). The *Heart and Stroke Foundation South Africa* reported that hypertension was the leading cause of CVD mortalities and is the leading risk factor for strokes in the country (Ntuli et al., 2015). In the year 1998, the prevalence of hypertension in South Africa was estimated at 21% (Steyn et al., 2001), with a greater prevalence in women (14%) than in men (11%) (Ntuli et al., 2015). Consequently, in 2016 the prevalence of hypertension had almost doubled since 1998, resulting in an increase from 25% to 46% in women and 23% to 44% in men (SADHS full report 2019, 2019). Similar trends are shown in data collected in the year 2015 from WHO, in which South African men (27.4%) displayed a higher prevalence of hypertension than women (26.1%), with an overall higher prevalence in hypertension (60%) in the country (World Health Organization, 2018).

Epidemiological evidence often highlights that black ethnic groups are most likely to have a higher prevalence of hypertension in comparison to other ethnic groups (Lackland, 2014; Lindhorst et al., 2007). Similar results were found in two cohorts determining the differences in CV measurements between black and white participants, particularly those living in the urban areas, where BP levels were determined to be much higher in black people who, in addition, had an onset of hypertension which developed much earlier when compared to their white counterparts (Lackland, 2014; Lindhorst et al., 2007). Not only is hypertension prevalence becoming a greater problem in South Africa, but research clearly highlights an additional presence of early vascular aging as well as an increased risk for CVDs amongst the black population (Schutte, 2019). This high prevalence of CVDs, along with the increased consumption of EDs by young adults, raises a question of the adverse effects of ED consumption on the already-at-risk CVS of young, black South African university students.

1.3 Aims and Objectives

The aim of this study was to determine the effects of acute ED exposure on the CVS of generally healthy, black, male university students in the context of potential risk for the development of hypertension.

The objectives of the study were as follows:

1. To evaluate changes in heart rate (HR, bpm) and arterial blood pressure (BP, mmHg) (systolic and diastolic) before and after the administration of ED in healthy, young black males.
2. To compare the effect of ED consumption on HR (bpm) and arterial BP (systolic and diastolic, mmHg) versus the effects of a placebo in healthy, young black males.
3. To determine whether any relationship exists between ED consumption and CV variables such as HR and BP.

1.4 Research Questions

1.4.1 Does the acute exposure of EDs on the CVS cause changes to HR and BP in healthy, young black males?

1.4.2 Is there any association between ED consumption and CV variables in a population that is already at risk of CVDs?

1.5 Assumptions

1.5.1 All participants have answered the survey questions with honesty, pertaining to their health, ED knowledge and consumption.

1.5.2 All participants have adhered to the pre-requisites for study participation: fasting for 10 hours prior to the study; no strenuous physical exercise; no ED and caffeine consumption for 24-hours prior.

1.5.3 The experiment was setup in such a way as to provide a quiet, calm environment. Thus, it is assumed that there were no influences of external factors to the CVSs of participants, at rest.

1.5.4 Drinks were prepared in such a way that participants were unable to differentiate the intervention from the placebo – through taste and visual observation.

1.6 Study limitations

Limitations of a study refers to the uncontrollable characteristics/variables which represent weakness of a design methodology (Ross and Bibler Zaidi, 2019), thus can compromise the study, and influence the interpretation and conclusion. The limitations of this study included:

- Participants' genetic predisposition and pharmacokinetic factors were not assessed, thus there may have been such variability in participants.
- The study sample was restricted to a limited range in age, as well as ethnicity (black). Hence, data cannot be generalised to represent the South African population.
- The duration of the experiment was restricted to 2-hours, thus effects after 2-hours in this sample is unknown.

1.7 Dissertation chapter overview

This dissertation is divided into five chapters.

- Chapter 1 introduces the concept of EDs, the brief evolution of sports and energy drinks, as well as the significance, motivation, and the aim of the study.
- Chapter 2 addresses the relevant literature on the ingredients and nutritional value of EDs, the use of EDs, and their market success, especially in the youth population. This chapter also reviews the adverse physiological and psychological effects documented in previous studies from ED consumption, as well as delve into the biochemistry of EDs and their bioactive constituents. Chapter 2 also highlights the adverse events relating to the CVS, and concludes by reviewing the prevalence of CVDs, especially hypertension, in the South African, black population.
- Chapter 3 describes the study design, study sample, as well as the experimental site, equipment, and methodology used for data collection and analysis.
- Chapter 4 presents the study's results and discussion pertaining to the changes observed in participants' CV variables before and after ED consumption, and for ED effects relative to the placebo. This chapter also reflects the assessment of whether any relationship exists between ED consumption and CV variables.
- Chapter 5 is the concluding chapter of the dissertation, summarising the study's results and concludes on the observations. This chapter also provides the recommendations based on the results from the study.

CHAPTER 2
LITERATURE REVIEW

2.1 Energy drinks: Ingredients, Nutritional Value and Usage

This chapter provides an overview of the relevant literature pertaining to the biochemical components of energy drinks (EDs) as well as the adverse physiological effects that have been reported in consumption studies. The chapter starts by discussing the nutritional values of EDs and their use by the youth population, with intent to highlight on the success of ED marketing, in the youth population specifically. It then reviews the adverse physiological effects documented in previous studies, followed by the discussion on the biochemical effects of ED consumption. The actions of the bioactive constituents in the beverage are also discussed to characterise the adverse physiological events observed, especially in the context of the cardiovascular system (CVS). The chapter then concludes by emphasising the prevalence of cardiovascular disease (CVDs) in South Africa, and highlights the racial disparities observed in CVDs.

2.1.1 Ingredients and Nutritional Value

Energy drinks, as defined in chapter 1, belong to a category of beverages that usually contain caffeine and other bioactive supplements (Corbo et al., 2014). These cold beverages are often distributed in the form of carbonated drinks which are packaged in tin cans – for the preservation of the acidity in the soft drink. Popular brands of such beverages in the global market, which can also be found in South African local stores, include *Red Bull®, Monster®, Switch®, Dragon®, Burn®, Play®, BioPlus®, Coca-Cola® Energy, Rockstar®* and South African local brand, *MoFaya®*. Common ingredients which can be found in various brands, such as that used in this study (Table 3), include caffeine; sugar; water; colourants; acidity regulators such as citric acid or sodium citrate; salts; vitamin B complex (niacin, pantothenic acid, vitamin B_6 and vitamin B_{12}); as well as bioactive compounds such as guarana extract, yerba mate, taurine, inositol, and glucuronolactone (Goldfarb et al., 2014; Nowak and Gośliński, 2020; Subaiea et al., 2019).

Table 3 Ingredients commonly found in EDs (adopted from the nutritional label of Monster® - Mucho Loco)

Monster® Energy Drink – Mucho Loco	
Ingredients/Nutritional value:	Amount present per serving (500ml):
Energy (kJ)	954
Protein (g)	0
Glycaemic carbohydrates (g)	57
• Of which total sugar (g)	54.1
Total fat (g)	0
• Of which total saturated fat (g)	0
Dietary fibre (g)	0
Total sodium (mg)	70
Vitamin B_2 (mg)	3.5 (269%)
Vitamin B_3 (mg)	42.5 (266%)
Vitamin B_6 (mg)	4 (235%)
Vitamin B_{12} (µg)	10 (417%)
Salt (g)	0.08
Taurine, 0.4% (mg)	80/100ml
Carbonated water (g)	Present
Fruit Juice from concentrate (white grape, mango, guava, apple, pineapple, passion fruit, apricot, peach, orange, lemon) (g)	Present
Citric acid (mg)	Present
L-Carnitine, L-Tartrate (0.004%) (mg)	42/100ml
Inositol (0.002%) (mg)	Present
Colourant (g)	Present
Acidity Regulators (Sodium citrate, potassium citrate) (mg)	Present
Preservatives (Potassium sorbate, Sodium benzoate) (mg)	Present
Maltodextrin (mg)	Present
Flavourings (mg)	Present
Caffeine (0.032%) (mg)	160
Stabilizers (Xanthan Gum, Sodium alginate) (mg)	Present
Non-nutritive sweeteners (Sucralose) (mg)	Present
Guarana seed extract (mg)	<300
Yerba mate (mg)	Present
Glucuronolactone (mg)	2/100ml
Panax ginseng root extract (mg)	82/100ml
Cyanocobalamin (mg)	Present

Although ED consumption has been proven to stimulate the activity of the brain and other vital organs (Breda et al., 2014; Jee et al., 2020), hence temporarily improving muscle performance and cognitive processes such as memory and elevating one's mood, multiple studies continue proving that ED consumption may be harmful to health

due to frequent and excessive caffeine consumption (Breda et al., 2014; Cappelletti et al., 2018; de Sanctis et al., 2017; Jee et al., 2020). The potential adverse effects of EDs, in relation to their ingredients, not only targets those that affect the CV and neurological systems but can result in gut and metabolic problems, as well as dental erosion over time (Alsunni, 2015; de Sanctis et al., 2017). Such conditions often occur and progress due to consumers being unaware of the true amount of caffeine consumed per a single serving (Subaiea et al., 2019).

Even though various ED manufacturers recommend a maximum caffeine dosage of 200mg per day (Gunja and Brown, 2012), a single serving of ED will likely contain a caffeine concentration higher than the recommended amount. Typically, the packaging of a 500ml can of ED will state that caffeine content is approximately 80-180mg, however, additional bioactive compounds and natural sources such as guarana, yerba mate and ginseng, also contain caffeine (Gunja and Brown, 2012).

The synergy of the various compounds in EDs results in caffeine reaching high concentrations of approximately 300mg per single serving (Gunja and Brown, 2012). These volumes are concerning, considering that mild adverse effects of caffeine on the CVS and CNS were documented with an ingestion of as little as 50mg (Subaiea et al., 2019).

2.1.2 The use of energy drinks: Youth consumption and market sales

With emphasis, the popularity of EDs stems from the desirable effects they produce such as feeling energised, inducing temporary increased alertness, and an improved memory (Hendricks et al., 2017), to name a few. This induced energy-boost in ED consumers allows them to experience an improvement in physical performance, thus allowing them to perform their desired tasks efficiently for sustained periods. These effects are required by consumers for several reasons. For instance, amongst the younger population, which predominantly comprises of students, athletes and young professionals, energy-boosting properties of EDs provide consumers with the physiological and psychological capabilities to deal with strenuous conditions such as academic pressure; pressure to perform in sports and recreational activities; as well as frequently engaging in social activities for extended periods (Hendricks et al., 2017).

In substantiation to the global increase of ED popularity and usage, a study was conducted by Alford et al., (2001) on the efficiency of a market-leading ED brand on

mental and physiological effects of 36 participants. Variables examined included: physical endurance, subjective alertness, and psychomotor performance (reaction time, concentration, and memory) (Alford et al., 2001). The study found that ED consumption significantly increased aerobic performance by 9% (maximum speed maintained) and anaerobic performance by 24%, on cycle ergometers. Additionally, the assessment of mental performance, which included memory; choice reaction; and focus, were all significantly enhanced, thus increasing subjective alertness in all participants (Alford et al., 2001).

One of the alarming uses for EDs, however, which is rapidly becoming a trend in the younger population, is to mix these energising beverages with alcoholic ones. Common alcoholic cocktails served in bars include Red Bull® and vodka or *Jagerbombs*, which is a combination shot of *Jagermeister*® and Red Bull® (Higgins et al., 2010). The combination of EDs with alcohol, according to Higgins et al., (2010), has been determined to reduce the effects of alcohol intoxication. Consumers find this convenient as it allows them to carouse and socialise for extended periods without experiencing the sensation of drunkenness. Due to these ED-alcoholic cocktails having been reported to disrupt cognitive functions and significantly reduce the depressant effects from excessive alcohol consumption, this results in consumers being unaware of their alcoholic intake and thus, are likely to increase consumption (Higgins et al., 2010). Consequently, this could potentially lead to increased harmful behaviours and the increased probability of getting involved in various life-threatening calamities, as well as the possible development of alcohol-dependency (Marczinski et al., 2012). These ED-alcoholic cocktails have also been reported to potentially induce and/or increase arrhythmias in patients with underlying cardiac diseases (Higgins et al., 2010). Although there are reported adverse effects that may occur from the consumption of these cocktails, this particular use of EDs is becoming increasingly popular in the adolescent group. For instance, in a study conducted amongst polish students aged between 12-20 years, 24% (n=631) of respondents reported consuming these cocktails (Nowak and Jasionowski, 2015). Although there was no significant comparison and influence on gender (p=0,204), the type of school that students attended showed a significant impact (p<0,01) with 37% of the respondents attending senior high school and 14% attending junior high school (Nowak and Jasionowski, 2015). However, there was a significant correlation (p<0,01) in age and the tendency

of consuming ED-alcoholic cocktails, especially amongst high school senior-students aged 17 (34%), 18 (44%), 19 (49%) and 20 (71%) years (Nowak and Jasionowski, 2015). The study further reported that of all respondents who admitted to consuming these cocktails, 4.4% (n=116) – which comprised of 60 males (5.2%) and 56 females (3.8%), reported experiencing side effects (Nowak and Jasionowski, 2015). The common side effects reported included headaches, vomiting, nausea, heart palpitations, dizziness, and gastrointestinal cramps (Nowak and Jasionowski, 2015).

2.1.2.1 Energy drinks - youth consumption

In South Africa, following its reintroduction to the global economy after democracy was granted in the year 1994 (Stacey et al., 2017), there has been a significant entry of multi-national food companies that have resulted in rapid, ever-changing diet preferences (Igumbor et al., 2012). This exposure is one of the primary reasons for EDs rising to prominence in daily diets of adolescents and young professionals (Miller, 2008). It was determined that EDs make up 20% of the total beverage market in convenience stores worldwide, with “Red Bull®” and “V” reaching 97% sales in the industry (Gunja and Brown, 2012). These statistics are echoed in the South African context, with EDs reaching high sales due to the success in advertising and branding activities being directed towards the younger audiences (Stacey et al., 2017).

South African university students, mostly those enrolled in medical and athletic programs, frequently seek dietary alternatives to deal with stressors such as academic pressure and efficient physical performance (Stacey et al., 2017). It is no surprise that this group was found to have a higher ED consumption in a 2017 cohort (Stacey et al., 2017). Similarly, in Ghana it was reported that 62.2% of students participating in athletics consumed at least one ED on a weekly basis (Reid et al., 2015), with the objective of meeting the high demands of physical performance over extended periods. These reports support the notion that ED brands are successfully being marketed to athletes through various brands sponsoring extreme sport activities. Undoubtedly, university students are frequently dealing with expectations of mastering large volumes of academic work in limited amounts of time (Reid et al., 2015). Consequently, a study reported that ED consumption elevated during extended periods of studying (Nowak and Jasionowski, 2015). Another study reported that university students (26.7%) also tend to frequently substitute sleep with the

consumption of EDs (Lee et al., 2009). In recent findings on the driving force behind increased ED usage among students, reports state that the use of EDs is mostly influenced by factors such as recommendations from physicians; engaging in physical activities; peer and social pressure; rehydration; as well as the promotion and marketing of ED brands (Reid et al., 2015). In addition to the increasing usage among young adults, research has also shown that many university students may be ignorant to the adverse effects of ED over-consumption (McDaniel et al., 2010).

In expansion to youth reliance on caffeine and EDs, in a study conducted by Temple (2009), it was reported that caffeine exposure starts in early stages of development with 2- to 11-year-old individuals consuming 0,4mg/kg and 12- to17-year old individuals consuming approximately 0,55mg/kg (Temple, 2009) per day. When you compare these findings to the average adult caffeine consumption of approximately 1,3mg/kg per day (Temple, 2009), they determine that children consume an average of half the concentration of caffeine that adults do. This is alarming, considering that paediatric ED consumption is increasing, while there have been reports of infants and children experiencing adverse physiological effects from caffeine and ED exposure (Alsunni, 2015). For instance, in a systematic review to determine fatal cases in which caffeine had been recognised as the exclusive cause, Cappelletti et al., (2018) identified 92 cases which reflected a higher frequency of caffeine-overdose related deaths in psychiatric patients (n=37; 39%), from as young as 21 years; infants (n=5; 5.4%), from as early as 5-weeks old; and athletes (n=4; 5%), from as young as 18 years of age (Cappelletti et al., 2018). The study further reported that the primary causes of death in those categories included suicide (n=36; 97.3%) in psychiatric patients, cardiac arrest due to ventricular fibrillation in all athletes (n=4; 100%), as well as child abuse and neglect in infants (n=3; 60%) accompanied by caffeine overdose (Cappelletti et al., 2018). In the instance of the youngest reported case (5-weeks old) of caffeine overdose, death resulted from persistent tachycardia and subarachnoid haemorrhages following an unknown method of caffeine administration of 5-12mg/L (Cappelletti et al., 2018).

This notable problem in young adults, regarding their ED consumption patterns, is immensely reflected in studies around the world, with 8.3% of participants of a cross-sectional survey stating that their consumption of EDs began before the age of 10 years (Reid et al., 2015). For perspective, a Polish study was conducted in 2015 with

the objective to analyse ED consumption and awareness in adolescents (n=2629) (Nowak and Jasionowski, 2015). Using questionnaire surveying amongst junior and senior high school students aged between 12-20 years, Nowak and Jasionowski (2015) reported 67% (n=1756) of respondents who consumed EDs, with more males (75%) reporting consumption than females (61%) (p<0,01). Amongst students who admitted to consuming EDs, 55% were enrolled as junior scholars, 45% were senior scholars, and 76% participated in sports (Nowak and Jasionowski, 2015). These alarming statistics showing more junior students consuming EDs than senior students (p=0,017) (Nowak and Jasionowski, 2015), stem as a result of little to no regulation of ED consumption and distribution (Schutte, 2019).

The study also analysed the frequency of ED consumption amongst the youth group. It was determined that 20% of the respondents consumed a single serving of EDs a month; more than 16% consumed more than one serving on a weekly basis; and more than 2% reported consuming at least one serving of ED per day (Nowak and Jasionowski, 2015). With regards to a 250ml serving of ED, containing approximately 80mg of caffeine (Nowak et al., 2018), 44% of students admitted to consuming a single serving daily, 12% reported consuming two servings (160mg caffeine) daily, and 3% reported consuming as much as three to four servings (240-320mg) daily, in addition to other caffeinated food and drinks consumed (Nowak and Jasionowski, 2015). The study concluded that an average daily intake of caffeine for adolescents was approximately 518,4mg/day, which included 205,1mg of caffeine derived solely from EDs (Nowak and Jasionowski, 2015).

2.1.2.2 Sales and consumption rate

The ED industry, worth billions of U.S. dollars, comprises over 600 brands that are readily available on the shelves of many supermarkets and major chain stores worldwide (Stacey et al., 2017). Like the global distribution, EDs in South Africa can be found in abundance on the shelves of chain stores, supermarkets, local food stalls, garage stations, sports centres and events, as well as restaurants and bars. In the year 2015, *Euromonitor International* (Euromonitor, 2015) reported that EDs reached the highest sales volume growth across all categories of beverages sold in South Africa (Stacey et al., 2017). Although many ED brands continue to modify their product through producing new flavours*, Red Bull®, Monster*® and *Rockstar*® continue to be

the top three best-selling EDs in the world (Miller, 2008). Even within the diversity of EDs one may find on the shelf, these beverages share similarities in their composition.

Due to their easy accessibility, relatively cheap cost, and persistent marketing on various media platforms and sports campaigns, the rate of ED consumption keeps multiplying. According to Subaiea et al., (2019), since their first appearance in Europe and Asia in the 1960's, the ED market has risen with approximately 500 new brands launched globally in the year 2006, with approximately 200 new brands launched in the U.S alone (Subaiea et al., 2019). Being the fastest growing beverage market (Seifert et al., 2011), the ED retail market in the U.S was estimated at $5,4 billion in the same year (2006-2007) and has shown trends of a similar annual growth rate (47%) over the same period. Even with the perturbing increase in ED consumption, manufacturers and distributors have remained profitable with the global ED market size being valued at approximately $53,01 billion in the year 2018, having the Asia-pacific and North America contributing 55% of the global ED market share – with the global ED market forecast to reach $86,01 billion by the year 2026 (Roy and Deshmukh, 2019). This echoes how the power of marketing, together with consumers' limited knowledge on EDs and their potential negative effects, continues to influence the desire and consumption of these beverages. Although ED popularity and consumption has infiltrated over 140 countries worldwide (Seifert et al., 2011), there are rising health and behavioural concerns for regular ED consumption, especially amongst hypertensive and pre-hypertensive individuals, and those suffering from CVDs.

2.2 The Adverse Effects of Energy Drink Consumption

Although ED consumption has been associated with positive and desirable physiological and psychological effects, as discussed previously, this section aims to solely focus on the adverse effects which have been reported post consumption.

2.2.1 Adverse physiological effects of energy drink consumption

The primary physiological effects that result from ED consumption, as stated in section 1.1.2, are due to the compounded caffeine stimulation from bioactive ingredients as well as high sugar concentrations (Marczinski and Fillmore, 2014). Plamondon (2013)

states that even moderate consumptions of caffeine resulted in people experiencing mild symptoms such as headaches, heart palpitations, muscles tremors, as well as gastrointestinal discomfort (Plamondon, 2013). Other adverse effects reported from ED consumption include muscle and chronic fatigue, diuresis, constipation, and chest pains (Subaiea et al., 2019). These symptoms, similar to other studies, were reported by youth respondents in a survey (n=2629) conducted in 2015 (Nowak and Jasionowski, 2015). Although 6% of the respondents reported not experiencing any adverse effects post ED consumption, 7% (n=195), which consisted of more females (n=109) than males (n=86), reported experiencing discomfort (Nowak and Jasionowski, 2015). Symptoms included gastrointestinal cramps (46%); hyperactivity (27%); anxiety and heart palpitations (15%); vomiting and nausea (15%); as well as over-excitement followed by sudden tiredness (8%) (Nowak and Jasionowski, 2015). Caffeinism, as discussed previously, can however, result in far more serious physiological events such as seizures, supraventricular and ventricular tachyarrhythmias, and even lead to death (Al-Shaar et al., 2017).

Since EDs contain vasoactive metabolites, the most common adverse physiological effects reported by patients are those affecting the CVS (Somers and Svatikova, 2020). The range of symptoms varies from mild HR and BP increase to severe conditions such as tachycardia and cardiac arrest (Somers and Svatikova, 2020). For instance, in a randomized crossover study conducted on 25 young, non-obese, healthy participants to determine the effects of acute consumption of Red Bull® versus tap water (355ml) on CV and haemodynamic variables (Grasser et al., 2014), the study reported an increase in both systolic and diastolic BP (SBP and DBP) (p>0,005), as well as associations with increased HR and cardiac output (p>0,005) after the acute administration of an ED (Grasser et al., 2014). Similar results were recorded for a study conducted on 68 young participants (Nowak et al., 2018), with the objective to assess the effects of acute ED consumption versus the consumption of water on arterial BP (SBP and DBP), HR and blood glucose (Nowak et al., 2018). Participants who consumed three portions of EDs (total: 240mg caffeine) presented a significant increase in DBP by over 8% (p=0,003), but no significant changes in SBP (p=0,809) or HR (p=0,750) were identified (Nowak et al., 2018). The findings of both studies reflect the potential for caffeine to induce changes in haemodynamic variables. It achieves this through two mechanisms of action which include the inhibition of

phosphodiesterase enzymes as well as antagonizing adenosine receptors in the CVS (Kurtz et al., 2013), as discussed in the following section. Other common CV clinical features identified from caffeinism, according to other studies (Turnbull et al., 2017; Willson, 2018), include bradycardia; myocardial infarction, myocardial ischemia, and atrioventricular block (Willson, 2018). These CV effects occur primarily due to positive inotropic effects of caffeine in the myocardium; its positive chronotropic effects in the sinoatrial node; as well as its ability to dilate peripheral blood vessels (Kurtz et al., 2013).

In addition to the effects of caffeine and sugars on the CVS, taurine has also been reported to have effects on vascular muscles (de Sanctis et al., 2017). In the smooth muscles found in the endothelial lining of vessels - which also play a significant role in the resistance of vessels (Grasser et al., 2014), taurine has influence on intracellular calcium concentrations which, in the CVS, may lead to coronary vasospasms (de Sanctis et al., 2017). Persistent vasospasms have been known to cause vasoconstriction and ischemia of affected tissue; angina in cardiac tissue; and may lead to death (Song, 2018). Thus, the consumption of EDs increases the probability of spastic coronary vasculature, since EDs contain approximately ten times more taurine than the required daily intake of 40-400mg (de Sanctis et al., 2017). In contrast to these findings, however, Somers and Svatikova (2020), reported that taurine can significantly reduce BP in pre-hypertensive participants. In the randomised, double-blind trial, the research team documented a reduction of 7,2mmHg in mean SBP and a reduction of 4,7mmHg in DBP following a 1,6g per day dosage of taurine administered to participants for a duration of 12 weeks (Somers and Svatikova, 2020).

There are no systematic studies that identify long-term consequences of frequent ED consumption; however, multiple anecdotal reports have identified the implications of ED consumption to produce adverse CV effects, triggered by autonomic, haemodynamic, and electrocardiographic responses (Somers and Svatikova, 2020).

2.2.2 Adverse psychological effects of energy drink consumption

Apart from the physiological effects observed, caffeine is also a psychostimulant purine-like alkaloid (Willson, 2018). The psychological stimulation outcome is relative to caffeine concentration (Richards and Smith, 2016), with positive CNS performance

and psychological effects being documented at low doses of approximately 250mg (Richards and Smith, 2016). In contrast, higher caffeine concentrations (>350mg), which can be achieved by consuming two or more servings of EDs, have been documented to increase risk-seeking behaviour and induce mental-health problems in individuals (Cappelletti et al., 2018), which include an induction or increase in anxiety, insomnia, psychomotor agitation, and restlessness (Cappelletti et al., 2018). Al-Shaar et al., (2017) further substantiated that due to high caffeine concentrations, there may be an association between increased ED consumption and mental conditions such as stress; depressive symptoms; irritability; as well as suicide ideation, plan, and attempt (Al-Shaar et al., 2017). In other studies, caffeine has also been reported to have an association with manic behaviour, cerebral vasculopathy, as well as seizures – particularly in individuals who are sleep deprived (Iyadurai and Chung, 2007; Richards and Smith, 2016). In extension to inducing mental and cognitive instability, EDs have also been reported to aggravate pre-existing neurological conditions (Grasser et al., 2014). In a randomized crossover study conducted in 2014 (Grasser et al., 2014), it was determined that the consumption of a single serving of Red Bull® (250ml) not only increased BP (SBP and DBP), but also reduced the velocity of cerebral blood flow and increased cerebrovascular resistance, observed especially in resting conditions (Grasser et al., 2014). Thus, increasing the potential to aggravate neurological conditions (Grasser et al., 2014).

As stated in section 2.1.2, EDs are commonly mixed with alcoholic beverages at social gatherings to reduce symptomatic lethargy that is often associated with drunkenness (Miller, 2008). This allows consumers of alcoholic-ED cocktails to perceive less impaired motor coordination and visual recreation time (Miller, 2008). Consequently, these cocktails allow individuals to continue drinking alcohol and exceed their intake due to having suppressed sensations of drunkenness and hangovers (Miller, 2008). Contrary to these findings, however, a more recent study that examined whether ED-alcohol cocktail mixes alter the “cognitive processing and subjective measures of intoxication” (Marczinski et al., 2012), the study reported the opposite. Using questionnaire surveying of 18 participants (age: 21-28 years), with an equal distribution of males and females, the study reported participants experiencing an impairment in simple and complex motor coordination due to alcohol to which, even

with an ED mixed in the beverage, induced no significant changes (Marczinski et al., 2012).

Many EDs commonly found in local supermarkets can contain up to an extra 100mg/L added caffeine (Gunja and Brown, 2012). Concluding that, in a worst-case scenario, by consuming as little as two 500ml cans of EDs per day, various psychological and physiological effects can possibly result in death, either from physiological damage to various organ systems or from psychological impairments which can result in reckless and fatal behaviour (Richards and Smith, 2016). The following section aims to expand on the investigation of EDs and the physiological responses reported from consumption, by analysing the biochemical reactions of the predominant bioactive constituents found in the beverage.

2.3 Biochemical effects of bioactive compounds in energy drinks

The physiological effects of EDs have been well documented, but research on the underlying biochemical mechanisms is limited. The purpose of this section is to briefly describe the biochemistry of compounds commonly found across the broad spectrum of ED brands available globally, in the context of their role for the potential induction of adverse physiological effects.

Biochemical compounds can be defined as "compounds which have the capability and ability to interact with one or more component(s) of living tissue by presenting a wide range of probable effects" (Guaadaoui et al., 2014). According to the National Cancer Institute (2020), bioactive compounds are usually known to trigger metabolic processes and alter biochemical activities in the body (NCI, 2020). These activities occur due to the physicochemical and biological characteristics of the compounds found in EDs (Kammerer et al., 2014). The derivation of bioactive compounds found in EDs can be categorised into two different groups: phytochemicals - which are those extracted from plants; and synthetic compounds - which are synthetically created using laboratory techniques. The term “bioactive” (*bios* – derived from Greek, meaning life; and *-activus* – derived from Latin, meaning with energy; dynamic; involves activity) is often associated with positive health effects (Sachdeva and Gupta, 2013). However, the physicochemical effects of these compounds can vary greatly from having the ability to maintain good health and even promote healing in living tissue, to showing

dangerous and fatal effects (Guaadaoui et al., 2014). The outcomes of physiological events that occur usually depend primarily on the dosage, frequency, and the type of biochemical compound administered (Sachdeva and Gupta, 2013).

2.3.1 The biochemistry of Caffeine

Caffeine (1,3,7-trimethylxanthine) is a methylxanthine alkaloid compound (Figure 2) structurally related to adenosine (van Dijk et al., 2018). Popular sources of caffeine include coffee, soft drinks, confectionery, and medication (primarily analgesics) (Plamondon, 2013). Natural caffeine can also be found in various teas, cacao beans, guarana mate, as well as seeds, leave and nuts of various plants native to East Asia and South America (PubChem, 2020), such as kola nuts (Wilson, 2018). Caffeine is absorbed into the blood stream at a high rate, with total absorption occurring within 45-minutes, following initial ingestion (Hori and Kitakaze, 1991). Its plasma concentration has been documented to reach a peak between 15- to 120-minutes with a half-life of 2,5- to 4,5-hours in younger consumers, and longer in elderly consumers (Sachdeva and Gupta, 2013). The preceding sub-sections highlight the effects of caffeine, primarily on the CNS, CVS, as well as other organ systems.

2.3.1.1 Action in the nervous system

Caffeine derives its stimulatory traits primarily from acting as an adenosine-receptor antagonist through competing to bind with adenosine receptors (ARs) A_1, A_2, A_3, and A_4 located throughout the body (Bolignano et al., 2007). Adenosine, produced from ecto-5'-nucleotidase, acts with extracellular adenosine-triphosphate (ATP) that is released from parenchymal (endothelial) tissue (Sachdeva and Gupta, 2013). The functions of adenosine include playing a role in the synthesis of nucleic acids, contributes to energy currency in the form of ATP and adenosine-diphosphate (ADP), and participates in molecular signalling in living cells through the formation of cyclic adenosine monophosphates (cAMP) (Berg et al., 2012). Additionally, adenosine can directly affect various synaptic processes and signalling pathways; and plays a role in the regulation of several neurotransmitters in the CNS (Sachdeva and Gupta, 2013).

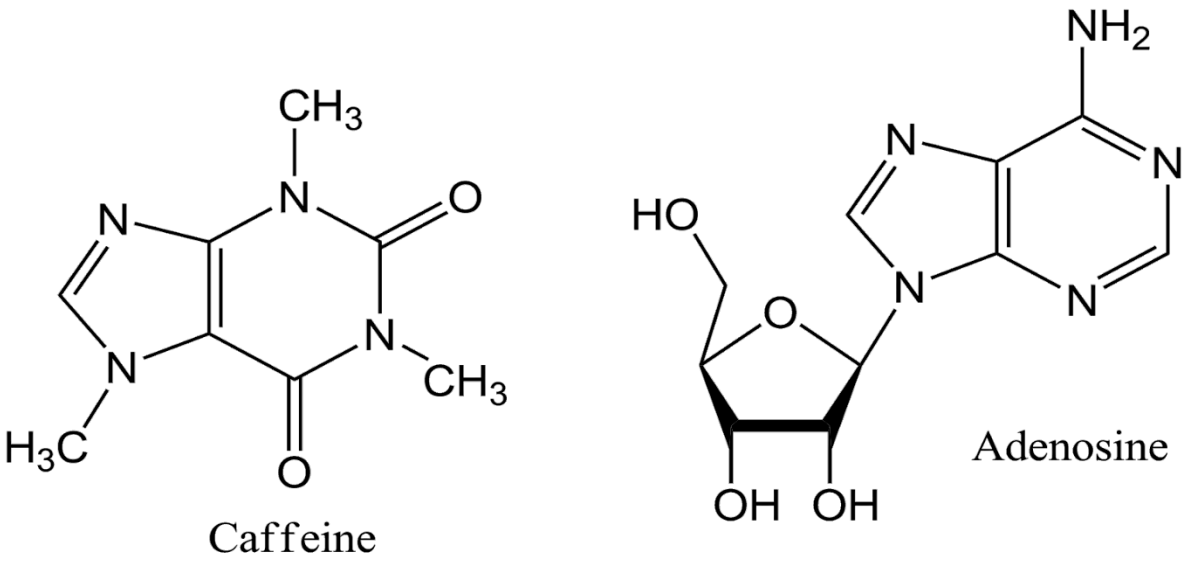


Figure 2 Molecular structures of Caffeine and Adenosine

Image from KindPNG, by Devanto Caramic, 2022, https://www.kindpng.com/imgv/hxhxJRm_caffeine-and-adenosine-adenosine-and-caffeine-structure-hd/

In neurons, caffeine blocks adenosine from binding to its receptors, which has been identified to inhibit sleep-promoting effects and promote the activation and speed of neuronal performance (Mustafa et al., 2009). This antagonistic action stimulates the medullary, vagal, vasomotor, and respiratory centers in the brain (PubChem, 2020), and promotes experiences of increased focus, alertness and feeling of optimism (Antes, 2018). Combined, these mechanisms are what provide caffeine its ability to stimulate neuronal pathways in the CNS and peripheral nervous system (PNS) (Cappelletti et al., 2018).

These stimulatory effects can be observed from consuming EDs with caffeine concentrations that are as little as 70mg/450ml. These findings deserve much attention, considering that EDs in South Africa are commonly distributed with caffeine concentrations that can exceed 250mg/500ml (Nowak et al., 2018).

2.3.1.2 Action in the cardiovascular system

It should be noted that the most extensive tissue in the human body is probably the endothelium, forming the functional and anatomical covering of arterial walls. Its permeability allows for the release of synthesized vasoactive molecular species that regulate the tonality of vascular smooth muscle cells (VSMC), through the balancing of vasoconstrictors such as renin and angiotensin, and vasodilators which include

nitric oxide (NO), bradykinins, and endothelium-derived hyperpolarizing factors (Sudano et al., 2006). Under normal physiological conditions, vasodilation, defined as the widening of a blood vessel due to the relaxation of its muscular walls (Ramanlal and Gupta, 2022), occurs in response to the release of endogenous vasodilators from tissues demanding a supply of oxygen and nutrients. The relaxation of the muscular wall is induced by the inhibition of contractility or by the removal of contractile stimuli such as adenosine, ATP, acetylcholine, and histamine. As a result, a decrease in vascular resistance occurs, accompanied by an increase in capillary perfusion (Ramanlal and Gupta, 2022).

Endothelial cells, forming the inner-most lining of the entire circulatory system, play a vital role in hemostasis. The cells achieve this by rearranging and remodeling the vasculature network, thus producing adequate blood flow that promotes growth and repair of tissues throughout the body. It has been reported that the function of the vascular endothelium and its permeability to extracellular constituents is in relation to changes of the intracellular concentrations of calcium ions, [Ca^{2+}] (Filippini et al., 2019). The maintenance of ideal physiological [Ca^{2+}] is driven by a complex mechanism that involves plasma membrane bound channels acting jointly with intracellular receptors distributed in the endothelial lining (Filippini et al., 2019).

Caffeine has the potential to improve/augment or abate endothelial function (Higashi, 2019). In endothelium, caffeine, and other xanthine molecules found in EDs, such as theobromine and theophylline, directly stimulate the production of NO (Umemura et al., 2006), through ryanodine channels of the sarcoplasmic reticula found in the VSMC (Echeverri et al., 2010). This occurs from the stimulation of the release of cytoplasmic Ca^{2+} due to the presence of caffeine, thus promoting the formation of a complex with calmodulin, a molecule involved in the synthesis of nitric oxide synthase (eNOs), taking place in the presence of Ca^{2+} (Umemura et al., 2006). Therefore, as a result, the presence of caffeine promotes the release of cytoplasmic Ca^{2+}, thus driving the expression of more NO which in turn, results in vasodilation (Echeverri et al., 2010). Interestingly, Öz et al., (2015) also reported that, although caffeine possesses pharmacological capabilities that enhance/induce vasodilation, it also appears to induce vasoconstriction (Öz et al., 2015). In a systematic review based on the assessment of coffee and caffeine effects on endothelial function of blood vessels, Higashi (2019) determined that caffeine effects on the endothelium proved

controversial, with some studies finding beneficial functions, whilst others determined more detrimental or no impact at all, following caffeine exposure (Higashi, 2019). It should be emphasized, that the heterogeneity observed in caffeine effects is in response to a multitude of factors, stemming from dietary choices and genetic predisposition, which include the amount/concentration of caffeine ingested; frequency of consumption; degree of absorption; and hepatic metabolism (Echeverri et al., 2010).

Contrary to vasodilation effects induced by caffeine, some studies in accordance with the present one (Green et al., 1996; Olas and Bryś, 2019; Öz et al., 2015; Shah et al., 2019), also determined that caffeine caused an increased BP. Hypertensive responses occur as result of vasoconstriction. As an inverse mechanism of vasodilation, vasoconstriction is the reduction in the circumference of blood vessels due to the contraction of the VSMC, thus producing higher pressures in blood flow. Caffeine can initiate vasoconstriction through various pathways. Since adenosine facilitates the inhibition of the release of norepinephrine – a molecule which, together with adrenaline, activates the SNS – by acting as an inhibitory neuromodulator of the noradrenergic neurotransmitter system (Green et al., 1996), the presence of caffeine, which competes for binding sites, therefore increases the release of norepinephrine. As a result, the CNS is positively stimulated (Dunwiddie, 1985), leading to the activation of the SNS. It should be noted that adenosine, together with ARs A_1 and A_2, provide an autacoid function in the CVS that regulates HR, coronary blood vessel dilation and blood flow, and a reduction of beta-adrenergic receptor-mediated increases in myocardial contractility (Zhang and Anderson, 2014). Adenosine is also capable of depressing both the sinoatrial and atrioventricular nodes (Kujawska, 2018). The overall effects of adenosine results in a parasympathetic response, especially in the CVS. Such events include reduced HR and conduction velocity, the promotion of vasodilation, increased myocardial strength and cardiac output (Mustafa et al., 2009). In the context of cardiology, the activation of the SNS promotes vasoconstriction in blood vessels, as well as an increased HR and myocardial contractility. Due to caffeine's ability to antagonize adenosine-mediated vasodilation (Chen et al., 2013), this aggregates its vasoconstrictive properties. As a result, in the context of HR, caffeine produces similar stimulatory actions such as those in BP, by binding to G-protein-coupled receptors located on the surface of the cardiac myocytes, thus

inducing a rise in intracellular cAMP and [Ca^{2+}] (Cannon et al., 2001). As a result, caffeine imitates the chronotropic and inotropic effects of adrenaline in the heart, therefore increased HR and cardiac contractility is observed (Shah et al., 2019).

In addition to its adenosine-antagonistic effects, caffeine in the renal system promotes the secretion of epinephrine, thus resulting in the activation of the sympathetic nervous system (SNS) (Gordan et al., 2015). In the CVS, the SNS activation results in peripheral and vasoconstriction; increased myocardial contractility; reduced venous capacitance; and increased cardio-acceleration (Kujawska, 2018). Over time, these effects contribute significantly to weakened cardiac tissue and can create the foundation for numerous CVDs. Consequently, caffeine produces its effects not only by acting through adenosine receptors, located in the brain, heart, lungs and spleen, but by also acting through ryanodine channels in myocardial tissue, the renin-angiotensin aldosterone axis; and through the activation of the autonomic nervous system (ANS) (Echeverri et al., 2010). In the context of BP and HR, the mechanisms of caffeine in the CVS are primarily attributed to aforementioned actions on cardiac muscle tissue, as well as on the endothelium and smooth muscle cells of the vascular system (Echeverri et al., 2010).

2.3.2 Additional bioactive compounds in EDs

- **Yerba mate**

Energy drinks not only comprise of significant concentrations of synthetic caffeine, but also have various extracts and ingredients which have been reported to increase the concentration of xanthine molecules in the beverages (Marczinski and Fillmore, 2014). For instance, yerba mate is said to contribute an approximated additional 80mg of caffeine to the already-present concentration of synthetic caffeine and other xanthine molecules available in a 250ml can of ED (Burris et al., 2012). In the context of EDs, yerba mate works by increasing these concentrations to, not only induce stimulatory effects on the CNS, but to also stimulate epinephrine receptors in the body and thus, significantly increase heart HR, BP, and cardiac contractility strength (Gordan et al., 2015).

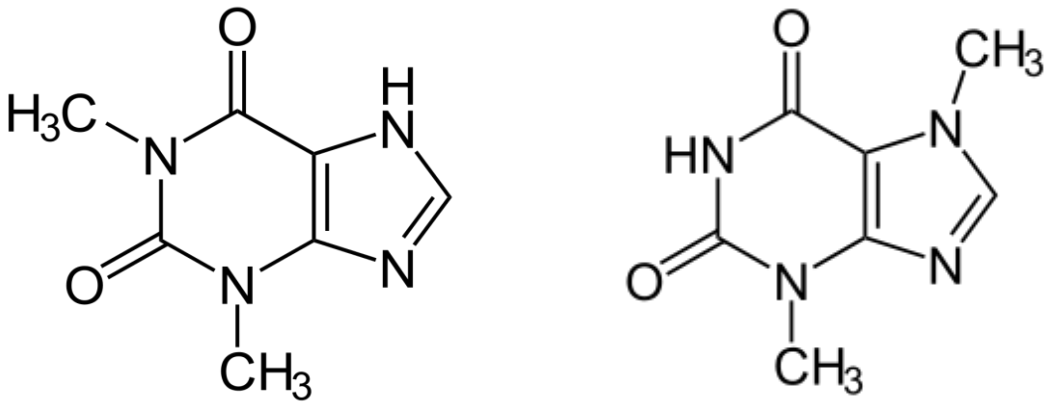
- **Sugars**

In addition, the presence of sugar, a calorie-containing constituent, in EDs may also contribute to haemodynamic changes. Unlike caffeine however, which stimulates the CVS primarily through vascular effects, sugar is able to exert similar effects on BP and HR through different haemodynamic pathways (Grasser et al., 2016). For instance, sugars could impact the CVS due to the generally observed increase in CV function which accompanies calorie consumption. Calorie ingestion is known to increase HR, cardiac output, and BP, by altering total peripheral resistance (Grasser et al., 2016).

Therefore, sugar-sweetened EDs can affect BP and HR through its effect on plasma insulin, as well as vascular resistance through the amplification of the SNS, thus producing observed disparities in both CV variables (Grasser et al., 2016).

- **Guarana and Taurine**

Additionally, guarana, a plant native to south America, also known as *Paullinia Cupana* (Al-Shaar et al., 2017), consists of bioactive and antioxidant properties that are attributed to the xanthine alkaloids present in the plant - namely: theophylline and theobromine (Silva et al., 2019). These two compounds share similar conformations (Figure 4) to that of caffeine (Figure 2) (Al-Shaar et al., 2017), thus, can produce caffeine-like biochemical effects in various organ systems.



Theophylline Theobromine

Figure 4 Molecular structures of Theophylline and Theobromine

Image from Pharmacy180.com, 2021, https://www.pharmacy180.com/article/xanthine-derivatives-2154/https://www.pharmacy180.com/article/xanthine-derivatives-2154/

In addition, guarana contains a significant amount of caffeine, with 1g of the plant (dry) containing approximately 40mg (Al-Shaar et al., 2017). Like yerba mate, the addition of guarana in EDs results in a significantly increased concentration of caffeine and other xanthine molecules, however, due to the slow releasing nature of the caffeine in guarana, the stimulating effects are thought to be less prominent while lasting longer (Silva et al., 2019). Subsequently, taurine (*2-aminoethanosulfonic acid*), a nonproteinogenic beta-amino sulfonic (Schaffer et al., 2010) derived from the metabolism of cysteine and methionine, is found in abundance in the human body (Diel and Khanferyan, 2018). In the CNS, taurine is responsible for neuromodulation, maintaining the integrity of cellular membranes, as well as the regulation of intracellular calcium concentrations (Diel and Khanferyan, 2018). Energy drinks contain an average of 200-400mg of taurine (Diel and Khanferyan, 2018). taurine has been reported to induce similar effects to that of caffeine in the VSMC, thus contributing to the resistance in vessels (Green et al., 1996), and regulating muscle contraction energy levels (Schaffer et al., 2010). Although extensive clinical research is required to confirm these reported effects, Somers and Svatikova (2020) did report that taurine triggers significant vasodilation, thus reducing BP, in pre-hypertensive participants (Somers and Svatikova, 2020), as discussed in the literature review. Similar findings were also reported by (Fujita et al., 1987). In dispute, however, Grasser et al., (2016) stated that EDs get their potency and stimulatory effects in the CVS from the concoction of caffeine with sugar, while other stimulants such as taurine and vitamin B-complexes play minor roles in the contribution to haemodynamic effects (Grasser et al., 2016).

Relating to HR, a study compared the effects of 500ml Red Bull ED, with and without taurine, on HR and stroke volume (Baum and Weiss, 2001). The study found that EDs with taurine resulted in a significant increase in cardiac contractility in the left atrium during a post-exercise recovery interval (Baum and Weiss, 2001), but no reports were made for HR changes. This study supports the notion of taurine being an anti-arrhythmic molecule (Shah et al., 2019). In contrast however, although no studies have been reported for increased BP and HR due to taurine concentrations, taurine has been determined to shorten the interval of the action potentials in cardiac tissue, as well as decelerate terminal repolarization of action potentials in the heart, thus

encouraging atrial and ventricular arrhythmias and possibly, cardiac arrest (Morin et al., 2012; Shah et al., 2019).

It should be noted that taurine, being the most abundant amino acid synthesized naturally in the human body, is primarily concentrated in the retina, as well as in cardiac and skeletal muscles (Yunusa and Ahmad, 2012). It is associated with a multitude of physiological functions, including cellular membrane stability, the modulation of intracellular Ca^{2+} levels, as well as neuromodulation (Brosnan and Brosnan, 2006). Thus, under normal circumstances, taurine has not been reported to induce any adverse effects in the CVS. Synthetic taurine, also found in EDs, would therefore be expected to produce similar physiological actions to that of taurine found in the body. In substantiation, a taurine analysis using various EDs found that even at different concentrations, varying between 375-8000mg/day, taurine resulted in no adverse physiological effects (Ikeda, 1977). Further analyses comparing the physiological impact of synthetic and natural taurine are required.

In summary, although the effects of taurine on the CVS are debated, caffeine and its possible amalgamation with sugar, taurine, and caffeine derivates found in EDs, such as guarana, may induce adverse influences on BP and HR through molecular mechanisms that act on endothelial and cardiac tissue. This may potentially induce or abet any underlying CVDs when considering long-term ED consumption.

In contrast, ginseng, a shrub from the plant family *Araliacae* indigenous to East Asian countries (Rokot et al., 2016; Yu et al., 2019), is known to significantly improve cognitive performance, and this is observed through improved memory, and a reduced decision-making time interval in consumers (Rokot et al., 2016; Yu et al., 2019). Although ginseng does not contribute to the caffeine and xanthine concentration of EDs, its ability to enhance cognitive processes adds to the effects of other biochemical contents in EDs which work to activate brain activity.

2.4 The Prevalence of Cardiovascular Disease in South Africa

The term ‘cardiovascular diseases’ refers to a vast range of non-communicable diseases (NCD) which compromise the general function of cardiac tissue and all blood vessels in the body (Kelley, 2014). Cardiovascular diseases can be triggered by abnormal heart rhythms, also known as arrhythmias; aortic diseases; structurally damaged or diseased major blood vessels and cardiac tissue; and abnormal genetic conditions affecting the CVS (Kelley, 2014). These triggers can be attributed to several factors ranging from lifestyle and diet, genetics, or injury.

With NCDs accounting for approximately 70% of all deaths globally (Jongen et al., 2019), most of these deaths are attributed to CVDs such as stroke and myocardial infarction (Jongen et al., 2019). This was reflected in 2008 when CVDs were reported to be responsible for almost half of the deaths in the world (Keates et al., 2017), with majority of the deaths occurring in low-to-middle income countries (>70% in individuals aged below 70 years) (Keates et al., 2017). In 2013, not only were CVDs the leading cause of death in the U.S (Kelley, 2014), but an estimated one million deaths in sub-Saharan Africa (SSA) were attributed to CVDs – which represented 5.5% of global CVD-related deaths, and 11.3% of all deaths in the African continent (Keates et al., 2017). With such an alarming global morbidity and mortality rate from CVDs, hypertension has been the major driving force, particularly in South Africa. In a systematic review of 33 studies which were conducted in 15 SSA countries, the prevalence for hypertension in black cohorts ranged between 15-70% from the year 1999 to 2003 (Addo et al., 2007). The study further explained that the urban areas of almost all the countries analysed had a much higher prevalence of hypertension, when compared to that of countries with well-established economies, than their rural counterparts (Addo et al., 2007). Globally, hypertension has affected over one billion people (Jongen et al., 2019), and South Africa remains one of the countries on the African continent that is most affected, having a prevalence of approximately 60% in the country’s population for hypertension (Jongen et al., 2019).

The increase in the prevalence of CVDs, as seen in South Africa, is attributed to multiple factors such as urbanization and epidemiological transitions (Keates et al., 2017), which are characterised by migration and significant changes in lifestyle and dietary preferences (Keates et al., 2017; Nnyepi et al., 2015). A South African cohort

of the THUSA study, which was conducted between the years 1996 to 2005, demonstrated that socioeconomic and education status contributed to a shift in lifestyle and behavioural changes (Keates et al., 2017). These changes include tobacco smoking; a shift from unrefined staple foods to processed and refined foods that are higher in salt and sugar content; as well as conducting sedentary lifestyles (Keates et al., 2017; Nnyepi et al., 2015). This has resulted in a higher rate of obesity, with South Africa being documented as one of the top ten African countries with the highest obesity rates (27%), particularly in black women (10-15%) than in their male counterparts (4-5%) (Keates et al., 2017). This is in correlation to the rise in hypertension statistics observed in the country (women: 68%; men: 31%) (Schutte, 2019). In a recent report, the age-standardised death rates for NCDs have surpassed that of HIV/AIDS and tuberculosis (TB) combined (Schutte, 2019). Subsequently, obesity increases the probability for the development of other NCDs. In addition to hypertension, other CVDs which have a significant impact on the morbidity and mortality rate in Africa include stroke, cerebrovascular disease, endomyocardial fibrosis, Takayasu arteritis, arteriosclerosis, atherosclerosis, coronary artery disease rheumatic heart disease, cardiomyopathies, as well as heart failure (Keates et al., 2017).

2.4.1 Racial differences in CVDs

The racial disparities in CVDs, especially hypertension, have long been recognised and reported in many countries. In the U.S, the contrast on life-expectancy for the black American population in relation to the Caucasian population is evident (Lackland, 2014). For instance, the probability for stroke mortality was reported to be twice as high, and end-stage renal disease five times higher in black American men and women and develops at a considerably earlier age than their white counterparts (Lackland, 2014). These findings are substantiated by another study using meta-analyses in black versus white populations both, in South Africa and the U.S (2007), in which it was found that hypertension was more prevalent and severe amongst the black population in comparison to their white counterparts, even in urban areas (Lindhorst et al., 2007). Additionally, the study further determined that the black population groups in both countries had significantly higher SBP and DBP than the white population group (Lindhorst et al., 2007).

The exact mechanisms that contribute to the differentiation in CVD prevalence amongst both populations are not fully understood. Aside from differences in BP, other factors according to various studies (Lackland, 2014; Lindhorst et al., 2007), include the differences in accessibility to hypertension treatment and general medical care. Other factors include black populations having increased salt sensitivity and renal sodium handling in comparison to their white counterparts (Lackland, 2014; Lindhorst et al., 2007), primarily due to genetic determinants; difference in body mass, especially in women, having a greater obesity rate and prevalence for being overweight; resistant and refractory hypertension occurring primarily in the black population; as well as dietary factors (Lackland, 2014; Lindhorst et al., 2007).

Knowing the prevalence of hypertension and CVDs in South African black populations, paired with the increasing use of EDs, particularly among young individuals, this study aims to investigate the use of EDs in black male university students and what possible effect this may have on an already at-risk CV profile. It should be noted that although studies on physiological effects of ED consumption have been reported for many countries, no such study has been conducted in the South African context, especially in black students. In the context of ED research in South Africa, Stacey et al., (2017) highlighted the trends in ED consumption and the influence of advertising (Stacey et al., 2017). The study provided extensive research into trends in ED consumption relative to age, gender, household income, and ethnicity; as well as ranked the leading television channels by ED expenditure and number of advertisements (Stacey et al., 2017). Consequently, being the only study of its nature in the country, the data served as inspiration to further research the physiological effects of ED consumption. This comes at a time where numerous studies have reported adverse effects from ED consumption. Furthermore, with South Africa's high prevalence of CVDs, this study is driven by a sense of urgency to investigate and provide empirical data on CVS responses to diet, such as the popular ED.

With acknowledgement to similar studies being conducted by researchers in several countries, this dissertation aims to contribute and/or substantiate to the knowledge of ED consumption and its effects, and to CV physiology, in South Africa.

CHAPTER 3: METHODOLOGY

This chapter outlines the procedures pertaining to the study design; sample selection; study location; the experimental procedure; data collection; the techniques for statistical analysis; ethical matters as well as measures which were applied to ensure the validity and reliability of the study.

3.1 Study Design

Following the positivist paradigm, we used the quantitative, experimental research design, with a randomised, controlled, cross-over approach (Kurtz et al., 2013). This study was conducted on generally healthy, male, black, university students aged between 18-29 years, to investigate the physiological effects of acute ED consumption on the CVS.

3.2 Study Location

The study was conducted at the University of South Africa (UNISA) Science campus, in the Food laboratory which is on the second floor of the Eureka building, situated in Roodepoort, Johannesburg. The study was conducted non-consecutively based on participant bookings on weekdays from mid-October 2020 to the end of June 2021.

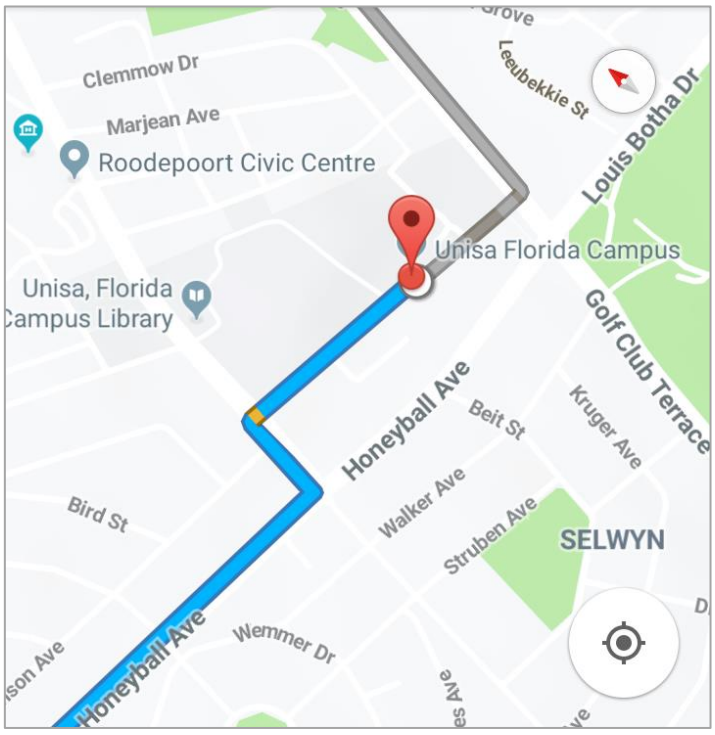


Figure 5 Map of Study Area (UNISA) (Source: Google Maps, 2019)

3.3 Population and Sampling Strategy

The study population consisted of black, male, university students studying at tertiary institutes in the Gauteng province of South Africa. A priori power analysis was performed *(G*Power 3.1.9.4)* (Faul et al., 2007), to determine the required sample size. To reach a power (1-β) of 0,80, effect size (f^2) of 0,05 and the significance level (α) of 0,05, the result indicated a total of 27 participants would be required.

Therefore, participants aged between 18 to 29 years were recruited from the UNISA Science campus using a single-stage sampling procedure (Creswell, 2014). Consecutive sampling was used to recruit participants at the different campus entrances. Each fieldworker responsible for recruitment handed out participant recruitment flyers (Appendix A) that briefly explained the study being conducted and what was required of the participant. In addition, digital copies of the recruitment flyer were distributed to students, on and off the UNISA campus, who showed interest for recruitment. Due to a low response rate during the COVID lockdowns, further snowball sampling was applied to male university students at any tertiary institute and who are residing in Gauteng. Prior to study enrolment, volunteering participants were screened using a checklist (Appendix B) based on the inclusion and exclusion criteria (Table 4).

Table 4 Study Inclusion and exclusion criteria

Inclusion Criteria for Participants	Exclusion Criteria for Participants
Black/African	Diagnosed with a chronic disease (such as hypertension, diabetes, epilepsy)
18 to 29 years of age	An office blood pressure ≥ 140/90 mmHg (for screening purposes, previously diagnosed hypertension)
Male	Recent use (within 48-hours) of prescribed or over-the-counter medication
Registered university student	Allergies to EDs or its contents
Student is a consumer of energy drinks	Diagnosed with conditions that are affected by caffeine such as arrhythmia, heart attack and stroke

3.4 Study Variables

Variables in quantitative research refer to the characteristics of an individual or organisation which are observable and measurable and tend to vary among the different individuals and organisations (Creswell, 2014). Data on the physiological effects of acute ED consumption on the CVS was collected through assessment of the following variables:

Independent variables:

Independent variables, defined as those that induce or influence the outcome of the dependent variables (Kumar, 2012), are also known as *predictor, antecedent or manipulated* variables (Creswell, 2014). In this study, independent variables consisted of 500ml of ED (intervention) and 500ml of carbonated diluted fruit juice (placebo) administered to participants.

Dependent variables:

The dependent variables of a study refer to the outcomes which stem from the manipulation and influence of independent variables (Creswell, 2014). These are also known as criterion, response, outcome, or effect variables. In this study, the dependent variables were BP (SBP, DBP, mean arterial pressure, pulse pressure expressed in mmHg), and HR (bpm) of the participants.

Moderating variables:

Moderating variables are defined as the independent variables which have an impact on the direction and/or strength of the relationship between the dependent and independent variables (Creswell, 2014), also called covariates. In this study, the moderating variables were participant weight, height, body mass index (BMI), waist circumference and age.

3.5 Study Procedure

Before proceeding with the study, all protocols were explained to the volunteers in English, in a private space as part of the consent procedures, using the information sheet and consent form (Appendix C). Upon obtaining written informed consent,

volunteers were enrolled as study participants and assigned two dates for administering the intervention and placebo in no ordered fashion.

Participants were assigned either a placebo or an ED in a single blind manner to prevent any psychological influence on the effects of the beverage. Blood pressure and HR were recorded at several time points. There was a washout period of at least 4 days following the first assessment after which participants were required to return for the second assessment period, where the alternate beverage was administered, and the same standard procedure of the experiment was followed.

Participants were required to adhere to the following before each experimental day:

- Fasting for 10-hours.
- No ED or caffeine consumption for at least 24-hours prior to the start of their experiment.
- No strenuous physical exercise for at least 24-hours prior to the start of their experiment.

3.6 Experimental Procedure and Data Collection

The experiment was conducted in the Food laboratories of the UNISA Science Campus. For this experiment, two beverages were used, a *Monster*® carbonated ED as the intervention drink (Figure 6) and a diluted (1:3) *Magalies*® fruit juice concentrate with *Schwepps*® *Soda Water* as the placebo.

Figure 6 Mucho Loco Monster® energy drink

Participants were single-blinded to which beverage they were consuming to prevent any psychological influence on the effects of the beverage consumed. The baseline CV measurements (0-minutes) were conducted prior to consumption of the beverage. Participants then consumed the first beverage within a grace period of 10-minutes, then CV measurements were recorded in the following time intervals: 10-minutes – indicating the first reading post beverage consumption; 30-; 60-; 90- and 120-minutes after consumption.

Any adverse events or physical discomfort experienced by participants were immediately reported verbally to the researcher and captured. Possible adverse events from ED consumption included headaches, restlessness, jitters, nausea, heart palpitations, dizziness, sweating, an upset stomach or diarrhoea (Lee et al., 2009). The UNISA clinic nurse was alerted and on stand-by should any participant require emergency medical assistance.

A washout period of at least 4 days following the first assessment (Kurtz et al., 2013) was granted, where participants could proceed with normal day-to-day activities. After the washout period, participants were required to return for the second assessment period where the alternative beverage was administered, and the same standard procedure of experiment was followed.

3.6.1 Cardiovascular Measurements

At each measuring interval, BP and HR were measured twice, 2-minutes apart, while participants were in a seated position. Measurements were performed using a calibrated, automated blood pressure monitor (OMRON MIT5 device: OMRON Healthcare; Kyoto, Japan) and an adjustable cuff. Measurements were performed on the left arm of each participant with the adjustable cuff placed on the brachialis for all respective readings. For each CV measurement taken, participants were requested to remain quiet and seated, rest their left arm and control their breathing. During the intervals between measurements, participants were given the option to stand, stretch their bodies and use the restroom. Measurements were conducted at baseline (0-minutes) then followed by the administration of the beverage over 10-minutes; 10-minutes, following initial beverage consumption; followed by measurements at 30-minute intervals for the remaining duration of 2-hours (30-, 60-, 90-, and 120-minutes). All measurements were performed in duplicate, and the mean of both recordings were

used. Separate data sheets (Appendix D) were used to capture time of beverage administration (experimental start time) together with the type and time of each CV measurement.

3.6.2 Questionnaires

During the waiting period for CV measurements, participants completed a self-administered questionnaire (Appendix E) to obtain data on the following data and to confirm inclusion in the study: participant demographics (which included age, ethnicity and qualification registered for at UNISA or any other tertiary institute in South Africa), caffeine consumption in the previous 24-hours, usual ED consumption, smoking and the use of over the counter and prescribed medication.

Additionally, study participants received a second questionnaire to complete during the waiting period (Appendix F). This modified questionnaire was adopted from the "The Use and Knowledge of Caffeine" questionnaire (Lee et al., 2009) with permission from Dr Larson (University of the Free State) (Appendix G). This questionnaire obtained data on the following: caffeine dosage and frequency; knowledge about caffeine side effects; and withdrawal symptoms of caffeine-containing products. The purpose of this second questionnaire was to describe the study sample in terms of habitual use of caffeine.

3.6.3 Anthropometric Measurements

Anthropometric measurements were taken prior to the first beverage consumption. The height was measured in centimetres (cm) using a freestanding metric stadiometer and all participants were required to take their shoes off prior to measuring. Weight of all participants was measured in kilograms (kg) using a MDW-250L scale (Adam Equipment Co. Ltd., Danbur, CT) while the participant was wearing light clothing and no shoes. The BMI was determined using the following calculation:

$$BMI\left(\frac{kg}{m^2}\right) = \frac{Weight}{Height}$$

3.7 Product and Dosage Selection

We compared a carbonated fruit-flavoured *Monster® ED (Mucho-Loco Flavour)* found in local and major supermarkets (Figure 6), to a placebo which was formulated by diluting a fruit juice concentrate (Magalies® – Peach and Mango) with carbonated water (Schwepps® Soda water) at a ratio of 1:3. These beverages were selected on the basis of similarities in appearance (orange liquid), consistency, and a fruity taste with the sensation of a carbonated beverage. This reduced the likelihood of distinction between the two beverages, as shown in Figure 7.

Figure 7 Physical comparison of EDs versus carbonated juice administered to participants

Table 5 Content ingredients of Magalies® Fruit Juice Concentrate vs. Monster® ED

Ingredients of Magalies® Fruit Juice concentrate	Ingredients of Monster® carbonated ED
Water	Carbonated Water
Orange concentrate (28%)	Fruit Juice from concentrate (white grape, mango, guava, apple, pineapple, passion fruit, apricot, peach, orange, lemon)
Peach concentrate (7%)	Sugar
Mango puree (7%)	Glucose
Plum concentrate (5%)	Citric acid
Sucrose	Taurine (0.4%)
Citric acid (E330)	Acidity Regulators (Sodium citrate, potassium citrate)
Stabilizers (E412, E440)	Flavourings
Flavouring	Maltodextrin
Preservatives [Sodium Benzoate (E211)]	Preservatives (Potassium sorbate, Sodium benzoate)
Sodium metabisulphite (E223)	Caffeine (0.032%)
Potassium sorbate (E202)	Stabilizers (Xanthan Gum, Sodium alginate)
Pimaricin (E235)	Nicotinamide (B3)
Flavour modulator	Non-nutritive sweeteners (Sucralose)
Non-nutritive sweeteners [sodium saccharin (E954)]	L-Carnitine, L-Tartrate (0.004%)
Sodium cyclamate (E952)	Salt
Acesulfame K (E950)	Inositol (0.002%)
Antifoaming agent	Vitamins B_2, B_6, B_{12}
Clouding agent	Colourant
Colourants (E110, E104, E122, E155)	Vegetable oils (Coconut, Rapeseed
	Starch (chemically modified)

Beverage contents listed were obtained from manufacturer's ingredient list.
Beverage contents were not analysed through independent analysis

Based on the ingredients of each beverage (listed in Table 5), the placebo contains flavouring, preservatives and sweeteners but lacks the caffeine and herbal extracts found in EDs. Thus, it is not expected that the placebo will result in significant changes

in HR and BP (Pham et al., 2019), making it an ideal control for determining the effect of EDs on the CVS.

Both beverages were served at room temperature and in doses of 500ml based on the typical ED serving size in South Africa. Beverages were obtained from local supermarkets and were administered to participants in plain unlabelled polystyrene cups (Figure 7) with the objective to yield a matching single-blinded product (Kurtz et al., 2013).

3.8 Quality Criteria

The quality control of a study is defined as the operational techniques and activities which are performed to fulfil the quality of specific samples and the analysis of reference materials (Bode, 2003). This section explains the procedures taken to produce experimental results which are appropriate and meaningful to the research.

3.8.1 Reliability

The reliability of a study refers to the extent to which a measurement process yields the same results when repeated under similar conditions and is subject to minor random measurement error (Gleason et al., 2010). Moreover, reliability measures the accuracy of an instrument (Heale and Twycross, 2015), and refers to the degree to which a researcher can depend on an instrument to produce accuracy in measurements when the study is conducted repeatedly. For this study, we ensured reliability by conducting duplicate measurements for HR (bpm) and BP (mmHg) for each participant at each time point, under the same environmental conditions. In addition, the weight scale internally calibrated every time it is switched on and would, therefore, calibrate for every measurement taken.

Furthermore, conditions for all measurements were maintained at a constant. Participants were requested to fast for 10-hours prior to the experiment (including over the counter medication), to avoid strenuous exercise for 48-hours prior to the experiment as well as to wear light clothing during all measurements.

3.8.2 Validity

Validity of a quantitative research is defined as a method to extract useful and meaningful inferences from values on the instruments (Creswell, 2014). For our instruments, we used construct validity, defined as a method to draw inferences about the test scores related to the concept being analysed (Heale and Twycross, 2015), by using convergence and theory evidence obtained from surveys as well as CV measurements. All machines used to obtain CV measurements were validated by manufacturers through assessment protocols that analysed the auscultation of the devices for accuracy and precision, as well as device calibration. Our surveys were adopted from a previous study thus, maintained validity.

3.9 Statistical Analysis

IBM SPSS Statistics 27 Software (IBM corp., NY, USA) were used for the statistical analyses in this study. The statistical analyses included the tests for outliers using a z-score of 3 or more, descriptive statistics (reported as frequency and percentages for categorical data and mean and ±standard deviation for continuous data with a normal distribution), dependent t-tests, as well as single (unadjusted) correlations and partial (adjusted) correlations for the analysis of CV changes before and after ED consumption, as well as to compare ED effects against the placebo. An analysis of dependencies was performed using the two-way repeated measures analysis of variance (ANOVA), to determine whether a relationship between ED consumption and CV changes exists. Microsoft Excel and IBM SPSS were used for data representation in the form of tables and line graphs. Table 6 summarises the research methodology and data analysis procedures by stating the objectives of the study as well as the study variables investigated, and instruments used.

Table 6 Summary Table of Research Methodology and Data Analysis

Study objectives	Variables	Instruments	Statistical analysis
To evaluate changes in HR (bpm) and BP (mmHg) before and after intervention administration	Dependent: HR (bpm) and BP (SBP, DBP, PP and MAP; mmHg) Independent: Beverage administered and time	Calibrated blood pressure machine Data sheets	*IBM SPSS Program* – Descriptive statistics Dependent t-tests
To compare effects of ED consumption on HR (bpm) and arterial BP (mmHg) to a placebo	Dependent: HR (bpm) and BP (SBP, DBP, PP and MAP; mmHg) for placebo and intervention Independent: Beverage administered and time	Calibrated blood pressure machine Data sheets	*IBM SPSS Program* – Dependent t-tests
To determine whether a relationship exists between ED consumption and CV variables	Dependent: HR (bpm) and BP (SBP, DBP, PP and MAP; mmHg) Independent: ED consumption Co-variates: BMI, waist circumference, smoking, age	Questionnaires, anthropometry, CV measurements	*IBM SPSS Program* - Single correlations, partial correlations and repeated measures ANOVA

3.10 Ethical Consideration

3.10.1 Ethical clearance

Ethical clearance was obtained from the University of South Africa, College of Agricultural and Environmental Sciences (CAES) Health Research Ethics committee. Once relevant permission was granted, additional ethical clearance was obtained from the registrar of UNISA for the permission to conduct a study specifically on students at the institute under the following ethical clearance number: 2019/CAES_HREC/187

3.10.2 Informed consent

Prior to the commencement of the study, the researcher and fieldworkers described the objectives of the study, the experimental procedures as well as what is expected of the potential participants. Consent forms (Appendix C) were handed to volunteers and thoroughly explained by the research team prior to potential participants completing and signing the forms. It was made clear to all participants that they may withdraw from the study at any time point without any consequences. Participants

were assisted in this process and given the opportunity to raise concerns and ask questions. Participants were provided with R250 as a reimbursement of travelling expenses in two payments of R150 and R100 after the completion of each experiment respectively.

3.10.3 Data storage

All consent forms, questionnaires and personal information were dealt with in confidence and privacy. The hard copies were safely stored by the researcher in a locked cabinet in Roodepoort, Gauteng, where they will be stored for a maximum of 5 years. All documentation were scanned, and the digital copies were stored on Cloud storage on Google Drive under the researcher's personal account. Datasets were managed by the researcher and supervisors and were only distributed to individuals involved in the study to be used for the duration of the research project.

3.10.4 Participant safety

The experiment was conducted in the Food laboratories of the UNISA Science Campus with a certified campus nurse who was on-call for any potential medical emergencies (Appendix H). The primary researcher, Ayanda Nyalela, a postgraduate student at Unisa was appropriately trained by qualified and experienced researchers to perform standardised CV and anthropometric measurements accurately. Fieldworkers executed all tasks in a manner that expressed the participant's safety as a priority and handled all participant information with confidentiality as stated in the Confidentiality Agreement (Appendix I).

CHAPTER 4: RESULTS AND DISCUSSION

4. Introduction

The following sections present the results of the analyses as per the study objectives, which were to evaluate changes in HR and arterial BP, systolic and diastolic – before and after the administration of an ED; to compare changes in CV variables for ED consumption versus the administration of a placebo; and to determine whether any relationship exists between ED consumption and changes observed in CV variables, in healthy, young, black male participants – the results of the tests conducted are presented in section 4.2 and 4.3, and were primarily conducted for the comparison of the population's mean values for CV variables, before and after the administration of the ED; as well as for the comparison of mean values in CV changes for the administration of the ED versus that of the placebo. To determine whether any relationship existed between ED consumption and changes in CV variables, a two-way repeated measures analysis of variance (ANOVA) was conducted, as reported in section 4.4.

In each sub-section of the results, data has also been diagrammatically depicted to visually represent the correlations of variables. This allows for a clear interpretation of how CV variables were influenced from the exposure to EDs or the placebo, thus illustrating the mean changes in the CVS of participants over a total duration of 2-hours, from baseline (0-minutes), for both beverages. The chapter starts by presenting the demographic data for the study participants, together with the data on their use of caffeinated beverages (Table 7), followed by the analysis of CV variables before and after ED consumption (section 4.2), and analyses in CV changes for ED consumption versus the placebo in section 4.3. Section 4.3 also discusses the possible physiological mechanisms for observed changes under each variable. The chapter concludes with the analysis of possible relationships which may exist between CV changes and ED consumption in section 4.4.

4.1 Socio-Demographic Description of the Participants

4.1.1 Participant characteristics

From a total of 30 participants in the study, four participants' data were excluded from statistical analyses due to their CV variables being identified as outliers. Thus, data was obtained from 26 individuals with a mean age of 25,9±2,9 years, BMI of

22,7±4,4kg/m² (depicted in Table 7) and a 42% prevalence of smoking in the group. In accordance with the research question of this study, all the participants were enrolled in tertiary education, primarily in undergraduate level (34,6%).

Table 7 Descriptive characteristics of participants

characteristics	mean±SD	number (n)	percentage (%)
age (years)	25,9±2,9		
BMI (kg/m²)	22,7±4,4		
smoking		11	42,3
washout period (days)	6,8±5,47		
education			
diploma		5	19,2
undergraduate		9	**34,6**
honours		6	23,1
masters		4	15,4
PhD		2	7,7
consumption frequency of caffeinated beverages			
sparingly		2	7,7
< 7 servings/week		7	26,9
7-13 servings/week		4	15,4
≥ 14 servings/week		13	**50,0**
purpose for caffeine consumption			
academic purposes		16	**61,5**
vigilance for driving		10	38,5
athletic performance		8	30,8
social drinking		12	46,2
alcohol recovery		6	23,1
beverage enjoyment		8	30,8
Preferred caffeine beverage			
Coffee		11	42,3
Tea		4	15,4
Cool drink		12	46,2
Energy tonics/shots		1	3,9
Energy drinks		20	**77**
Other (specify)		1(alcohol)	3,9

SD, standard deviation; BMI, body mass index
Descriptive data expressed as arithmetic mean± standard deviation
Bold text indicates largest data value per descriptive sub-category

Interestingly, although all participants consume caffeine, our study found that 60,4% of participants consumed a minimum of 7 servings of caffeinated beverages per week,

with half of the sample group consuming 14 or more servings per week (Table 7). The main purposes for caffeine consumption amongst our study participants was attributed to academic purposes (61,5%), followed by social drinking (46,2%). This is in agreement with research in this field, that suggests that the primary motivation for university students to consume EDs and other caffeinated beverages may be to enhance academic performance (Bertasi et al., 2021).

The most preferred caffeinated beverage consumed in our sample group was EDs and although the quantities of beverage servings were not specified in the questionnaire, it is presumed that a single serving ranges between 250-500ml based on the quantities served in South Africa. This infers that the regular caffeine users could be consuming anything between 1750-7000ml of caffeinated beverages in a single week. These numbers are worrisome, considering that the current recommended limit of caffeine for young adults is 2,5mg/kg body weight/day or 100-175mg/day, which is equivalent to 0,1-0,175ml caffeine/day (Mitchell et al., 2013). In the context of the current study, these values can be equated to consuming a maximum of one 500ml ED can per day, which contains 0,16mg of caffeine. Thus, individuals who consume more than a single serving per day could be at risk of caffeinism, which has been observed from caffeine plasma concentrations of as little as 0,015ml/L (Cappelletti et al., 2018).

4.2 Comparative analysis for changes in CV variables before and after energy drink administration

As stated in chapter 1, the first objective of this study was to evaluate the changes in CV variables before and after the administration of an ED intervention. Our study showed that consuming a single 500ml serving of an ED significantly increased SBP, DBP, pulse pressure and MAP from 30-minutes post ED consumption, with *p*-values of <0,001, 0,015, 0,043, <0,001 and 0,015, respectively (Table 8 & Table 10). Additionally, HR showed a significant decrease relative to baseline at 10-minutes post consumption, with a *p*-value of 0,015 (Table 9). For the remaining duration of the experiment, HR remained below the baseline value, however, an increase greater than baseline was observed at 90-minutes. The results for all CV variables are illustrated and discussed in the sub-sections that follow.

To avoid repetition, all possible physiological reasons for the changes observed in CV variables assessed in this study are briefly highlighted at the end of each sub-section for all variables, in relation to the placebo, under section 4.3

Mean systolic and diastolic blood pressure

The changes in BP over the 120-minute assessment period following ED consumption are depicted in Table 8 below. The mean baseline SBP for the sample group was 121,0±9,0mmHg, and the mean baseline DBP was 80,8±8,4mmHg, falling well within the normal BP ranges for both BP variables (Ovbiagele et al., 2008).

Table 8 Changes observed in mean SBP and DBP (mmHg), before and after ED administration, over 120-minutes in black, male participants

		SBP (mmHg)			***DBP (mmHg)***		
Intervals	Time (mins)	Mean±SD	interval mean± SD	interval p-value	Mean±SD	interval mean± SD	interval p-value
1	0	121±9,0	0,7±7,3	0,612	80,8±8,4	0,9±12,7	0,714
	10	121,6±8,8			81,7±10,1		
2	0	121±9,0	8,4±7,8	<0,001*	80,8±8,4	4,8±9,4	0,015*
	30	129,3±9,3			85,6±6,0		
3	0	121±9,0	7,3±12,1	0,005*	80,8±8,4	5,2±13,7	0,064
	60	128,2±12,7			86,0±12,3		
4	0	121±9,0	4,0±12,1	0,104	80,8±8,4	3,6±9,5	0,064
	90	124,9±10,2			84,4±6,2		
5	0	121±9,0	2,7±10,1	0,187	80,8±8,4	2,2±8,4	0,186
	120	123,5±5,5			83,0±5,2		

Data expressed as mean ± standard deviation (SD). SBP, systolic blood pressure; DBP, diastolic blood pressure.
p-values less than 0,05 are considered significant (*)
Results derived using a Dependent T-test

In comparison to baseline, SBP and DBP significantly increased 30-minutes post intervention (SBP = p<0,001; DBP, p=0,015) with the increase in SBP persisting to 60-minutes post intervention (p=0,005). Although statistical significance is only evident in two intervals, BP was consistently higher than baseline readings. In addition, the study also determined that although a reduction in BP was observed from 60-minutes post intervention, all BP values up 120-minutes were still greater than initial BP at baseline, with a 2,7±10,1mmHg increase in SBP, and a 2,2±8,4mmHg increase in

DBP at 120-minutes. These results suggest that increases in BP from ED consumption persist for more than 2-hours, relative to baseline.

These results reiterate those of several studies (Kurtz et al., 2013; Shah et al., 2019; Tauseef et al., 2017). A study conducted by Steinke et al., (2009) analysed cardiac effects induced by a 500ml commercially available ED in healthy participants (mean age 25,9±5,9 years) over a period of 4-hours (Steinke et al., 2009). Steinke and colleagues (2009) determined that ED consumption increased BP, with SBP reaching its maximum at the 4-hour interval (9,6% increase, p<0,001), and DBP reaching its maximum at the 2-hour mark (7,8% increase, p=0,063) (Steinke et al., 2009). The results are similar to those of a study conducted by Shah and colleagues (2019), where it was found that SBP initially declined from 60- to 150-minutes before a significant increase occurred, lasting approximately 60-minutes (150- to 210-minutes), before reaching a plateau (Shah et al., 2019). Although our study was limited to a period of 2-hours, the increases in BP were observed earlier than results reported by Steinke et al., (2009) and Shah et al., (2019), with a decline in BP from 60-minutes, as reported by Shah et al., (2019).

In a study by Tauseef et al., (2017) changes in CV variables were assessed over the same duration as our study while also investigating the effect of serving size. Interestingly, Tauseef and colleagues (2017) determined that both SBP and DBP were significantly increased after 1-hour (p<0,001 and p=0,002 respectively), and 2-hours (both p<0,001), however these results were visible when administering twice the serving size as that of our study's (1000ml), possibly suggesting that an increase in serving size may play a compounding effect on BP (Tauseef et al., 2017). Although the proposed reason for this occurrence may be difficult to distinguish, taurine and caffeine are the two predominant constituents in EDs that have been emphasized for their impact on SBP and DBP (Tauseef et al., 2017). While the peak BP values were higher than baseline, they were still within normotensive ranges which may suggest that the adverse effects of EDs on BP could potentially be more significant in persons who are prehypertensive or at risk for CVDs, especially when consuming large quantities of EDs.

4.2.1 Mean heart rate

In contrast to the changes observed in BP, changes in mean HR displayed an irregular trend, starting with a mean value of 77,2±13,5bpm at baseline before reaching a significant reduction at 10-minutes post ED consumption (*p*=0,015). These values, presented in Table 9 below, also indicated that although an irregular trend occurred, changes in mean HR were still within the range of 73-77,2bpm, considered a normal range for an adult resting HR (British Heart Foundation, 2021). Statistically, the first interval was the only interval that showed a change in mean HR which had significance.

Table 9 Changes observed in mean HR (bpm), before and after ED administration, over 120-minutes, in black, male participants.

HR (bpm)				
Intervals	Time (mins)	Mean±SD	interval mean±SD	interval p-value
1	0	77,2±13,5	-4,2±8,1	0,015*
	10	73,0±8,2		
2	0	77,2±13,5	-1,5±9,0	0,414
	30	75,7±11,9		
3	0	77,2±13,5	-1,9±10,0	0,345
	60	75,3±10,3		
4	0	77,2±13,5	0,5±11,9	0,883
	90	77,5±8,1		
5	0	77,2±13,5	-1,9±11,7	0,427
	120	75,3±8,2		

Data expressed as mean ± standard deviation (SD). HR, heart rate.
p-values less than 0,05 are considered statistically significant (*)
Results derived using a Dependent T-test

Following the initial mean HR of 77,2±13,3bpm for the sample group, a large decrease of 4,2±8,1bpm in HR, with statistical significance (p=0,015), was observed at 10-minutes, thus reaching 73,0±8,2bpm post ED consumption. This was the lowest HR value recorded over the 120-minute duration (Table 9). For the remaining experimental duration, mean HR experienced a fluctuation, diagrammatically depicted in Figure 8, that was kept within a range of 75,0-77,5pbm, falling under the standard resting HR range for adults which ranges between 60-100bpm (British Heart Foundation, 2021). Following ED consumption, the peak value for mean HR was observed at 90-minutes (77,5±8,1bpm), being the only data point that exceeded the initial value of mean HR at baseline. Although the trend was inconsistent for mean HR post ED consumption, similar to findings by Green et al., (1996), our study also determined that HR was predominantly reduced relative to baseline, as depicted in Figure 8, with the largest decline, showing statistical significance (p=0,015), observed at 10-minutes. The data also demonstrated that apart from the significant decline observed at 10-minutes post consumption, there were no significant changes in HR for the remaining experimental duration (10-120minutes).

In contrast, however, some studies determined that ED consumption increased HR (Steinke et al., 2009; Tauseef et al., 2017; Öz et al., 2015; Shah et al., 2019). In a study comparing changes in CV variables in relation to caffeine and ED dosage, Tauseef et al., (2017) found that the group consuming the highest volume of ED (2 servings=500ml) showed the highest increase in pulse rate (16,1%) after 1-hour of consumption of an ED and was significantly different from group A (control group) (3,5%, p<0,001) and B (group consuming 250ml) (6,5%, p<0,008) (Tauseef et al., 2017). The study further determined that in relation to the control group, change in HR for group B was 7,3% (p=0,015) at the 1-hour interval and 1,4% (p<0,001) at the 2-hour interval, whereas in group C, HR was at 17,3% (p<0,001) at 1-hour and 16.4% (p<0,001) at 2-hours (Tauseef et al., 2017). Similar findings were also reported by Öz et al., (2015) and Yeragani et al., (2005), who reported a significant increase in HR and the high-frequency component of the HR variability (Yeragani et al., 2005). These results were attributed to ED consumption as well as the reciprocal increases in sympathetic tone and the decrease in vagal drive, especially during exercise (Yeragani et al., 2005). Other studies also found results in contrast to the predominant decline in HR observed in the current research. For instance, Grasser et al., (2014) reported

findings from a randomized crossover study that assessed CV changes for the consumption of Red Bull (114mg caffeine) versus tap water. The study determined that Red Bull increased cardiac output and HR by approximately 3,7bpm, amongst other CV variables (Grasser et al., 2014). Furthermore, in a subsequent study conducted for Red Bull versus tap water, the same researchers found that Red Bull increased HR by 7bpm (Grasser et al., 2016). Steinke et al., (2009) also noted a significant increase in HR of 7-11% as well as an increase in the QTcentral (QTc) interval by approximately 5%, following ED consumption (Steinke et al., 2009).

Although the literature consensus suggests that EDs may stimulate the activity in the CNS and PNS neuronal pathways due to caffeine and thus increase HR, some studies reported opposing results, similar to that of the present study. For instance, Nishijima et al., (2002) reported no changes in HR following ED consumption for individuals at rest (Nishijima et al., 2002). However, in a non-controlled study conducted by Kozik et al., (2016) they found that 57% of participants had a QTc (corrected QT interval) >500ms following the consumption of 32oz (946,4ml) of an ED (Kozik et al., 2016), indicating a reduction in HR. Both studies, similar to the present, were conducted on a small study sample consisting of young (average age: 25,5years) male participants.

4.2.2 The assessment of pulse pressure and mean arterial pressure

The pulse pressure, which serves as a measurement for a decreased artery compliance in larger arteries (Steinke et al., 2009), is mathematically defined as the difference between SBP and DBP (Homan et al., 2021), and was derived from the direct readings of the electronic BP cuff, which were applied to the following calculation, using Microsoft Excel.

$$Pulse\ pressure\ (mmHg) = SBP - DBP$$

Prior to ED consumption (0-minutes), the mean pulse pressure for the sample group was measured at 40±8,2mmHg, as reflected in Table 10, falling within the optimal pulse pressure range (Homan et al., 2021). Additionally, the recordings for MAP,

defined as the average pressure in the arteries of an individual during one cardiac cycle (Smeltzer et al., 2010), were also derived directly from the readings off the electronic BP cuff, which were then applied to the calculation below, using Microsoft Excel.

$$MAP = \frac{SBP + 2DBP}{3}$$

With the initial MAP reading of 94,2±7,7mmHg for the sample group, the mean MAP readings for the sample group are also reflected below in Table 10.

Table 10 Changes observed in mean pulse pressure and MAP (mmHg), over 120-minutes, in black, male participants.

		Pulse pressure (mmHg)			***MAP (mmHg)***		
Intervals	Time (mins)	Mean±SD	interval mean± SD	interval p-value	Mean±SD	interval mean± SD	interval p-value
1	0	40,0±8,2	-0,2±8,8	0,912	94,2±7,7	0,9±10,4	0,677
	10	39,9±10,1			95,0±8,4		
2	0	40,0±8,2	3,6±8,6	0,043*	94,2±7,7	6,0±7,9	<0,001*
	30	43,7±8,0			100,2±6,2		
3	0	40,0±8,2	2,1±8,4	0,21	94,2±7,7	8,0±12,6	0,024*
	60	42,2±9,6			100,1±11,6		
4	0	40,0±8,2	0,4±11,7	0,868	94,2±7,7	3,4±8,9	0,041*
	90	40,4±9,5			98,0±6,4		
5	0	40,0±8,2	0,5±10,1	0,817	94,2±7,7	2,4±7,6	0,124
	120	40,5±5,4			96,5±4,7		

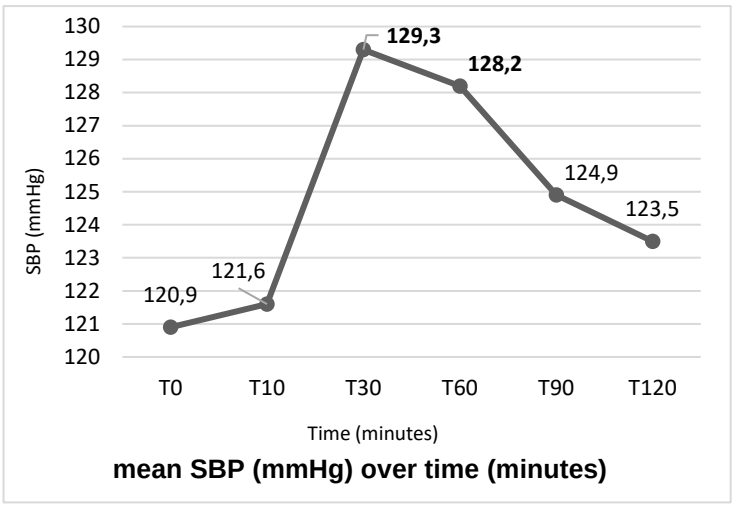
Data expressed as mean ± standard deviation (SD). MAP, mean arterial pressure.
p-values less than 0,05 are considered significant (*)
Results derived using a Dependent T-test

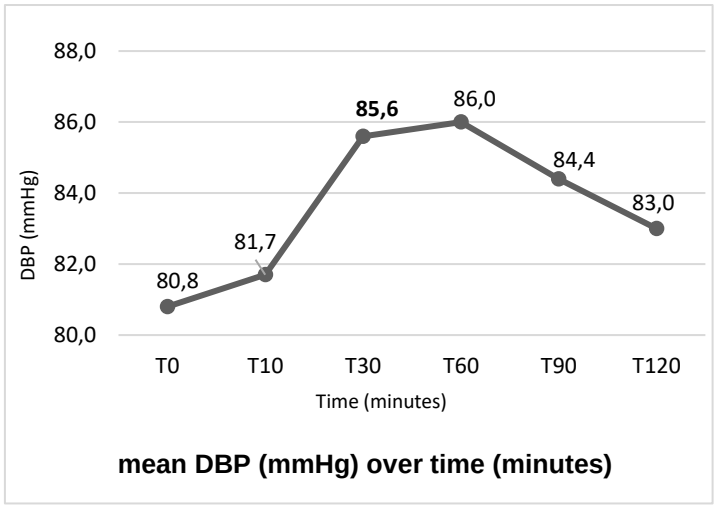
In relation to the mean pulse pressure of the sample group, the administration of a 500ml ED resulted in a 0,2±8,8mmHg reduction 10-minutes post consumption. This was followed by a large increase of 3,6±8,6mmHg in pulse pressure, with statistical significance, observed at 30-minutes (43,7±8,0mmHg, *p*=0,043), being the peak value. As depicted in Figure 8, following the peak value at 30-minutes, a reduction in pulse pressure occurred, lasting approximately 60-minutes (reflected in intervals 3 and 4, Table 10). Although a slow reduction was observed, all data points following 10-minutes were higher than the value at baseline. Thus, our study showed that ED consumption resulted in a significant (*p*=0,043) increase in pulse pressure between 10- and 30-minutes post consumption, before a gradual reduction occurred. Additionally, with the exclusion of the 10-minutes interval (interval 1, Table 10), ED consumption resulted in an overall increase in pulse pressure, relative to non-consumption, reflected at baseline. Although the mean pulse pressure presented a spike from 10-minutes, graphically depicted in Figure 8, the variable fluctuated within a range of 39,9-43,7mmHg, reaching values higher than the normal standard value of 40mmHg (Homan et al., 2022). A “narrowed” pulse pressure is defined as <25% of the SBP, while a “widened” pulse pressure is defined as >100 mmHg (Tang et al., 2020). A widened pulse pressure is associated with several diseases, including aortic sclerosis, aortic regurgitation, severe iron deficiency anaemia, and arteriosclerosis, while a narrowed pulse pressure can be an indication of blood loss, heart failure, cardiac stenosis (reduced stroke volume), and cardiac tamponade (reduced cardiac filling time) (Homan et al., 2023). It should be noted that although an increase was identified in mean pulse pressure, the highest value recorded in our study was 43,7mmHg at 30-minutes (Figure 8), thus may not pose any medical risk when temporarily experienced, especially due to ranging at values significantly less than 100mmHg (Tang et al., 2020).

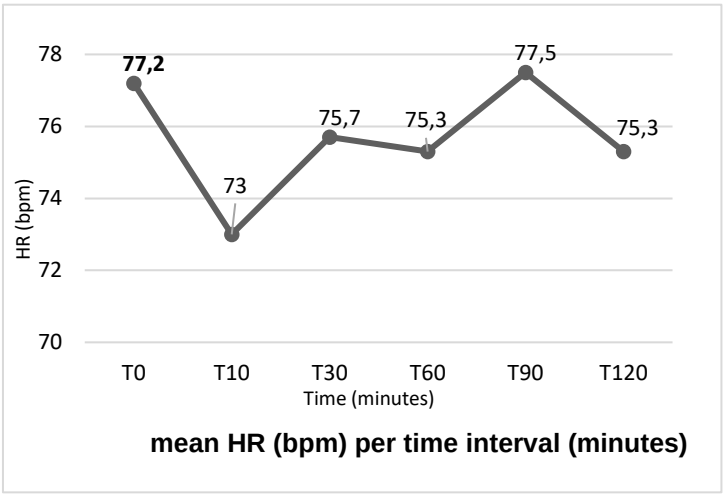
Regarding changes in the mean MAP for the sample group, ED administration resulted in an increase of 0,9±10,4mmHg from 94,2±7,7mmHg, with no statistical significance, from as early as 10-minutes post consumption. An increase in MAP, with statistical significance however, following ED consumption was observed at 30-minutes (100,2±6,2mmHg, *p*<0,001); 60-minutes (100,1±11,6mmHg, *p*=0,024); and at 90-minutes (98,0±6,4mmHg, *p*=0,041). As depicted in Figure 8, the peak value was reached at 30-minutes (100,2±6,2mmHg, *p*<0,001), followed by a partial plateau that

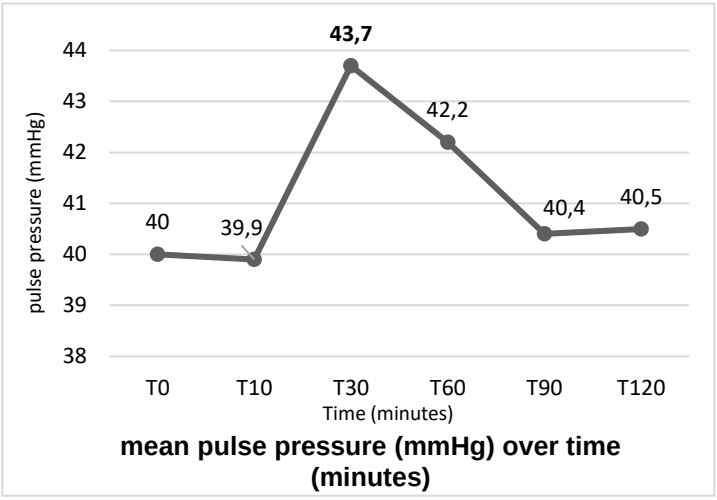
lasted for an additional 30-minutes (60- to 90-minutes), before a gradual decline occurred. Similar to the changes observed in pulse pressure and BP, all data points following ED consumption were higher than the value at baseline, showing statistical significance for approximately 80-minutes of the experimental duration. Therefore, our study showed that ED consumption not only resulted in an increased MAP from as early as 10-minutes post consumption, but that the increase in MAP had statistical significance, relative to baseline, for two thirds of the experimental duration (approx. 80/120minutes).

The reference ranges for MAP vary from normotensive (<93mmHg); elevated BP (between 93 and <97mmHg); stage-1 hypertension (97 to <107mmHg); and stage-2/severe hypertension (≥107mmHg) (Melgarejo et al., 2021). Consequently, MAP is considered normal by physicians when it falls within a range of 70-100mmHg for an individual (Nall, 2021), with a minimum MAP of approximately 60mmHg considered the standard to be maintained for the perfusion of vital organs (DeMers and Wachs, 2022). On that basis, the changes observed in mean MAP in the current study presented normotensive values, followed by an elevated BP, with the largest increase occurring between 10- and 30-minutes (p<0,001). This was followed by a gradual decline between 60- to 120-minutes, dropping back to the normotensive range.









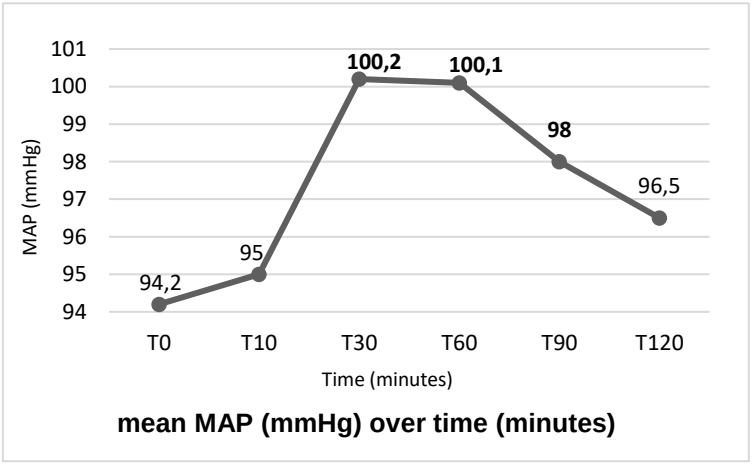


Figure 8 Diagramatic depiction of mean Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Pulse pressure (PP) Mean arterial pressure (MAP) and Heart rate (HR) across 120 minutes post intervention.
Bold texts indicate values with statistical significance (*p*-value≤0,005)

4.3 Comparative analysis of cardiovascular variables for energy drink versus the placebo

In a similar fashion depicted in section 4.2, this section is written in correspondence to the second sub-aim of the experiment, which was to assess the changes in CV variables for the consumption of an ED versus that of the placebo carbonated juice mix. As shown below, Table 11 statistically describes the changes observed in both, the mean SBP and DBP following the consumption of an ED or placebo at each time point, for a total duration of 2-hours.

4.3.1 The assessment of systolic and diastolic blood pressure

Table 11 Descriptive data for changes in SBP (mmHg) and DBP (mmHg), for the administration of the intervention (ED) versus placebo over 120-time in black, male participants, using a cross-over study design

time (mins)	SBP (mmHg)			DBP (mmHg)		
	ED means±SD	placebo means±SD	p-value	ED means±SD	placebo means±SD	p-value
0	120,9±9,0	115,0±8,3	<0,001*	80,8±8,4	75,0±8,8	<0,001*
10	121,6±8,8	115,7±6,8	<0,001*	81,7±10,1	76,8±8,3	0,022*
30	129,3±9,3	114,0±9,7	<0,001*	85,6±60,0	77,4±7,0	<0,001*
60	128,2±12,7	115,7±10,0	<0,001*	86,0±12,3	75,7±7,5	<0,001*
90	124,9±10,2	115,2±10,2	<0,001*	84,4±6,2	77,6±8,8	<0,001*
120	123,5±5,5	116,2±11,1	0,003*	83,0±5,2	79,6±8,4	0,037*

Comparative data expressed as mean ± standard deviation (SD). SBP, systolic blood pressure; DBP, diastolic blood pressure (mmHg)
p-values less than 0,05 are considered significant (*)
Results derived using a Dependent T-test

With reference to Table 11, the findings of the study revealed that there was an increase in mean SBP for the ED in relation to its placebo counterpart. The baseline SBPs for intervention and placebo days were at 120,9±9,0mmHg and 115,0±8,3mmHg, respectively, thus producing statistical significance (p<0,001) at 0-minutes in relation to each other. The variation at baseline for the sample group may be a result from numerous factors. For instance, participants had to walk to the experimental site, thus physical activity could have induced an increased BP and HR. It is possible that psychological factors such as feeling nervous for the first

measurement may have played a role. Furthermore, although measures were taken to ensure that the experimental and control beverages have a similar appearance, it may have been possible that participants were able to distinguish one beverage from the other, especially if they were familiar with the ED brand and flavour from previous use.

As depicted in Figure 9, following baseline, mean SBP increased at 10-minutes post ED consumption (121,6±8,8mmHg), whereas for the administration of the placebo, mean SBP resulted in a minor increase of 0,7mmHg to 115,7±6,8mmHg, for the same time point. Furthermore, mean SBP experienced another reduction at 30-minutes post placebo consumption, with no statistical significance. With reference to Figure 9, placebo consumption resulted in a relatively stable SBP with minor changes in BP ranging between 114±9,7-116,2±11,1mmHg. Therefore, the placebo induced no significant changes to mean SBP. On the contrary, ED consumption induced an increase in mean SBP from 10-minutes post consumption, as shown in Figure 9. The increase in mean SBP lasted for approximately 50-minutes, before a gradual decline was observed from 60-minutes post consumption. Although mean SBP and DBP were different for both experimental days at baseline, the findings of the study revealed that for ED consumption, mean SBP and DBP were greater than that of the placebo for the full experimental duration (p<0,001 – 0,037).

In reference to Table 11, like changes observed in SBP, ED consumption also induced an increase in mean DBP in relation to the placebo. The initial mean DBP values for both experiments were at 80,8±8,4mmHg and 75±8,8mmHg, respectively. Thus, also producing statistical significance which was observed at 0-minutes (p<0,001). Similar reasoning for the differences in baseline for SBP is applied for mean DBP baseline variation, primarily highlighting physical exercise and psychological factors as attributes. Following the administration of the placebo, mean DBP maintained a relatively constant trend, ranging between 76,8-77,4mmHg from 10- to 30-minutes, as shown in Figure 9, before a gradual decline occurred from 60-minutes (75,7±7,5mmHg). The overall trend for mean DBP for the administration of a placebo ranged between the pressures of 76,8±8,3mmHg-79,6±8,4mmHg (Table 11). For ED consumption, however, an increase in mean DBP was identified from as early as 10-minutes post consumption (81,7±10,1mmHg), producing p-values indicating significance, in relation to DBP changes for the placebo. Figure 9 illustrates that

changes in mean DBP post ED consumption remained higher for all time points relative to the placebo. The study, therefore, found that ED consumption increased both mean SBP and DBP, with statistical significance (p<0,001), from as early as 10-minutes post consumption, and remained higher than that of the placebo counterpart for the full experimental duration.

Physiological mechanisms of caffeine and taurine, as described in the literature review, could be the potential reasons for the increased changes observed in BP from as early as 10-minutes post ED consumption, attributed to the bilateral capabilities of caffeine to produce vasoconstriction and vasodilation. Contrary to its vasodilatory effects, some studies in accordance with the present one (Green et al., 1996; Olas and Bryś, 2019; Öz et al., 2015; Shah et al., 2019), also determined that caffeine caused an increased BP. These hypertensive responses occur as result of vasoconstriction. Caffeine can initiate vasoconstriction through various pathways. For instance, in the CNS, caffeine has neurotransmission effects, primarily due to its antagonistic action on the adenosine receptors (Green et al., 1996). Since adenosine facilitates the inhibition of the release of norepinephrine – a molecule which, together with adrenaline, activates the SNS – by acting as an inhibitory neuromodulator of the noradrenergic neurotransmitter system (Green et al., 1996), the presence of caffeine, which competes for binding sites on the A2A receptors, therefore increases the release of norepinephrine. As a result, the CNS is positively stimulated, at the central (cerebral cortex and locus coeruleus) and peripheral sites (Dunwiddie, 1985). This leads to the activation of the SNS. In the context of cardiology, the activation of the SNS promotes vasoconstriction in blood vessels, thus an increased BP will be observed over time. Taurine, a nonproteinogenic beta-amino sulfonic (Schaffer et al., 2010) has been reported to induce similar effects to that of caffeine in vascular smooth muscle cells, thus contributing to the resistance in vessels (Green et al., 1996), and regulating muscle contraction energy levels (Schaffer et al., 2010). In contrast, regarding the placebo, a lack of active ingredients, excluding sugar, may be a result of the decreased BP observed following initial consumption. However, the placebo also induced minor increases to BP. The formulated placebo drink for this study contained a dilute glucose concentrate (1:3) in carbonated water. Since glucose is a vaso-stimulant, it can potentially be the cause for the gradual increase in mean DBP that was observed from 60- to 120-minutes following administration, as depicted in

Figure 9. Similarly, in the study conducted by Shah et al., (2019), the composition of the placebo was similar to that of the present study and was formulated using lime juice, carbonated water, and cherry flavouring (Shah et al., 2019). Shah et al., (2019) reported similar results for SBP post placebo administration where they found that, not only did EDs A and B increase SBP to values ranging between 140-160mmHg for 8 and 9 participants respectively, but the placebo induced similar temporary-hypertensive reactions in 2 participants, even with the absence of caffeine and other bioactive compounds found in EDs (Shah et al., 2019). Energy drinks differed from the placebo for various studies, including ours, due to containing bioactive compounds such as taurine, B-group vitamins, glucuronolactone, and ginseng, to name a few, whereas the placebo primarily consisted of glucose, being the single ingredient with vaso-reactive properties. It should be noted, however, that glucose may not be the sole nor predominant cause of changes observed in CV variables. For instance, in a study conducted by Worthley et al., (2010), BP increased by approximately 4% following the administration of 250ml glucose-free ED (80mg caffeine) in relation to 250ml of carbonated water, which was the control (Worthley et al., 2010).

In other studies, including those that used water (carbonated or still) as a control (Worthley et al., 2010, Nowak et al., 2018), SBP and DBP were significantly greater for ED consumption relative to the placebo. As discussed previously, the plausible explanation for these effects, according to literature, is due to various ingredients in EDs and high-caffeine concentrations which influence CV control through the stimulation of the CNS and PNS. Another study proved that EDs continued to produce significant increases in BP relative to a placebo that consisted of the same amounts of caffeine (Franks et al., 2012). The study, which was conducted in a duration of 24-hours reported that BP was significantly higher even after 24-hours post consumption, relative to the placebo (132,2 vs. 117,4mmHg; 73,6 vs. 68,2mmHg, respectively) (Franks et al., 2012). Thus, proving the synergistic effects of ingredients found in EDs, such as taurine and inositol as previously discussed in the literature review.

4.3.2 The assessment of heart rate

The assessment for changes in the mean HR (bpm) for the sample group is shown statistically in the descriptive data captured in Table 12, below. Unlike other CV variables analysed thus far, the changes in mean HR displayed fluctuating trends for

both beverages. It should be noted, however, that although similar trends were determined for changes in HR following the administration of either beverage, mean HR presented a larger decline for ED versus the placebo in the first 10-minutes of the experiment. This was followed by an increase in HR for the ED group while the placebo group further declined for the same time interval (Figure 9). As depicted in Table 12, HR remained higher for the ED group relative to the placebo group, experiencing a partial plateau between 30-60 minutes before reaching its peak value, post consumption, at 90-minutes. On the contrary, the placebo group experienced an increase between 30-60 minutes before declining to its lowest values at 90-minutes, thus producing a statistically significant (p<0,001) difference when comparing the two groups.

Table 12 Descriptive data for changes in HR (bpm), for the administration of the intervention (ED) versus placebo over time (minutes), in black, male participants, using a cross-over study design

time (mins)	HR (bpm)		
	ED means±SD	placebo means±SD	p-value
0	77,2±13,5	74,0±10,0	0,212
10	73,0±8,2	72,6±8,7	0,776
30	75,7±11,9	72,1±7,7	0,132
60	75,3±10,3	75,1±7,8	0,932
90	77,5±8,1	71,3±9,1	<0,001*
120	75,3±8,2	72,0±11,3	0,148

Comparative data expressed as mean ± standard deviation (SD). HR (bpm), heart rate.
p-values less than 0,05 are considered significant (*)
Results derived using a Dependent T-test

The initial mean HR for the sample group was at 77,2±13,5bpm and 74±10bpm for both experimental trials, with no statistical significance relative to one another. Following the consumption of both beverages, there was a significant decline in HR 10-minutes post consumption (-4,2±8,1bpm, p=0,015) (Table 9), with ED consumption resulting in a larger decline in relation to that of the placebo (Figure 9). Although the decline in HR, following ED consumption, resulted in statistical significance relative to baseline (Table 9), it was, however, not significant in relation to the results of the placebo. As depicted in Figure 9, the trends in mean HR for both beverages resulted in fluctuations ranging from 73-77,5bpm for ED and 71,3-75,1bpm for the placebo. It

should also be noted that although not all measurements were statistically significant, ED consumption maintained a generally higher HR relative to the placebo (Table 12), with the HR reaching its peak value at 90-minutes (77,5±8,1bpm), which is the only time point higher than baseline (77,2±13,5bpm) (Table 12). Interestingly, although the mean HR continued to decline until the 30-minute interval, following the administration of the placebo, and reaching the value of 72,6±8,1bpm, ED administration resulted in an increase in HR for the same time interval (75,7±11,9bpm) (Table 12). Likewise, a similar trajectory was also identified at 60- to 90-minutes, where HR increased for ED consumption (from 75,3±10,3bpm to 77,5±8,1bpm), and decreased for the placebo (from 75,1±7,8bpm to 71,3±9,1bpm), respectively. Consequently, these changes resulted in statistical significance being identified at 90-minutes (p<0,001), with mean HR being higher for ED consumption. Thus, the study showed that although both the ED and the placebo reduced mean HR, for ED consumption, mean HR reached its peak at 90-minutes while the placebo group reached its lowest mean HR for the same time interval, thus producing statistical significance when both groups are compared.

With reference to other studies, the effects of EDs on HR are inconsistent. For instance, Nowak et al., (2018) found that although EDs caused a significant increase in DBP and blood glucose levels, no significant changes were observed in HR relative to the placebo (p=0,705) (Nowak et al., 2018). Similar effects in HR were also recorded by Worthley et al., (2010). On the contrary, another study conducted on twelve participants demonstrated an increase in HR by 2bpm when compared to the placebo (del Coso et al., 2012). Substantially, Steinke et al., (2009) also demonstrated an increased HR by 7.8% in their study after 4-hours following ED consumption, relative to the placebo (Steinke et al., 2009). These findings are similar to that of our study when comparing ED effects to the placebo. Although mean HR was reduced for both beverages relative to baseline values, the increase in HR for ED consumption, from 10-minutes, relative to the placebo may be attributed to its caffeine content. In contrast, however, a randomized double-blind study investigating CV responses to EDs versus a placebo beverage reported increases in the readings for the placebo (Franks et al., 2012). The study further explained that the placebo increased plasma glucose (+50mg/dL), SBP (3mmHg), plasma norepinephrine (+30%), and HR (7pbm) due to potent excitatory effects from sugar (Franks et al., 2012).

It should be considered that in the present study, the difference in the readings for mean HR at baseline for both beverages, which were recorded on two non-consecutive days, may be a result of unintentional physical exercise that may have occurred during the time when participants were required to walk up a flight of stairs to the experimental site. As described by Stewart et al., (2007), the exercise pressor reflex is characterized by an increased HR and BP (Stewart et al., 2007). Another reason for these observations can be attributed to the unreported consumption of food and drink by participants, prior to the experiment.

Interesting observations were made in the changes for mean HR for the sample group, following ED consumption. Although HR changes remained in the margin of what is considered the normal range for a resting adult, 60-100bpm, a fluctuation with no apparent direction occurred, starting from as early as <5-minutes, as shown in Figure 8. In several studies, changes observed reported either a significantly increased HR (Green et al., 1996; Olas and Bryś, 2019; Öz et al., 2015; Shah et al., 2019); a decreased HR (Myers, 1998; Whitsett, 1984; Grasser et al., 2016); induced arrhythmias (Mattioli et al., 2016; Hanif et al., 2020; Acampa et al., 2021), or no changes at all (Nowak et al., 2018). It should be noted, however, that the studies are heterogenous and varied in ED brands, which vary in their active ingredients and concentration; as well as differences in volume of consumption by participants, ranging between 200-500ml.

For ED consumption, trends in mean HR, relative to the placebo group, presented more peaks; increases occurred earlier; remained elevated for longer periods; and reached its maximum value at 90-minutes. Whereas for the placebo group, trends in mean HR presented more stability; remained relatively lower; and reached its minimum value at 90-minutes. In context to ED consumption, the stimulatory effects may be attributed to caffeine and its action on G-protein-coupled receptors located on cardiac myocytes, thus imitating the effects of adrenaline on HR. Additionally, changes in HR and cardiac contractility could also be due to sugar, as observed in changes from placebo consumption. These mechanisms are substantiated in the literature review. Therefore, as shown in the present study, although mean HR was reduced for both beverages, the slight increase in HR for ED consumption relative to the placebo may be attributed to its caffeine content. As shown in Figure 9, mean HR declined for the ED group between 90-120 minutes. Although the half-life of EDs has not been

confirmed in literature, the mean half-life of caffeine in healthy adults is 5-hours (Institute of Medicine, 2001). In addition, caffeine's elimination half-life varies, starting from as early as 1,5-hours (90-minutes) (Brachtel and Richter, 1992). Thus, could be a possible reason for the decline observed from 90-minutes for the ED group.

4.3.3 The assessment of pulse pressure and mean arterial pressure

The assessment for the changes in BP for the study also included the investigations of pulse pressure and MAP in all participants. The results for mean pulse pressure and MAP, following the consumption of the ED or placebo are depicted in Table 13, showing significant increases for ED consumption relative to that of the placebo, for both CV variables assessed.

Table 13 Descriptive data for changes in pulse pressure (mmHg) and MAP (mmHg), for the administration of the intervention (ED) versus placebo over time (minutes), in black, male participants, using a cross-over study design

time (mins)	pulse pressure (mmHg)			MAP (mmHg)		
	ED means±SD	placebo means±SD	p-value	ED means±SD	placebo means±SD	p-value
0	40,0±8,2	40,0±9,5	0,967	94,2±7,7	88,4±7,4	<0,001*
10	40,0±10,1	38,9±7,8	0,682	95,0±8,4	89,7±6,9	0,002*
30	43,7±8,0	36,6±7,7	<0,001*	100,2±6,2	89,6±7,2	<0,001*
60	42,2±9,6	40,1±6,9	0,190	100,1±11,6	89,0±7,8	<0,001*
90	40,4±9,5	37,6±5,6	0,184	98,0±6,4	90,2±8,9	<0,001*
120	40,5±5,4	36,7±6,7	0,016*	96,5±4,7	91,8±8,8	0,009*

Comparative data expressed as mean ± standard deviation (SD). MAP, mean arterial pressure (mmHg)
p-values less than 0,05 are considered significant (*)
Results derived using a Dependent T-test

Using the same cross-over study design to assess for the mean pulse pressure and MAP in the sample group, when exposed to an ED versus the placebo, similar trends such as those observed in the assessment of SBP/DBP can be identified. For instance, as shown in Table 13, the mean pulse pressure of participants for ED consumption was significantly higher than that of the consumption of the placebo at 30- and 120-minutes. When relating the changes observed for both beverages, as shown in Figure 9, ED consumption resulted in an increased pulse pressure from as early as 10-minutes post consumption, thus resulting in its peak value at 30-minutes

(43,7±8,0mmHg), while placebo consumption resulted in a decline for the same time interval (36,6±7,7mmHg). Consequently, statistical significance (p<0,001) was observed at 30-minutes when relating pulse pressure changes for both beverages, with ED consumption resulting in a higher pulse pressure than that of the placebo (Table 13). Following the peak value at 30-minutes post ED consumption, pulse pressure experienced a gradual decline, lasting approximately 60-minutes, reaching the value of 49,4±9,5mmHg at 90-minutes (Table 13). Furthermore, as depicted in Figure 9, a gradual increase in pulse pressure occurred from 90-minutes to the end of the experimental trial (120-minutes: 40,5±5,4mmHg). In contrast, however, following its decline at 30-minutes, placebo consumption resulted in a gradual increase lasting approximately 30-minutes, thus reaching its peak value of 40,1±6,9mmHg at 60-minutes post consumption (Figure 9). This was followed by a gradual decline that lasted for 60-minutes, reaching the value of 36,7±6,7mmHg at 120-minutes. As a result, statistical significance (p=0,016) was observed at 120-minutes due to an increase in pulse pressure for ED consumption, whilst the placebo resulted in a decrease for the same time interval. It should also be noted that pulse pressure, following ED consumption, was greater than that of the placebo, at every time interval following the consumption of the beverages. Therefore, the study showed that mean pulse pressure was higher for ED consumption, in relation to the placebo, resulting in statistical significance and 30- and 90-minutes.

A high pulse pressure (>60mmHg) is considered a risk factor and is often associated with several CVDs as stated in section 4.2.3 (Homan et al., 2022). The results of this study, however, do not suggest that the chronic consumption of EDs could potentially result in the prolonged increase of pulse pressure, ultimately leading to the CVDs mentioned. This is due to mean pulse pressure changes which ranged between 40±8,2-43,7±8,0mmHg, thus remaining in the normotensive spectrum of 40-60mmHg (Homan et al., 2022). There is little to no literature on the effects of EDs on pulse pressure thus, further investigation is required.

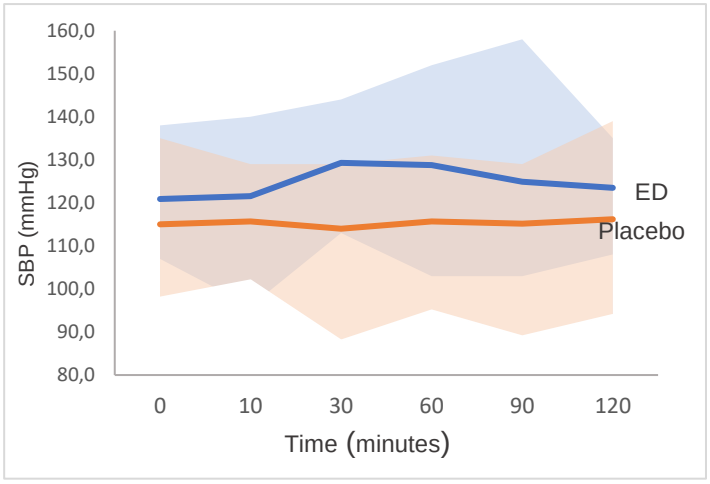
With reference to changes observed in MAP, initial mean readings for both experimental trials were at 94,2±7mmHg and 88,4±7,4mmHg, respectively. As a consequent, statistical significance (p<0,001) was identified at baseline when relating both readings to one another (Table 13). As depicted in Table 13, all data points were significantly higher for ED consumption than its placebo counterparts, for the full

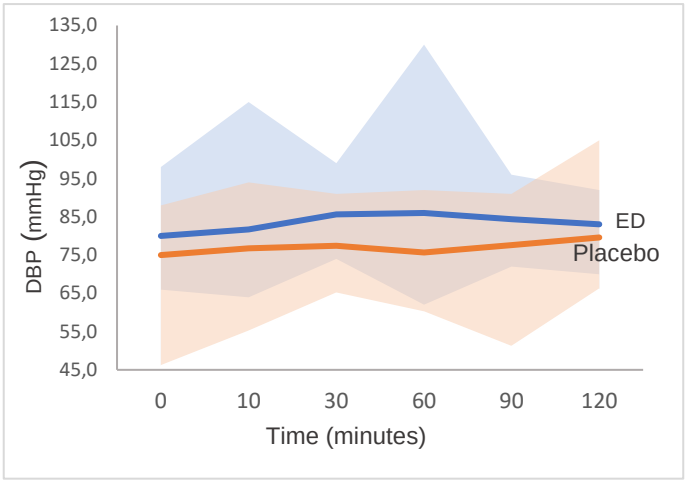
duration of the experiment. This is due to MAP increasing from 10-minutes post ED consumption, reaching a peak value at 30-minutes (100,2±6,2mmHg), while MAP for the consumption of the placebo resulted in a minor decline for the same time interval (89,6±7,2mmHg) (Table 13). As shown in Figure 9, the trend for MAP remained relatively stable following the administration of a placebo, for the full duration of the experiment, ranging between 88,4±7,4-91,8±8,8mmHg, which is considered normotensive (<93mmHg) (Melgarejo et al., 2021). In contradictory fashion, a significant increase was observed following the administration of the ED. Although a decline occurred in MAP for EDs, from its peak at 30-minutes to the end of the experiment, it should be noted that MAP remained significantly higher from 10-minutes post consumption, before reaching a relatively close value to that of the baseline at 120-minutes (96,5±4,7mmHg). Furthermore, the increase in MAP post ED consumption resulted in an elevated BP (93mmHg - <97mmHg) (Melgarejo et al., 2021) from 10-minutes before temporary stage-1 hypertension was observed (97mmHg - <107mmHg) (Melgarejo et al., 2021) from 60- to 120-minutes of the experiment. Therefore, the study showed that the acute administration of 500ml ED induced a significantly increased MAP which lasted for approximately 110-minutes in the sample group, from as early as 10-minutes post consumption, resulting in statistical significance (p<0,001) in relation to that of the placebo. Although the study did not determine whether ED induced changes in MAP which may suggest any medical risk in the short-term, it did, however, prove that MAP can reach values that may lead to temporary stage-1 hypertension, lasting approximately 60-minutes, following an elevated BP that occurred from 10-minutes post ED consumption. Similar to pulse pressure, there is little literature on the long- and short-term effects of EDs on MAP thus, further investigation is also required.

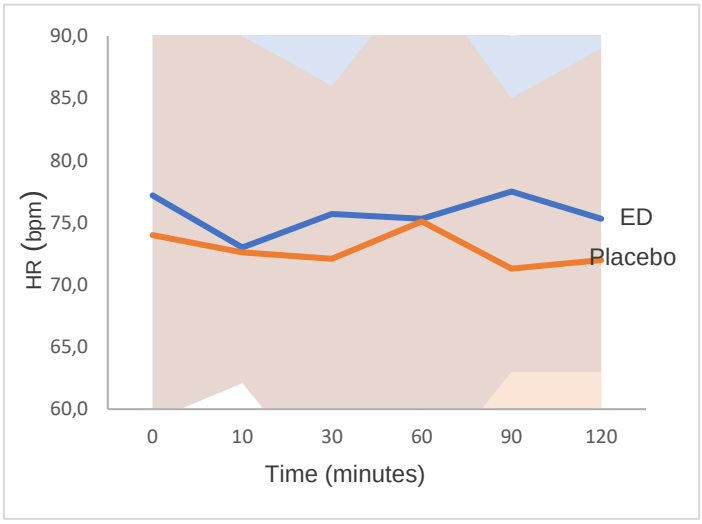
Öz et al., (2015) reported that, although caffeine possesses pharmacological capabilities that enhance/induce vasodilation, it also appears to induce vasoconstriction (Öz et al., 2015). It should be emphasized, that the heterogeneity observed in caffeine effects is in response to a multitude of factors, namely its ability to intervene as an antagonist of adenosine and gamma-aminobutyric acid (GABA) receptors located in the brain, heart, lungs and spleen; acting as an inhibitor of phosphodiesterase enzymes; its ability to alter the sensitivity of calcium liberation channels in the vasculature (Daly, 2007); and acting through ryanodine channels in

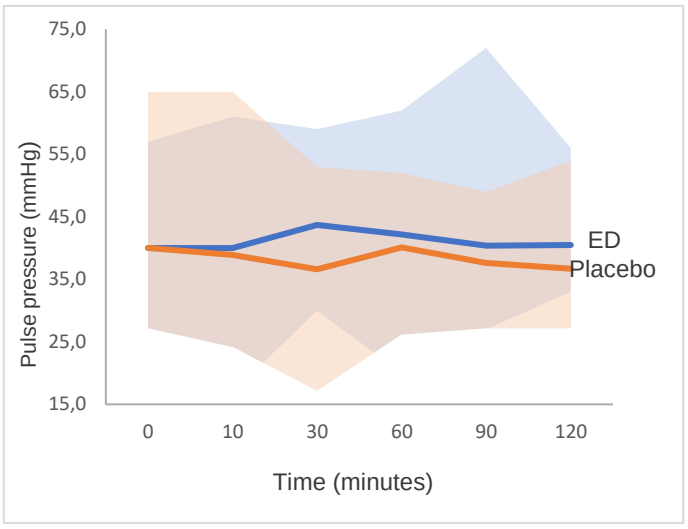
myocardial tissue, the renin-angiotensin aldosterone axis; and through the activation of the autonomic nervous system (ANS) (Echeverri et al., 2010). In relation to pulse pressure and MAP, these mechanisms which trigger vasoconstriction and vasodilation, together with the half-life on caffeine in an individual, may be the possible explanations to the increases and declines observed.

Thus, looking at the five assessed CV variables collectively, there was a significant increase to mean BP, pulse pressure, and MAP for the exposure of ED relative to that of the carbonated juice mix. In contrast, the mean HR was reduced following administration of both beverages, and significance was observed only at 90-minutes. Although HR was reduced for both beverages, the study also found that generally, HR was still higher for ED consumption for most of the experimental duration following 10-minutes post consumption, except at 60-minutes, relative to the placebo.









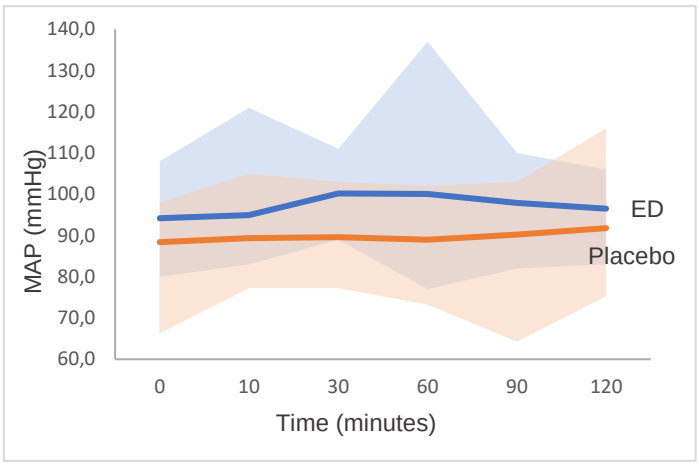


Figure 9 Diagramatic depiction of comparative changes in mean systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), pulse pressure, and mean arterial pressure (MAP) across 120-minutes, post intervention or placebo administration

4.4 The analysis of whether any relationship exists between energy drink consumption and cardiovascular variables

In response to the third, and final, objective of the current study, which was to assess for whether any relationship exists between energy drink (ED) consumption and CV variable changes, a two-way repeated measures analysis of variance (ANOVA) was performed, with statistical significance being acknowledged at $p<0,05$, to test for the interaction between the independent and dependent variables. Partial significance was determined for *p*-values ranging between 0,051-0,06. A Greenhouse-Geisser correction was made throughout as Mauchly's Tests of sphericity yielded violations of $p>0,05$.

The effects of the independent variables, which included drink type (ED and placebo) and time points (minutes), were analysed against the dependent variable, being the changes observed in all CV variables (SBP, DBP, HR, pulse pressure, and MAP) measured. A post hoc repeated-measures ANOVA, which assumes a compound symmetry covariance structure (Shah et al., 2019), was performed for the possible influence of the main effects of drink type and time points, individually; as well as for the interaction of drink type and time. The null hypotheses (H_0) of the study, in relation to all CV variables assessed, are as follows:

- H_0: Drink type will have no significant main effect on mean CV variables; SBP, DBP, HR, pulse pressure, and MAP
- H_0: Time change (time points) will have no significant main effect on mean CV variables; SBP, DBP, HR, pulse pressure, and MAP
- H_0: The interaction between drink type and time points will have no significant main effect on mean CV variables; SBP, BDP, HR, pulse pressure and MAP

4.4.1 The interaction between drink type, time points and mean blood pressure (SBP/DBP)

For the analysis of the main effects of drink type and time points, using the two-way ANOVA, in relation to changes in participants' mean SBP, the study found that drink type had a statistically significant main effect on changes observed in mean SBP, ($F(1, 25) = 51.6$, $p<0{,}001$, $\eta p^2 = 0{,}67$). When analysing for changes in mean SBP across different time points, for each drink type, our study found that time points had no significant main effects in mean SBP changes, ($F(3.40, 84.9) = 2.04$, $p=0{,}106$, $\eta p^2 = 0{,}75$). The analysis of the interaction between both independent variables, as shown in Figure 10 below, found that the interaction had a statistically significant main effect in mean SBP changes, $F(3.88, 97.1) = 5.28$, $p<0{,}001$, $\eta p^2 = 0{,}17$.

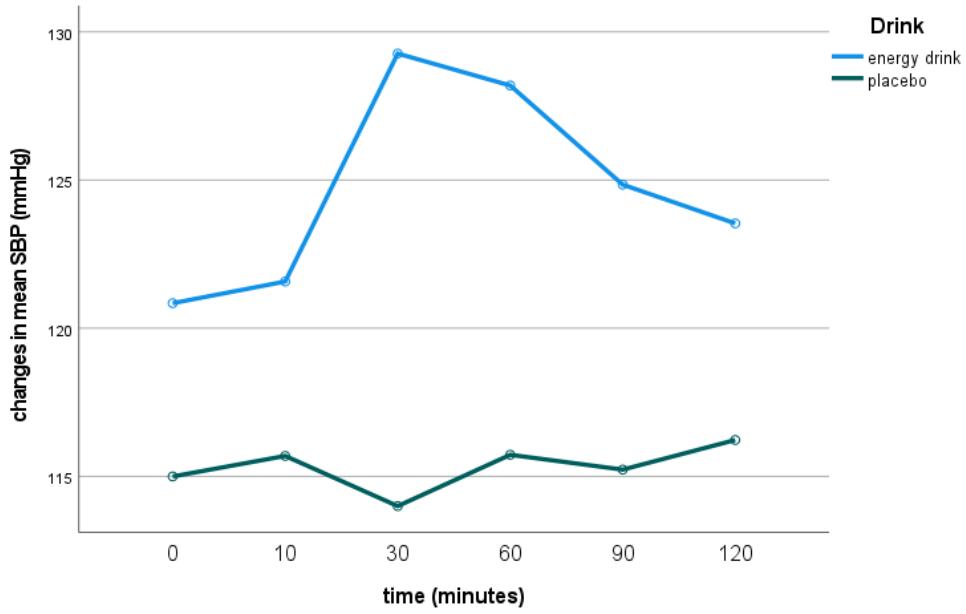


Figure 10 Analysis of variance for mean SBP

Mean systolic blood pressure changes observed from baseline, then at 10, 30-, 60-, 90-, and 120-minutes post beverage consumption in young, healthy, black male participants. Statistical significance ($p<0{,}05$) was determined using a two-way repeated measures ANOVA for the influence of drink type and time points on SBP

Remaining in the context of BP, the independent variables for this analysis were also measured against the changes in participants' mean diastolic pressure.

Our analysis for the individual main effects of drink type and time points against mean DBP determined that drink type had a statistically significant main effect, ($F(1, 25) = 42.6$, $p<0{,}001$, $\eta p^2 = 0{,}630$). In relation to the changes in mean DBP across different time points, the study found that time points had no significant main effect on mean DBP changes, ($F(4.21, 105)= 2.13$, $p=0{,}079$, $\eta p^2 = 0{,}078$). Furthermore, we found that there was a partially significant main effect of the interaction of drink type and time points, in relation to changes observed in mean DBP, ($F(3.58, 89.5)= 2.46$, $p=0{,}057$, $\eta p2 =0{,}090$), as shown in Figure 11 below.

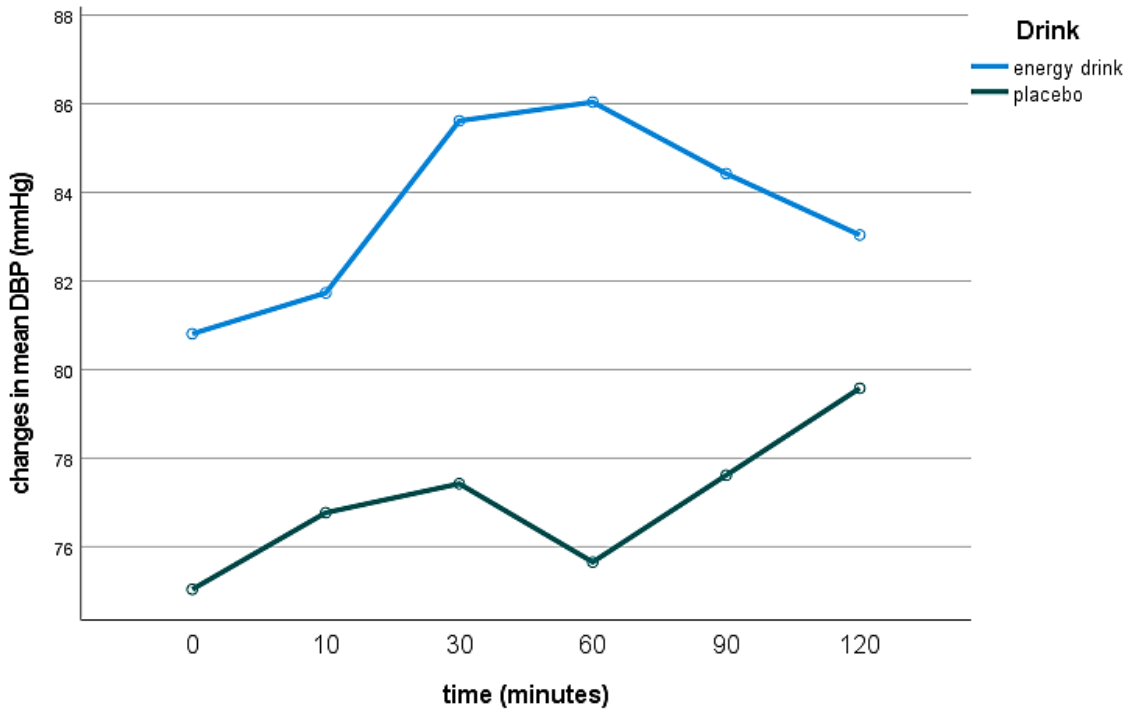


Figure 11 Analysis of variance for changes in mean DBP

Mean diastolic blood pressure changes observed from baseline, then at 10, 30-, 60-, 90-, and 120-minutes post beverage consumption in young, healthy, black male participants. Statistical significance ($p<0{,}05$) was determined using a two-way repeated measures ANOVA for the influence of drink type and time points on DBP

In summary, when looking at the analyses of variance for drink type and time points against changes observed in BP, the study found that changes in both SBP and DBP were significantly different over time for both drink types, as shown by the significant main effect of drink types, as well as by the significant main effect of the interaction against mean SBP, with partial significance for DBP. Therefore, the study not only determined that drink type had a significant main effect on changes in mean BP, but that ED consumption significantly increased both SBP and DBP in relation to the

placebo, as shown by the estimated marginal mean differences for ED and the placebo in Figures 8 and 9.

4.4.2 The interaction between drink type, time points, and mean heart rate

For the assessment of participants' mean HR against drink type and time points, the study found that only drink type had a partial significant effect on changes observed in mean HR, ($F(1, 25)= 4.16$, p=0,052, $\eta p^2 = 0,143$). On the contrary, time points showed no significant main effect on mean HR,($F(2.94, 73.5)= 1.14$, p=0,338, $\eta p^2 = 0,044$). The final analysis, conducted for the assessment of the interaction, found that the interaction of drink type and time points had no significant effect on changes in mean HR, ($F(3.81, 95.3)= 2.08$, p=0,092, $\eta p^2 =0,077$), as shown below in Figure 12.

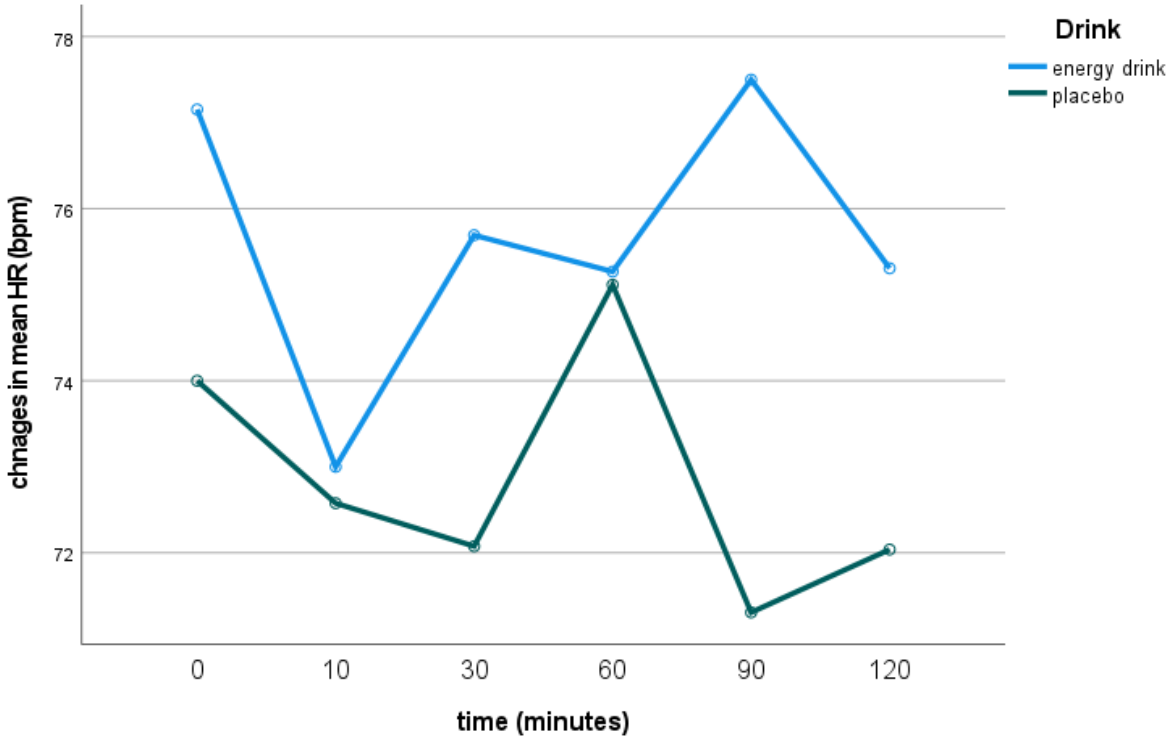


Figure 12 Analysis of variance for changes in mean HR

Mean heart rate changes observed from baseline, then at 10, 30-, 60-, 90-, and 120-minutes post beverage consumption in young, healthy, black male participants. Statistical significance (p<0,05) was determined using a two-way repeated measures ANOVA for the influence of drink type and time points on HR

Through the two-way ANOVA, our study thus determined that only drink type had a partially significant main effect on changes observed in participants' mean HR,

whereas time points and the interaction between the two independent variables against mean HR, showed no significance.

4.4.3 The interaction of drink type, time points, and mean pulse pressure

When assessing for the participants' mean pulse pressure against drink type and time points, our study found that drink type had a statistically significant main effect on changes in mean pulse pressure, ($F(1, 25)=10.3$, $p=0{,}004$, $\eta p^2 = 0{,}291$). For time points, however, the study found that there was no significant main effect in changes for pulse pressure, ($F(3.57, 89.1)= 0.88$, $p=0{,}471$, $\eta p^2 = 0{,}034$). when analysing for the interaction of both independent variables against pulse pressure, as shown in Figure 13 below, the study found that the interaction had no statistically significant main effect in mean pulse pressure changes, ($F(4.12, 103)= 2.04$, $p=0{,}093$, $\eta p^2 = 0{,}075$).

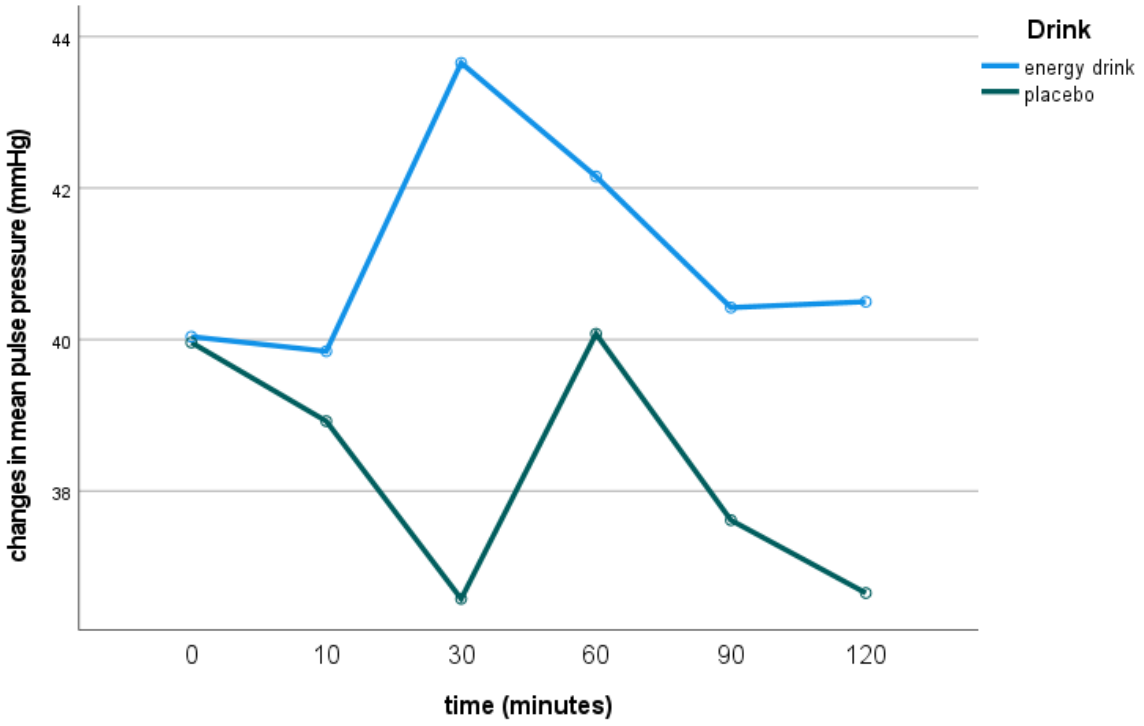


Figure 13 Analysis of variance for changes in mean pulse pressure

Mean pulse pressure changes observed from baseline, then at 10, 30-, 60-, 90-, and 120-minutes post beverage consumption in young, healthy, black male participants. Statistical significance ($p<0{,}05$) was determined using a two-way repeated measures ANOVA for the influence of drink type and time points on pulse pressure

In relation to participants' mean pulse pressure against drink type and time points, the study thus found that only drink type had a main effect on changes observed in mean pulse pressure. Furthermore, although mean pulse pressure was almost identical at

baseline, ED consumption resulted in a significantly increased mean pulse pressure in relation to the placebo, as reflected by the different estimated marginal means of both beverages in Figure 13. The independent variable of time points, together with the interaction of both variables, had no significant main effect on changes in mean pulse pressure.

4.4.4 The interaction between drink type, time points and mean arterial pressure

For the assessment of the final CV variable, participant's mean MAP, against drink type and time points, the analysis of variance found that drink type had a statistically significant main effect on changes in MAP, ($F(1, 25)= 52.4$, $p<0{,}001$, $\eta p^2 = 0{,}677$). Regarding the second independent variable, however, the study found that time points had no significant main effect in changes of MAP, ($F(4.15, 104)= 2.40$, $p=0{,}053$, $\eta p^2 = 0{,}087$). We also determined that although no significant main effect for time points was present, the interaction of both independent variables had a statistically significant main effect on changes in MAP,($F(3.30, 82.5)= 3.99$, $p= 0{,}008$, $\eta p^2 = 0{,}138$), as shown below in Figure 14.

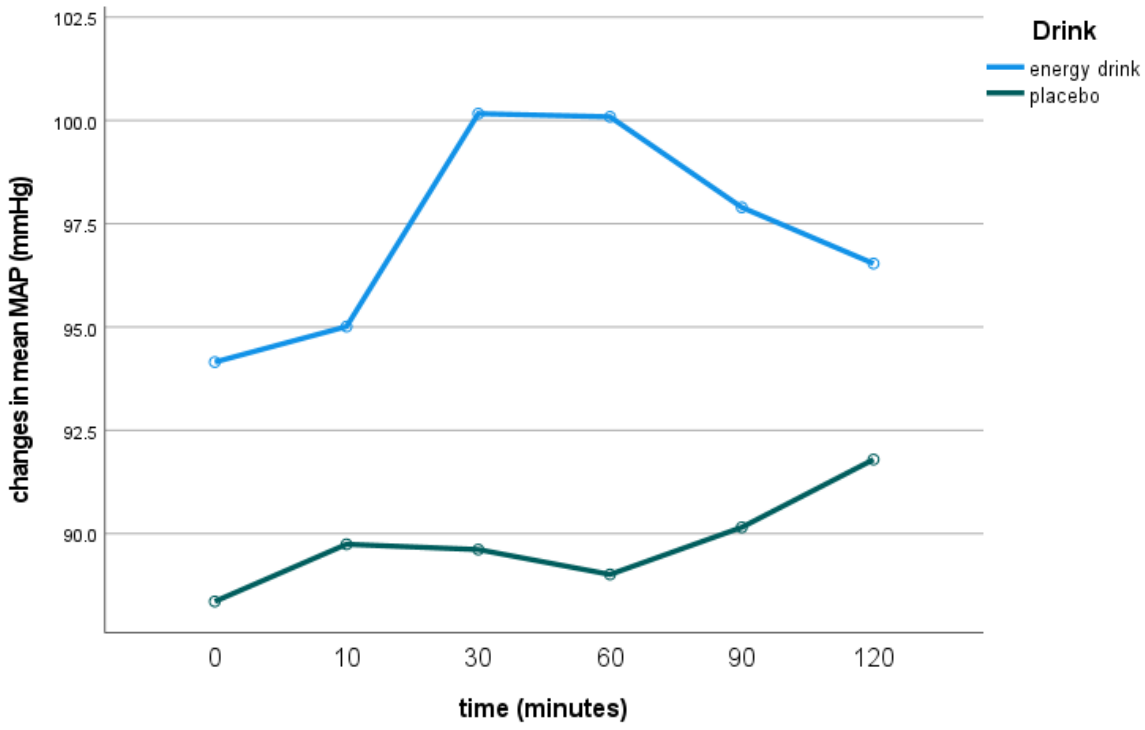


Figure 14 Analysis of variance for changes in mean pulse pressure
Mean arterial pressure changes observed from baseline, then at 10, 30-, 60-, 90-, and 120-minutes post beverage consumption in young, healthy, black male participants. Statistical significance ($p<0{,}05$) was determined using a two-way repeated measures ANOVA for the influence of drink type and time points on MAP

Therefore, our study showed that changes in participants' MAP were significantly different over time for drink type, as shown statistically by its main effect, as well as the significant main effect of the interaction on MAP.

4.4.5 Discussion for the two-way ANOVA

When using the two-way analysis of variance to test for the study's null hypotheses (H_0), we found that drink type had a significant effect on the changes observed for all CV variables assessed, namely mean SBP, DBP, pulse pressure and MAP, while a partial significant effect was reported for changes in HR, respectively. Furthermore, regarding the analysis of BP, our study showed that mean SBP, DBP and MAP had significantly higher-pressure measurements for ED consumption in relation to the placebo counterpart, over the full experimental duration. As per results in previous sub-sections of this chapter, it should be noted that ED consumption significantly induced temporary, and potentially adverse, CV effects such as an increased BP (Figures 8 and 9) that could potentially result in prehypertension in non-hypertensive individuals, and/or hypertension in prehypertensive individuals. In contrast to the escalations observed in BP, however, mean HR was significantly reduced for ED consumption in relation to the placebo. As discussed in section 4.4 previously, this could potentially be a result of taurine and its anti-arrhythmic action. Therefore, our first H_0, which states that drink type will have no significant effect on mean CV variables, is rejected.

In response to the study's second H_0, which states that time points will have no significant effect on mean CV variables, the study found that time points had no significant effect on any changes observed for all CV variables. Therefore, the study's second H_0 is accepted.

Relating to the final H_0 of the study, which states that the interaction will have no significant effect on the mean CV variables, the two-way ANOVA found that the interaction between drink type and time points had a statistically significant effect on the changes in mean SBP and MAP, with *p*-values of <0,001 and 0,008, respectively. Additionally, the interaction between drink type and time points also had an influence on mean DBP, with a partially significant main effect (*p*=0,057). In contrast, however, the interaction of both independent variables had no significant effect on mean HR

(*p*=0,092) and mean pulse pressure (*p*=0,093). Therefore, the final H_0 is partially accepted.

- H_1: The interaction between drink type and time points will have a significant effect on mean systolic BP and mean arterial pressure, and a partially significant effect on diastolic BP
- H_1: The interaction between drink type and time points will have no significant effect on mean HR and pulse pressure

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The current study aimed to identify whether acute ED consumption influences the CVS of a young, healthy population. The central questions of this research were as follows:

1. Does the acute exposure of EDs on the CVS cause changes to HR and BP in healthy, young, black males?
2. Is there any association between ED consumption and CV variables in a population that is already at risk of CVDs?

All CV readings were collected using an electric BP cuff from the left arm of participants at rest. Cardiovascular data was analysed using dependent t-tests for the assessment of ED consumption relative to baseline and the placebo. Furthermore, a two-way repeated measures ANOVA was conducted for the determination of whether a relationship exists between ED consumption and changes observed in CV variables. The objectives of this study, together with the corresponding results are depicted below in Figure 15.

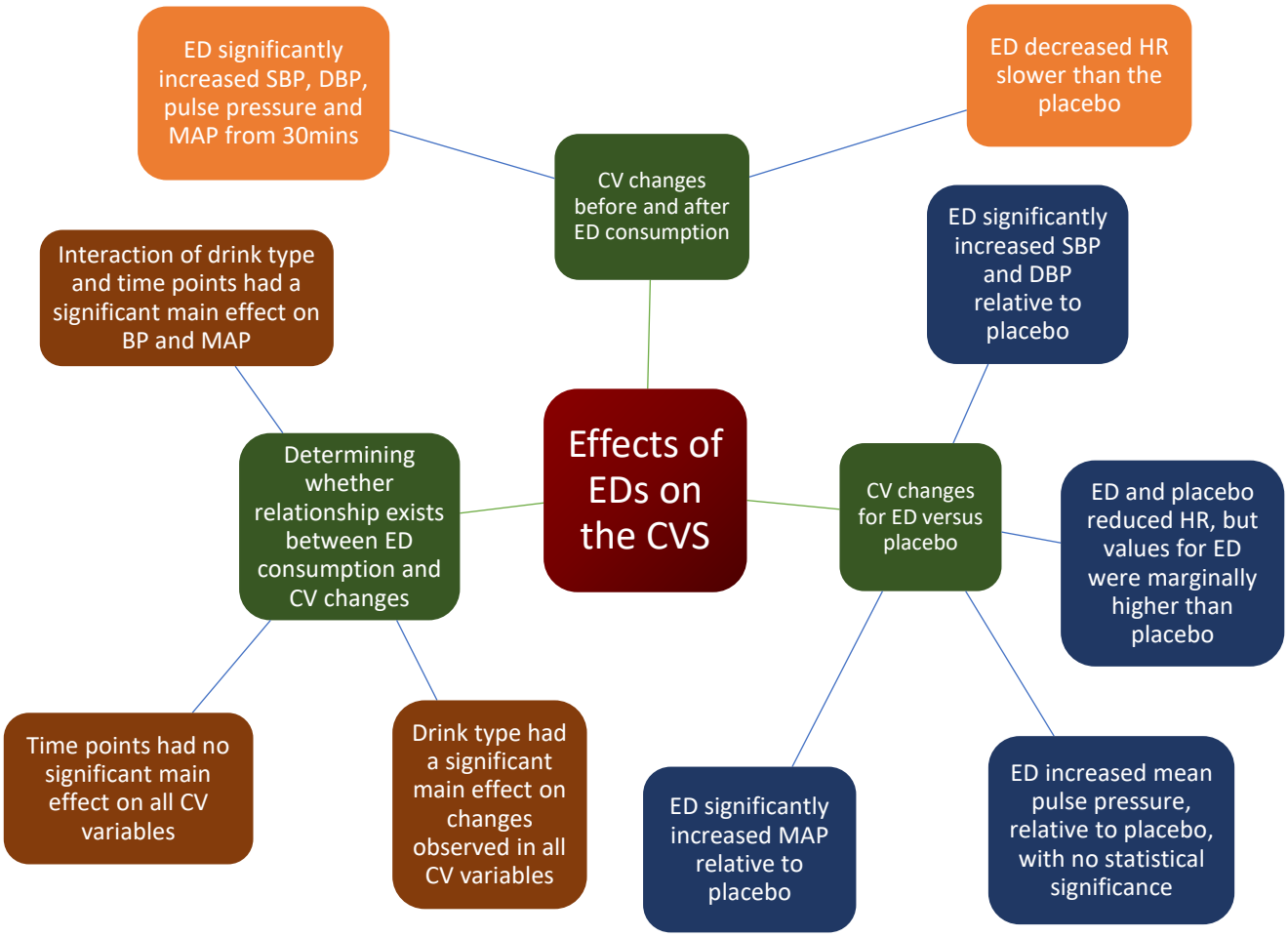


Figure 17 Diagrammatic summary of study objectives and corresponding results

In substantiation to Figure 15, our study demonstrated a significant temporary elevation of mean SBP, DBP, HR, pulse pressure, and MAP in young, healthy, black male participants post ED consumption. For both mean SBP and DBP, peak values occurred between 30-60 minutes post ED consumption when comparing to baseline, showing statistical significance from 30-minutes (SBP: p<0,01; DBP: p=0,015), as recorded in Table 8. Relative to placebo intake, mean BP also demonstrated an increase of up to 5,9mmHg in SBP and 4,9mmHg in DBP at 10-minutes post ED consumption. This event was followed by significant differences 15,3mmHg (p<0,001) for mean SBP at 30-minutes, and 10,3mmHg (p<0,001) for mean DBP at 60-minutes, when comparing ED effects to the placebo (Table 11). Additionally, mean pulse pressure and MAP were also significantly increased from ED consumption, with mean pulse pressure reaching 43,7±8,0mmHg and MAP at 100,2±6,2mmHg 30-minutes post consumption (Table 10). These values resulted in a 3,6±8,6mmHg and 6,0±7,9mmHg difference for pulse pressure and MAP respectively, when comparing to the values at baseline. In relation to placebo administration, mean pulse pressure only showed statistical significance (p<0,001) at 30-minutes, with maximum difference of 7,1mmHg for both drinks (Table 13). Although mean MAP presented statistical significance for the full duration of the experiment, with ED resulting in higher pressure measurements, the maximum difference between both beverages was identified at 30-minutes, with a difference of 10,6mmHg (p<0,001) (Table 13).

When assessing changes in HR, our study found that mean HR was significantly reduced by 4,2±8,1bpm (p=0,015) from as early as 10-minutes post ED consumption, in relation to baseline (Table 9). For the remaining experimental duration, mean HR fluctuated, reaching a peak value of 77,5±8,1bpm at 90-minutes. Consequently, when compared to the placebo, ED consumption induced a marginally higher HR, with statistical significance (p<0,001) being identified at 90-minutes due to the maximum difference of 6.2bpm between the two beverages (Table 12).

In relation to the objective of determining whether there is an association between ED consumption and CV variables, in a population that is already at risk of CVDs, our study found that drink type had a significant main effect on mean SBP (p<0,001), DBP (p<0,001), pulse pressure (p=0,004) and MAP (p<0,001) and a partial significant main effect on mean HR (p=0,052). Although time points showed no significant effect on changes in CV variables, the interaction of drink type and time points had a significant

main effect on mean SBP (p<0,001) and MAP (p=0,008) and had a partial significant effect main on DBP (p=0,057).

In conclusion, the study showed that ED consumption significantly increased BP, with maximum measurements being observed 30-minutes post consumption for mean SBP, DBP, pulse pressure and MAP, as described in Figure 16. Our study also determined that ED consumption decreased mean HR. Although both ED and placebo resulted in an overall decreased mean HR, ED consumption produced a higher HR, with exceptions at 10- and 60-minutes (Figure 9). Therefore, in response to the first research question, acute exposure of EDs on the CVS caused changes to HR and BP in healthy, young, black males. Additionally, when assessing for the association between ED consumption and CV variables, our study found that drink type had a significant effect in the changes observed for in all CV variables overtime. Furthermore, the interaction of drink type and time points also had a significant main effect on changes observed in mean SBP, DBP and MAP over time. It should be emphasized that in this analysis, ED consumption resulted in higher marginal CV means in relation to the placebo.

As discussed in the previous chapter, the increase in BP variables was attributed to caffeine's activation of the SNS through its antagonistic actions on neuromodulator adenosine in the cerebral cortex and locus coeruleus (Dunwiddie, 1985), located in the CNS. It is this mechanism that caffeine can induce physiological responses to the activation of the SNS. In the context of CV physiology, these include and increased BP and HR. on the contrary, caffeine also acts on the vascular endothelium, and vascular smooth muscle cells by promoting the increase in $[Ca^{2+}]$ and encouraging the expression of nitric oxide, resulting in vasodilation (Echeverri et al., 2010). These contrasting mechanisms thus produce caffeine's bilateral capability to induce vasodilation and vasoconstriction.

Similarly, caffeine's actions on numerous pathways that affect BP also influence HR. For instance, although both ED and the placebo decreased mean HR, ED consumption induced changes in HR that were marginally higher than that of the placebo. This can be attributed to caffeine's stimulatory effects in the SNS, thus inducing an increased HR. Furthermore, caffeine also binds to G-protein-coupled receptors found on cardiac myocytes, inducing inotropic and chronotropic effects

(Cannon et al., 2001) which may lead to the observed increased HR. Although a significant reduction was observed in mean HR briefly, following beverage administration, it appeared that, overall, HR was not significantly impacted by both beverages. Relative to ED consumption, a reduction in HR, followed by a fluctuation, could be attributed to high concentrations of taurine in the beverage. It should be emphasized that EDs contain ten times the amount of taurine than the required daily intake (40-400mg) (de Sanctis et al., 2017). Therefore, various physiological mechanisms of caffeine, taurine and sugar could be the explanation for a reduced HR, as well as the increased HR for ED consumption relative to the placebo.

The observed changes in BP and HR following ED consumption, are worrisome when considering that EDs are food products sold with no restriction to quantity consumption. When evaluating the effects of acute ED consumption on the CVS, no clinical concerns were identified in participants. However, an increased BP and cardiac arrhythmias were identified, which if persistent, could lead to serious adverse effects. Even though the present study investigated the acute consumption of EDs, it provides empirical evidence which supports the reported adverse CV effects that occur in the CVS from long-term ED exposure. It should be emphasized that the list of common pathologies and adverse CV events resulting from ED consumption is extensive, and has no discrimination to age, gender, BMI, or vitality. Adverse events include hypertension, heart palpitations, increased cardiac output and stroke volume, tachycardia, ectopic beats, ventricular and atrial fibrillation, myocardial infarction, cardiac arrest, and death (Breda et al., 2014; Cappelletti et al., 2018; Lee et al., 2009; Shah et al., 2019). These adverse effects tend to accompany ED consumption due to the bioactive constituents in the beverage influencing the autonomic and haemodynamic effects on the CVS (Somers and Svatikova, 2020).

When evaluating changes in CV variables and possible physiological mechanisms post ED consumption, in relation to South Africa, it raises the concern that ED consumption may exacerbate the already-at-risk CVS of the general population. As described in chapter 1, South Africa has one of the highest hypertension prevalence (60%) globally (World Health Organization, 2018), with CVDs being the second leading cause of fatalities in the country (Ntuli et al., 2015). Ntuli et al., (2015) also reported that hypertension was the leading risk factor for CVDs (Ntuli et al., 2015). When considering these statistics in relation to hypertensive results from ED

consumption recorded in the present study, one may assume that a further increase in the prevalence of hypertension can be expected, in association with the ever-increasing ED consumption globally (Reid et al., 2015). Furthermore, not only did the World Health Organization (WHO) report that South African men had a higher prevalence of hypertension (27.4%) that that of the women (26.1%) (World Health Organization, 2018), but additional epidemiological evidence also suggests that in relation to other ethnic groups, the black ethnic population had a higher prevalence of hypertension (Lackland, 2014; Lindhorst et al., 2007). These findings, together with those reporting that male counterparts consume more EDs than the females (Reid et al., 2015), statistically describe a pattern and likeliness of the black, male population, who are already high risk for CVDs, consuming EDs and potentially increasing their CVD risk. Even outside the black, male South African population, this trend could lead to an increased number of premature fatalities due to underlying CVDs. Due to the lack in regulations of ED distribution and consumption in South Africa, CV risks may occur in the general population from early age groups, thus impacting the youth population, who is already victim of aggressive ED marketing (Stacey et al., 2017).

In summary, the adverse effects of ED consumption remain a global concern, with increasing consumption due to the successful marketing and the perceived benefits of EDs. This study not only provided empirical data for acute ED consumption, clearly indicating an increased BP and cardiac arrhythmias, but also supports the notion that long term ED consumption can lead to numerous CVDs, and possible death. It is on that basis, that we aspire to raise awareness of risks in ED consumption, and to encourage various institutions and individuals to significantly reduce or terminate consumption. The recommendations, as described in the following sub-section, are in accordance with this notion.

Recommendations

The recommendations that can be made based on the results, and limitations, of this study are as follows:

- In future, studies assessing physiological changes from EDs should extend their experimental trials to a duration of +4-hours, based on the statistically significant changes reported in other studies, referenced in this dissertation, which were observed between 3- to 6-hours.
- Strict adherence and measures of control/discipline should be implemented for participant activities, such as ensuring that the resting and fasting periods are followed correctly. This is to ensure the removal of or significant reduction of external factors, such as exercise or dietary influences on CV variables.
- Further research on the effects of EDs on the CVS are required, with consideration to participant's genetic predisposition and lifestyle factors. Such results are important for authorities in regulatory decisions, such as warning labels on food and beverage items.
- Although bioactive contents of EDs have been studied, further research is required to bridge the gap in understanding the impacts of, not only the individual compounds, but their concentrations on the CVS. Thus, research in ED constituents, focusing on specific measurements for each bioactive compound, is required.
- In the context of studies pertaining to EDs and their effects on human health, long-term effects need to be assessed, especially in populations with a high prevalence of CVDs, such as in South Africa, to provide knowledge that can contribute towards the mitigation of potential CV adverse events and fatalities.

References

Acampa, M., Cerrone, M., Alberto Keller, G., Cao, D. X., Maiton, K., Nasir, J. M., Mark Estes, N. A., & Shah, S. A. (2021). Energy Drink-Associated Electrophysiological and Ischemic Abnormalities: A Narrative Review. Frontiers in Cardiovascular Medicine | Www.Frontiersin.Org, 8(679105), 1–12. https://doi.org/10.3389/fcvm.2021.679105

Addo, J., Smeeth, L., & Leon, D. A. (2007). Hypertension in sub-Saharan Africa: A systematic review. Hypertension, 50(6), 1012–1018. https://doi.org/10.1161/HYPERTENSIONAHA.107.093336

Akinmolusun, O., Bezabih, Y., Kaunissaari, S., & Mugambi, A. (2012). Detrimental Effects of Energy Drink Consumption on Adolescents. Turku University of Applied Sciences. https://core.ac.uk/download/pdf/38069247.pdf

Alford, C., Cox, H., & Wescott, R. (2001). The effects of Red Bull Energy Drink on Human Performance and Mood. Amino Acids, 21, 139–150. https://doi.org/10.1007/s007260170021

Al-Shaar, L., Vercammen, K., Lu, C., Richardson, S., Tamez, M., & Mattei, J. (2017). Health Effects and Public Health Concerns of Energy Drink Consumption in the United States: A mini-review. Frontiers in Public Health, 5(225), 1–6. https://doi.org/10.3389/fpubh.2017.00225

Alsunni, A. A. (2015). Energy Drink Consumption: Beneficial and Adverse Health Effects. International Journal of Health Sciences, 9(4), 469–474. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4682602/

Antes, J. P. D. (2018). Gets You Going: Lipovitan Needs an Energy Booster in the Japanese and Global Market. Business Development, Merger and Crisis Management of International Firms in Japan, 3–17. https://doi.org/10.1142/9789813234222_0001

Barnes, J. A., & Devries, S. G. (1999). The Effects of Water or Sports Drink Ingestion Prior to Exercise on the Performance of Middle Distance, Amateur Runners in a Thermoneutral Environment [Master of science in physical therapy, Grand Valley State University]. http://scholarworks.gvsu.edu/theseshttp://scholarworks.gvsu.edu/theses/338

Baum, M., Weiss, M. (2001). The Influence of a Taurine Containing Drink on Cardiac Parameters Before and After Exercise Measured by Echocardiography. Amino Acids, 20(1), 75-82. doi: 10.1007/s007260170067. PMID: 11310932.

Bertasi, R. A. O., Humeda, Y., Bertasi, T. G. O., Zins, Z., Kimsey, J., & Pujalte, G. (2021). Caffeine Intake and Mental Health in College Students. Cureus, 13(4), 1–8. https://doi.org/10.7759/cureus.14313

Bode, P. (2003). Chapter 2 Quality Assurance and Quality Control in Research Development. [Delft University of Technology]. https://www.semanticscholar.org/paper/CHAPTER-2-QUALITY-ASSURANCE-AND-QUALITY-CONTROL-IN-Bode/b1cd3b1cb97379c0f223c992949451c727856be6

Bolignano, D., Coppolino, G., Barillà, A., Campo, S., Criseo, M., Tripodo, D., & Buemi, M. (2007). Caffeine and the Kidney: What Evidence Right Now? Journal of Renal Nutrition, 17(4), 225–234. https://doi.org/10.1053/j.jrn.2007.02.006

Brachtel, D., Richter, E. (1992). Absolute Bioavailability of Caffeine from a Tablet Formulation. Journal of Hepatology, 16(3), 385. doi: 10.1016/s0168-8278(05)80676-2. PMID: 1487618.

Breda, J. J., Whiting, S. H., Encarnação, R., Norberg, S., Jones, R., Reinap, M., & Jewell, J. (2014). Energy Drink Consumption in Europe: A Review of the Risks, Adverse Health Effects, and Policy Options to Respond. Frontiers in Public Health, 2(134), 1–5. https://doi.org/10.3389/fpubh.2014.00134

Brosnan, J. T., & Brosnan, M. E. (2006). 5th Amino Acid Assessment Workshop. Journal of Nutrition, 136, 1636–1640. https://academic.oup.com/jn/article/136/6/1636S/4664439

Burris, K. P., Harte, F. M., Davidson, P. M., Stewart, C. N., & Zivanovic, S. (2012). Composition and Bioactive Properties of Yerba Mate (Ilex paraguariensis A. St.-Hil.): A Review. Chilean Journal of Agricultural Research, 72(2), 268–274. http://dx.doi.org/10.4067/S0718-58392012000200016

Cappelletti, S., Piacentino, D., Fineschi, V., Frati, P., Cipolloni, L., & Aromatario, M. (2018). Caffeine-Related Deaths: Manner of Deaths and Categories at Risk. Nutrients, 10(611), 1–13. https://doi.org/10.3390/nu10050611

Cannon, M.E., Cooke, C.T., McCarthy, J.S. (2001). Caffeine-Induced Cardiac Arrhythmia: An Unrecognised Danger of Healthfood Products. The Medical Journal of Australia, 174(10), 520-521. doi: 10.5694/j.1326-5377.2001.tb143404.x. PMID: 11419773.

Centre for Science in the Public Interest, 2021. Caffeine Chart. Viewed: 03/12/2021. https://cspinet.org/eating-healthy/ingredients-of-concern/caffeine-chart

Chen, J.-F., Eltzschig, H. K., & Fredholm, B. B. (2013). Adenosine Receptors as Drug Targets-What are the Challenges?. Nature Review Drug Discovery, 12(4), 265–286. https://doi.org/10.1038/nrd3955

Corbo, M. R., Bevilacqua, A., Petruzzi, L., Casanova, F. P., & Sinigaglia, M. (2014). Functional Beverages: The Emerging Side of Functional Foods: Commercial Trends, Research, and Health Implications. Comprehensive Reviews in Food Science and Food Safety, 13(6), 1192–1206. https://doi.org/10.1111/1541-4337.12109

Creswell, J. W. (2014). Research Design: Qualitative, Quantitative, and Mixed Methods Approaches. (V. Knight, J. Young, K. Koscielak, B. Bauhaus, & M. Markanich, Eds.; 4th ed., Vol. 4). SAGE Publications Ltd.

Daly, J.W. (2007). Caffeine Analogs: Biomedical Impact. Cellular and Molecular Life Sciences, 64(16), 2153–2169. PMID: 17514358 doi: 10.1007/s00018-007-7051-9

De Sanctis, V., Soliman, N., Soliman, A. T., Elsedfy, H., Maio, S. Di, Kholy, M. El, & Fiscina, B. (2017). Caffeinated Energy Drink Consumption Among Adolescents and Potential Health Consequences Associated with Their Use: a Significant Public Health Hazard. Acta Biomedica, 88(2), 222–231. https://doi.org/10.23750/abm.v88i2.6664

Del Coso, J., Salinero, J. J., González-Millán, C., Abián-Vicén, J., & Pérez-González, B. (2012). Dose Response Effects of a Caffeine-Containing Energy Drink on Muscle Performance: A Repeated Measures Design. Journal of the International Society of Sports Nutrition, 9(21), 1–10. https://doi.org/10.1186/1550-2783-9-21

Diel, F., & Khanferyan, R. A. (2018). Sports and energy drinks. Foods and Raw Materials, 6(2), 379–391. https://doi.org/10.21603/2308-4057-2018-2-379-391

Dobmeyer, D.J., Stine, R.A., Leier, C.V., Greenberg, R., Schaal, S.F. (1983). The Arrhythmogenic Effects of Caffeine in Human Beings. The New England Journal of Medicine, 308(14), 814-816. doi: 10.1056/NEJM198304073081405. PMID: 6835272.

Dunwiddie, T.V. (1985). The Physiological Role of Adenosine in the Central Nervous System. International Review of Neurobiology, 1985(27), 63-139. doi: 10.1016/s0074-7742(08)60556-5. PMID: 2867982.

Echeverri, D., Montes, F. R., Cabrera, M., Galán, A., & Prieto, A. (2010). Caffeine's Vascular Mechanisms of Action. International Journal of Vascular Medicine, 2010, 1–10. https://doi.org/10.1155/2010/834060

Faul, F., Erdfelder, E., Lang, A., & Buchner, A. (2007). G*Power 3: A Flexible Statistical Power Analysis Program for the Social, Behavioral, and Biomedical Sciences. Behavior Research Methods, 39(7), 175–191. https://doi.org/10.3758/bf03193146

Filippini, A., D'amore, A., & D'alessio, A. (2019). Molecular Sciences Calcium Mobilization in Endothelial Cell Functions. International Journal of Molecular Sciences, 20(4525), 1–13. https://doi.org/10.3390/ijms20184525

Foran, M., Strickland, F., Perkins, K., Smith, J.A. (2011). Excessive Intraoperative Bleeding with Chronic Energy Drink Consumption. Journal of Oral and Maxillofacial Surgery, 70(6), 1439-1441. doi: 10.1016/j.joms.2011.03.067. Epub 2011 Aug 3. PMID: 21813226.

Franks, A.M,, Schmidt, J.M., McCain, K.R., Fraer, M. (2012). Comparison of the Effects of Energy Drink Versus Caffeine Supplementation on Indices of 24-hour Ambulatory Blood Pressure. Annals of Pharmacotherapy, 46, 192-199. doi: 10.1345/aph.1Q555

Fujita, T., Ando, K., Noda, H., & Sato, Y. (1987). Effects of Increased Adrenomedullary Activity and Taurine in Young Patients with Borderline Hypertension. Circulation, 75(3), 525–532. https://doi.org/10.1161/01.cir.75.3.525

Gleason, P. M., Harris, J., Sheean, P. M., Boushey, C. J., & Bruemmer, B. (2010). Publishing Nutrition Research: Validity, Reliability, and Diagnostic Test Assessment in Nutrition-Related Research. Journal of the American Dietetic Association, 110(3), 409–419. https://doi.org/10.1016/j.jada.2009.11.022

Goldfarb, M., Tellier, C., & Thanassoulis, G. (2014). Review of Published Cases of Adverse Cardiovascular Events After Ingestion of Energy Drinks. American Journal of Cardiology, 113(1), 168–172. https://doi.org/10.1016/j.amjcard.2013.08.058

Gordan, R., Gwathmey, J. K., & Xie, L.-H. (2015). Autonomic and Endocrine Control of Cardiovascular Function. World Journal of Cardiology, 7(4), 204–214. https://doi.org/10.4330/wjc.v7.i4.204

Grasser, E. K., Miles-Chan, J. L., Charrière, N., Loonam, C. R., Dulloo, A. G., & Montani, J. P. (2016). Energy Drinks and Their Impact on the Cardiovascular System: Potential Mechanisms. Advances in Nutrition, 7(5), 950–960. https://doi.org/10.3945/an.116.012526

Grasser, E. K., Yepuri, G., Dulloo, A. G., & Montani, J. P. (2014). Cardio- and Cerebrovascular Responses to the Energy Drink Red Bull in Young Adults: A Randomized Cross-over Study. European Journal of Nutrition, 53(7), 1561–1571. https://doi.org/10.1007/s00394-014-0661-8

Greden, J. F. (1974). Anxiety or Caffeinism: A Diagnostic Dilemma. American Journal of Psychiatry, 131(10), 108–109. https://doi.org/10.1176/ajp.131.10.1089

Green, P. J., Kirby, R., & Suls, J. (1996). The Effects of Caffeine on Blood Pressure and Heart Rate: A Review. Annals of Behavioral Medicine, 18(3), 201–216. https://doi.org/10.1007/BF02883398

Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., & Hamal, A. (2014). What is a Bioactive Compound? A Combined Definition for a Preliminary Consensus. International Journal of Food Sciences and Nutrition, 3(3), 17–179. https://doi.org/10.11648/j.ijnfs.20140303.16

Gunja, N., & Brown, J. A. (2012). Energy drinks: Health Risks and Toxicity. Medical Journal of Australia, 196(1), 46–49. https://doi.org/10.5694/mja11.10838

Guo, M. (2009). Chapter 8 - Sports Drinks. In Functional Foods: Principals and Technology, 2009, 279–298). Elsevier. https://doi.org/10.1533/9781845696078.279

Hanif, M., Saleem, S., Naz, S., & Sundas, F. (2020). Energy Drinks and Atrial Fibrillation: An Unusual Case of Caution. Cureus, 12(10), 1–3. https://doi.org/10.7759/cureus.10807

Heale, R., & Twycross, A. (2015). Validity and Reliability in Quantitative Studies. Evidence-Based Nursing, 18(3), 66–67. https://doi.org/10.1136/eb-2015-102129

Hendricks, L., Jabrah, A., & Simpson, C. (2017). The Effects of Energy Drinks on College Students Overview on Energy Drinks American Journal of Drug Delivery and Therapeutics. American Journal of Drug Delivery and Therapeutics, 4(1), 1–2. https://www.primescholars.com/articles/the-effects-of-energy-drinks-on-college-students-103580.html

Higashi, Y. (2019). Coffee and Endothelial Function: A Coffee Paradox?. Nutrients, 11(2104), 1–21. https://doi.org/10.3390/nu11092104

Higgins, J. P., Tuttle, T. D., & Higgins, C. L. (2010). Energy beverages: Content and safety. Mayo Clinic Proceedings, 85(11), 1033–1041. https://doi.org/10.4065/mcp.2010.0381

Hori, M., & Kitakaze, M. (1991). Adenosine, the Heart, and Coronary Circulation. Hypertension, 18(5), 565–574. https://doi.org/10.1161/01.hyp.18.5.565

Igumbor, E. U., Sanders, D., Puoane, T. R., Tsolekile, L., Schwarz, C., Purdy, C., Swart, R., Durão, S., & Hawkes, C. (2012). "Big Food," the Consumer Food Environment, Health, and the Policy Response in South Africa. PLoS Medicine, 9(7), 1–7. https://doi.org/10.1371/journal.pmed.1001253

Ikeda, H. (19770. Effects of Taurine on Alcohol Withdrawal. Lancet, 3;2(8036), 509. doi: 10.1016/s0140-6736(77)91636-1. PMID: 70719. https://pubmed.ncbi.nlm.nih.gov/70719/

Institute of Medicine (US) Committee on Military Nutrition Research. Caffeine for the Sustainment of Mental Task Performance: Formulations for Military Operations. Washington (DC). National Academies Press (US), 2001(2), Pharmacology of Caffeine. https://www.ncbi.nlm.nih.gov/books/NBK223808/#:~:text=The%20mean%20half%2Dlife%20of,et%20al.%2C%201989).

Iyadurai, S. J. P., & Chung, S. S. (2007). New-onset Seizures in Adults: Possible Association with Consumption of Popular Energy Drinks. Epilepsy and Behavior, 10(3), 504–508. https://doi.org/10.1016/j.yebeh.2007.01.009

Jee, H. J., Lee, S. G., Bormate, K. J., & Jung, Y. S. (2020). Effect of Caffeine Consumption on the Risk for Neurological and Psychiatric Disorders: Sex Differences in Human. Nutrients, 12(10), 1–19. https://doi.org/10.3390/nu12103080

Jongen, V. W., Lalla-Edward, S. T., Vos, A. G., Godijk, N. G., Tempelman, H., Grobbee, D. E., Devillé, W., & Klipstein-Grobusch, K. (2019). Hypertension in a Rural Community in South Africa: What They Know,

What They Think They Know and What They Recommend. BMC Public Health, 19(341), 1–10. https://doi.org/10.1186/s12889-019-6642-3

Kammerer, M., Jaramillo, J. A., García, A., Calderón, J. C., & Valbuena, L. H. (2014). Effects of Energy Drink Major Bioactive Compounds on the Performance of Young Adults in Fitness and Cognitive Tests: A Randomized Controlled Trial. Journal of the International Society of Sports Nutrition, 11(44), 1–7. https://doi.org/10.1186/s12970-014-0044-9

Keates, A. K., Mocumbi, A. O., Ntsekhe, M., Sliwa, K., & Stewart, S. (2017). Cardiovascular Disease in Africa: Epidemiological Profile and Challenges. Nature Reviews Cardiology, 14(5), 273–293. https://doi.org/10.1038/nrcardio.2017.19

Kelley, D. (2014). Heart Disease: Causes, Prevention, and Current Research. JCCC Honors Journal, 5(2), 1–15. https://scholarspace.jccc.edu/cgi/viewcontent.cgi?article=1047&context=honors_journal

KindPNG, 2022. Caffeine and Adenosine – Adenosine and Caffeine Structure, HD [Online]. Shenzhen: Devanto Ceramic. Available from: https://www.kindpng.com/imgv/hxhxJRm_caffeine-and-adenosine-adenosine-and-caffeine-structure-hd/ [Accessed: 16/11/2022]

Kozik T.M., Shah, S., Bhattacharyya, M., Franklin, T.T., Connolly, T.F., Chien, W., Charos, G.S., Pelter, M.M. (2016). Cardiovascular Responses to Energy Drinks in a Healthy Population: The C-energy Study. American Journal of Emergency Medicine, 34, 1205-1209. DOI: 10.1016/j.ajem.2016.02.068, PMID: 27162113

Kujawska, M. (2018). Nutraceutical Ingredient or Conveyor for the Intake of Medicinal Plants? Evidence from Paraguayan Folk Medicine. Evidence-Based Complementary and Alternative Medicine, 2018(1), 1–18. https://doi.org/10.1155/2018/6849317

Kumar, D. (2012). Get Along with Quantitative Research Process. International Journal of Research in Management, 2(2), 1–16. https://www.researchgate.net/publication/259359212

Kurtz, A. M., Leong, J., Anand, M., Dargush, A. E., & Shah, S. A. (2013). Effects of Caffeinated Versus Decaffeinated Energy Shots on Blood Pressure and Heart Rate in Healthy Young Volunteers. Pharmacotherapy, 33(8), 779–786. https://doi.org/10.1002/phar.1296

Lackland, D. T. (2014). Racial Differences in Hypertension: Implications for High Blood Pressure Management. American Journal of the Medical Sciences, 348(2), 135–138. https://doi.org/10.1097/MAJ.0000000000000308

Laquale, K. (2007). Red Bull®: The Other Energy Drink and its Effect on Performance. Athletic Therapy Today, 12(2), 43–45. https://doi.org/10.1123/att.12.2.43

Lee, A., Lim, W., Kim, S., Khil, H., Cheon, E., An, S., Hong, S., Lee, D. H., Kang, S. S., Oh, H., Keum, N., & Hsieh, C. C. (2019). Coffee Intake and Obesity: A Meta-analysis. Nutrients, 11(6), 1–10. https://doi.org/10.3390/nu11061274

Lee, J. H., Yoon, J. H., Kim, S. S., Ma, S. K., Kim, S. W., & Bae, E. H. (2017). Panax Ginseng Induces Toxic Hepatitis and Acute Kidney Injury. Chonnam Medical Journal, 53(2), 168. https://doi.org/10.4068/cmj.2017.53.2.168

Lee, K. H., Human, G. P., Fourie, J. J., Louw, W. A. N., Larson, C. O., & Joubert, G. (2009). Medical Students' Use of Caffeine for "Academic Purposes" and their Knowledge of its Benefits, Side-effects and Withdrawal Symptoms. South African Family Practice, 51(4), 322–327. https://doi.org/10.1080/20786204.2009.10873872

Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R. (2002). Age-Specific Relevance of Usual Blood Pressure to Vascular Mortality: A Meta-Analysis of Individual Data for One Million Adults in 61 Prospective Studies. Prospective Studies Collaboration, Lancet, 360(9349), 1903-1913. doi: 10.1016/s0140-6736(02)11911-8. Erratum in: Lancet. 2003 Mar 22;361(9362):1060. PMID: 12493255. https://pubmed.ncbi.nlm.nih.gov/12493255/

Lindhorst, J., Alexander, N., Blignaut, J., & Rayner, B. (2007). Differences in Hypertension Between Blacks and Whites: An Overview. Cardiovascular Journal of Africa, 18(4), 241–247. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4170224/

Marczinski, C. A., & Fillmore, M. T. (2014). Energy Drinks Mixed with Alcohol: What are the Risks? Nutrition Reviews, 72(S1), 98–107. https://doi.org/10.1111/nure.12127

Marczinski, C. A., Fillmore, M. T., Henges, A. L., Ramsey, M. A., & Young, C. R. (2012). Effects of Energy Drinks Mixed with Alcohol on Information Processing, Motor Coordination and Subjective Reports of Intoxication. Experimental and Clinical Psychopharmacology, 20(2), 129–138. https://doi.org/10.1037/a0026136

Mattioli, A.V., Pennella, S., Manenti, A., Farinetti, A. (2016). Energy Drink Overconsumption Can Trigger Atrial Fibrillation. Journal of Cardiovascular Medicine, 17(12), 902-904. doi: 10.2459/JCM.0000000000000416

Melgarejo, J. D., Yang, W. Y., Thijs, L., Li, Y., Asayama, K., Hansen, T. W., Wei, F. F., Kikuya, M., Ohkubo, T., Dolan, E., Stolarz-Skrzypek, K., Huang, Q. F., Tikhonoff, V., Malyutina, S., Casiglia, E., Lind, L., Sandoya, E., Filipovský, J., Gilis-Malinowska, N., Zhang, Z. Y. (2021). Association of Fatal and Nonfatal Cardiovascular Outcomes With 24-Hour Mean Arterial Pressure. Hypertension, 77(1), 39–48. https://doi.org/10.1161/HYPERTENSIONAHA.120.14929

Miller, K. E. (2008). Energy Drinks, Race, and Problem Behaviors Among College Students. Journal of Adolescent Health, 43(5), 490–497. https://doi.org/10.1016/j.jadohealth.2008.03.003

Morin, D. P., Saad, M. N., Shams, O. F., Owen, J. S., Xue, J. Q., Abi-Samra, F. M., Khatib, S., Nelson-Twakor, O. S., & Milani, R. V. (2012). Relationships Between the T-peak to T-end Interval, Ventricular Tachyarrhythmia, and Death in Left Ventricular Systolic Dysfunction. Europace, 14(8), 1172–1179. https://doi.org/10.1093/europace/eur426

Mustafa, S. J., Morrison, R. R., Teng, B., & Pelleg, A. (2009). Adenosine Receptors and the Heart: Role in Regulation of Coronary Blood Flow and Cardiac Electrophysiology. Handbook of Experimental Pharmacology, 2009(193), 161–188. https://doi.org/10.1007/978-3-540-89615-9_6

NICE Guideline. (2019). Hypertension in Adults: Diagnosis and Management. NICE guideline, 2019. www.nice.org.uk/guidance/ng136

Nishijima, Y., Takeshi, A. E., Ae, I., Takamatsu, M., Kiso, Y., Hiroshi, A. E., Ae, S., Fushiki, T., & Moritani, T. (2002). Influence of Caffeine Ingestion on Autonomic Nervous Activity During Endurance Exercise in Humans. European Journal of Applied Physiology, 2002(87), 475–480. https://doi.org/10.1007/s00421-002-0678-1

Nnyepi, M. S., Gwisai, N., Lekgoa, M., & Seru, T. (2015). Evidence of Nutrition Transition in Southern Africa. Proceedings of the Nutrition Society, 74(4), 478–486. https://doi.org/10.1017/S0029665115000051

Nowak, D., & Gośliński, M. (2020). Assessment of Antioxidant Properties of Classic Energy Drinks in Comparison with Fruit Energy Drinks. Foods, 9(1), 1–12. https://doi.org/10.3390/foods9010056

Nowak, D., Gośliński, M., & Nowatkowska, K. (2018). The Effect of Acute Consumption of Energy Drinks on Blood Pressure, Heart Rate and Blood Glucose in the Group of Young Adults. International Journal of Environmental Research and Public Health, 15(3), 1–11. https://doi.org/10.3390/ijerph15030544

Nowak, D., & Jasionowski, A. (2015). Analysis of the Consumption of Caffeinated Energy Drinks Among Polish Adolescents. International Journal of Environmental Research and Public Health, 12(7), 7910–7921. https://doi.org/10.3390/ijerph120707910

Ntuli, S. T., Maimela, E., Alberts, M., Choma, S., & Dikotope, S. (2015). Prevalence and Associated Risk Factors of Hypertension Amongst Adults in a Rural Community of Limpopo Province, South Africa. African Journal of Primary Health Care and Family Medicine, 7(1), 1–5. https://doi.org/10.4102/phcfm.v7i1.847

Oberhoffer, F. S., Li, P., Jakob, A., Dalla-Pozza, R., Haas, N. A., & Mandilaras, G. (2022). Energy Drinks: Effects on Blood Pressure and Heart Rate in Children and Teenagers. A Randomized Trial. Frontiers in Cardiovascular Medicine, 9(862041), 1–9. https://doi.org/10.3389/fcvm.2022.862041

Olas, B., & Bryś, M. (2019). Effects of Coffee, Energy Drinks and their Components on Hemostasis: The hypothetical Mechanisms of their Action. Food and Chemical Toxicology, 127(1), 31–41. https://doi.org/10.1016/j.fct.2019.02.039

Orrù, S., Imperlini, E., Nigro, E., Alfieri, A., Cevenini, A., Polito, R., Daniele, A., Buono, P., & Mancini, A. (2018). Role of Functional Beverages on Sport Performance and Recovery. Nutrients, 10(1470), 1–21. https://doi.org/10.3390/nu10101470

Öz, F., Üniversitesi, İ., Tıp Fakültesi Kardiyoloji Anabilim Dalı, İ., Elitok, A., Panc, C., Sarıkaya, R., Sezikli, S., Pala, Y., Sinem Bugan, Ö., Ateş, M., Parıldar, H., Buğra Ayaz, M., Atıcı, A., & Oflaz, H. (2015). Acute Effects of Red Bull Energy Drink on Ventricular Repolarization in Healthy Young Volunteers: A Prospective Study. Anatolian Journal of Cardiology, 15(11), 919–922. https://doi.org/10.5152/akd.2015.5791

Ozen, A. E., Pons, A., & Tur, J. A. (2012). Worldwide Consumption of Functional Foods: A Systematic Review. Nutrition Reviews, 70(8), 472–481. https://doi.org/10.1111/j.1753-4887.2012.00492.x

Pagano, C.W., Wu, M., Wu, L. (2017). Acute Visual Loss and Intraretinal Hemorrhages Associated to Energy Drink Consumption. International Ophthalmology, 37(6),1349-1351. doi: 10.1007/s10792-016-0399-9. Epub 2016 Dec 1. PMID: 27904988.

Pangarkar, N., & Agarwal, M. (2013). The Wind Behind Red Bull's Wings. www.forbes.com. Accessed 22/09/2022.

Pharmacy180.com, 2021. Xanthine Derivatives: Synthesis and Drug profile. Medicinal Chemistry, CNS Stimulant [Online]. https://www.pharmacy180.com/article/xanthine-derivatives-2154/. [Accessed: 21/10/2021]

Plamondon, L. (2013). What is Topo? Energy drinks: Threatening or commonplace? An update. Topo, 1(6), 1–10. https://www.inspq.qc.ca/pdf/publications/1669_BoissonsEnergisantes.pdf

Ragsdale, F. R., Tyler, A. E., Gronli, D., Narjes, A. E., Ae, B., Haight, N., April, A. E., Ae, M., Mcmahon, E. C., Thomas, A. E., Ae, W. N., Ae, C. M. M., Sell, C. J., Patrick, A. E., Mccann, J., Gary, A. E., Kastello, M.,

Tisha, A. E., Ae, H., & Wilson, T. (2010). Effect of Red Bull Energy Drink on Cardiovascular and Renal Function. Amino Acids, 38, 1193–1200. https://doi.org/10.1007/s00726-009-0330-z

Ramanlal, R., Gupta, V. (2023). Physiology, Vasodilation. 2023 Jan 23. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 January. PMID: 32491494. https://pubmed.ncbi.nlm.nih.gov/32491494/

Reid, J. L., McCrory, C., White, C. M., Martineau, C., Vanderkooy, P., Fenton, N., & Hammond, D. (2017). Consumption of Caffeinated Energy Drinks Among Youth and Young Adults in Canada. Preventive Medicine Reports, 5, 65–70. https://doi.org/10.1016/j.pmedr.2016.11.012

Reid, S. D., Ramsarran, J., Brathwaite, R., Lyman, S., Baker, A., Cornish, D. C., Ganga, S., Mohammed, Z., Sookdeo, A. T., & Thapelo, C. K. (2015). Energy Drink Usage Among University Students in a Caribbean Country: Patterns of Use and Adverse Effects. Journal of Epidemiology and Global Health, 5(2), 103–116. https://doi.org/10.1016/j.jegh.2014.05.004

Richards, G., & Smith, A. P. (2016). A Review of Energy Drinks and Mental Health, with a Focus on Stress, Anxiety, and Depression. Journal of Caffeine Research, 6(2), 49–63. https://doi.org/10.1089/jcr.2015.0033

Rokot, N. T., Kairupan, T. S., Cheng, K.-C., Runtuwene, J., Kapantow, N. H., Amitani, M., Morinaga, A., Amitani, H., Asakawa, A., & Inui, A. (2016). A Role of Ginseng and Its Constituents in the Treatment of Central Nervous System Disorders. Evidence-Based Complementary and Alternative Medicine, 2016, 1–7. https://doi.org/10.1155/2016/2614742

Sachdeva, S., & Gupta, M. (2013). Adenosine and its Receptors as Therapeutic Targets: An Overview. Saudi Pharmaceutical Journal, 21(3), 245–253. https://doi.org/10.1016/j.jsps.2012.05.011

Schaffer, S.W., Jong, C.J., Ramila, K.C., Azuma, J. (2010). Physiological Roles of Taurine in Heart and Muscle. Journal of Biomedical Sciences, 17(Suppl 1), 1-8. doi: 10.1186/1423-0127-17-S1-S2. PMID: 20804594; PMCID: PMC2994395.

Schutte, A. E. (2019). Urgency for South Africa to Prioritise Cardiovascular Disease Management. The Lancet Global Health, 7(2), e177–e178. https://doi.org/10.1016/S2214-109X(18)30476-5

Seifert, S. M., Schaechter, J. L., Hershorin, E. R., & Lipshultz, S. E. (2011). Health Effects of Energy Drinks on Children, Adolescents, and Young Adults. Special Articles Pediatrics, 127(3), 511–528. https://doi.org/10.1542/peds.2009-3592

Sereshti, H., & Samadi, S. (2014). A Rapid and Simple Determination of Caffeine in Teas, Coffees and Eight Beverages. Food Chemistry, 158, 8–13. https://doi.org/10.1016/j.foodchem.2014.02.095

Shah, S. A., Szeto, A. H., Farewell, R., Shek, A., Fan, D., Quach, K. N., Bhattacharyya, M., Elmiari, J., Chan, W., O'Dell, K., Nguyen, N., McGaughey, T. J., Nasir, J. M., & Kaul, S. (2019). Impact of High Volume Energy Drink Consumption on Electrocardiographic and Blood Pressure Parameters: A Randomized Trial. Journal of the American Heart Association, 8(11), 1–14. https://doi.org/10.1161/JAHA.118.011318

Silva, C. P. da, Soares-Freitas, R. A. M., Sampaio, G. R., Camargo, A. C. de, & Torres, E. A. F. S. (2019). Guarana as a Source of Bioactive Compounds. Journal of Food Bioactives, 6, 1–5. https://doi.org/10.31665/jfb.2019.6182

Somers, K. R., & Svatikova, A. (2020). Cardiovascular and Autonomic Responses to Energy Drinks—Clinical Implications. Journal of Clinical Medicine, 9(2), 431. https://doi.org/10.3390/jcm9020431

Song, J. K. (2018). Coronary Artery Vasospasm. Korean Circulation Journal, 48(9), 767–777. https://doi.org/10.4070/kcj.2018.0251

Stacey, N., van Walbeek, C., Maboshe, M., Tugendhaft, A., & Hofman, K. (2017). Energy Drink Consumption and Marketing in South Africa. Preventive Medicine, 105, S32–S36. https://doi.org/10.1016/j.ypmed.2017.05.011

Statistics South Africa, & Maluleke, R. (2016). Selected Health Indicators Among the Elderly: Findings from the SADHS 2016, GHS 2016 and MACoD 2016. Statistics South Africa, 3(13), 1–47. http://www.statssa.gov.za/publications/03-00-13/03-00-132016.pdf

Steinke, L., Lanfear, D. E., Dhanapal, V., & Kalus, J. S. (2009). Effect of "Energy Drink" Consumption on Hemodynamic and Electrocardiographic Parameters in Healthy Young Adults. Annals of Pharmacotherapy, 43(4), 596–602. https://doi.org/10.1345/aph.1L614

Stewart, J. M., Montgomery, L. D., Glover, J. L., & Medow, M. S. (2007). Changes in Regional Blood Volume and Blood Flow During Static Handgrip. American Journal of Physiology - Heart and Circulatory Physiology, 292(1), H214–H223. https://doi.org/10.1152/ajpheart.00681.2006

Subaiea, G. M., Altebainawi, A. F., & Alshammari, T. M. (2019). Energy Drinks and Population Health: Consumption Pattern and Adverse Effects Among Saudi Population. BMC Public Health, 19(1539), 1–12. https://doi.org/10.1186/s12889-019-7731-z

Tang, K. S., Medeiros, E. D., & Shah, A. D. (2020). Wide Pulse Pressure: A Clinical Review. International Journal of Clinical Hypertension, 22(11), 1960–1967. Blackwell Publishing Inc. https://doi.org/10.1111/jch.14051

Tauseef, A., Akmal, A., Hasan, S., Waheed, A., Zafar, A., Cheema, A., Mukhtar, Q., & Malik, A. (2017). Effect of Energy Drink on Reaction Time, Haemodynamic and Electrocardiographic Parameters. Pakistan Journal of Physiology, 13(1), 7–10. http://www.pps.org.pk/PJP/13-1/Ambreen.pdf7

Temple, J. L. (2009). Caffeine Use in Children: What we Know, What we Have Left to Learn, and Why we Should Worry. Neuroscience and Biobehavioral Reviews, 33(6), 793–806. https://doi.org/10.1016/j.neubiorev.2009.01.001

Turnbull, D., Rodricks, J. V., Mariano, G. F., & Chowdhury, F. (2017). Caffeine and Cardiovascular Health. Regulatory Toxicology and Pharmacology, 89, 165–185. https://doi.org/10.1016/j.yrtph.2017.07.025

Umemura, T., Ueda, K., Nishioka, K., Hidaka, T., Takemoto, H., Nakamura, S., Jitsuiki, D., Soga, J., Goto, C., Chayama, K., Yoshizumi, M., & Higashi, Y. (2006). Effects of Acute Administration of Caffeine on Vascular Function. American Journal of Cardiology, 98(11), 1538–1541. https://doi.org/10.1016/j.amjcard.2006.06.058

Ünal, S., Şensoy, B., Yilmaz, S., Ünal, G.G., Süleymanoğlu, M., Şen, F., Açar, B., Balcı, M.M. (2015). Left Main Coronary Artery Thrombosis and Acute Anterior Myocardial Infarction Related to Energy Drink. International Journal of Cardiology, 179, 66-67. doi: 10.1016/j.ijcard.2014.10.073. Epub 2014 Oct 22. PMID: 25464415. https://pubmed.ncbi.nlm.nih.gov/25464415/

Urdampilleta, A., Gómez-Zorita, S., Soriano, J. M., Martínez-Sanz, J. M., Medina, S., & Gil-Izquierdo, A. (2015). Hidratación e Ingredientes Químicos en el Deporte: Seguridad Alimentaria en el Contexto Europeo. Nutricion Hospitalaria, 31(5), 1889–1899. https://doi.org/10.3305/nh.2015.31.5.7867

van Dijk, R., Ties, D., Kuijpers, D., van der Harst, P., & Oudkerk, M. (2018). Effects of Caffeine on Myocardial Blood Flow: A Systematic Review. Nutrients, 10(8), 1–14. https://doi.org/10.3390/nu10081083

WHO Expert committee. (1995). Physical status: the Use of and Interpretation of Anthropometry, Report of a WHO Expert Committee. WHO Technical Report Series, 845, 1–452. https://apps.who.int/iris/bitstream/handle/10665/37003/WHO_TRS_854.pdf?sequence=1&isAllowed=y

World Health Organisation, 2021. Noncommunicable Diseases. Published 13 April 2021. Viewed 10 May 2022. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

Worthley, M.I., Prabhu, A., De Sciscio, P., Schultz, C., Sanders, P., Willoughby, S.R. (2010). Detrimental Effects of Energy Drink Consumption on Platelet and Endothelial Function. American Journal of Medicine, 123(2), 184-7. doi: 10.1016/j.amjmed.2009.09.013. PMID: 20103032.

Willson, C. (2018). The Clinical Toxicology of Caffeine: A Review and Case Study. Toxicology Reports, 5, 1140–1152. https://doi.org/10.1016/j.toxrep.2018.11.002

Whitsett, T.L., Manion, C.V., Christensen, H.D. (1984). Cardiovascular Effects of Coffee and Caffeine. American Journal of Cardiology, 53(7), 918-22. doi: 10.1016/0002-9149(84)90525-3. PMID: 6702647. https://pubmed.ncbi.nlm.nih.gov/6702647/

Yu, H., Zhao, J., You, J., Li, J., Ma, H., & Chen, X. (2019). Factors Influencing Cultivated Ginseng (Panax Ginseng C. A. Meyer) Bioactive Compounds. PLoS ONE, 14(10), 1–17. https://doi.org/10.1371/journal.pone.0223763

Yunusa, I., & Ahmad, I. (2012). Energy- Drinks: Composition and Health Benefits. Bayero Journal of Pure and Applied Sciences, 4(2), 186–191. https://doi.org/10.4314/bajopas.v4i2.38

Zhang, D. Y., & Anderson, A. S. (2014). The Sympathetic Nervous System and Heart Failure. Cardiology Clinics, 32(1), 1–20. https://doi.org/10.1016/j.ccl.2013.09.010

Appendices

Appendix A

Participant Recruitment Flyer

University of South Africa (UNISA) – Department of Life and Consumer Sciences

Researcher: Ayanda Nompumelelo Nyalela

Contact details: tel. 079 4757764

Email. 54233550@mylife.unisa.ac.za

Title: The Effects of Acute Energy Drink Consumption on the Cardiovascular System of University Students

1. **Purpose of the study:**

The high prevalence of hypertension development in South Africa, along with the increased consumption of Energy Drinks (EDs) by young adults raises a question of the adverse effects of ED consumption on the already-at-risk cardiovascular system of young, black South African university students. The purpose of this study is to determine the effects of acute ED exposure on the cardiovascular system of generally healthy, black, male university students, aged between 18-29 years, in the context of potential risk for hypertension development. This will be achieved through the assessment of any cardiovascular changes observed – heart rate and blood pressure – following the administration of the intervention.

2. **Control Conditions:**

Prior to your testing, you're requested to adhere to the following conditions: no strenuous physical exercising for 48 hours prior to testing; no consumption of energy drinks for at least 6 days prior to testing; and you're required to fast for 10 hours prior to testing.

3. **Intervention:**

Two beverages will be administered in a blind manner; the placebo (control) will be formulated by diluting a fruit juice concentrate/syrup (Magalies – Peach and Mango) with carbonated water at a ratio of 1:3. And the intervention is a carbonated fruit-blended Monster energy drink (Mucho-Loco Flavour).

4. **Experimental procedure:**

- You will receive 2 dates for administering the intervention and placebo in no particular order. You will then be assigned either a placebo or the intervention in a blind manner in order to prevent any psychological influence on the effects of the beverage.

- The baseline cardiovascular measurements (0 minutes) will be conducted prior to consumption of the beverage. You will then be given a period of 10 minutes to which you have to completely ingest the beverage.
- Following the administration, you will then be placed in a relaxed, sitting position then the researcher/team member will proceed to record your heart rate and blood pressure measurements using a blood pressure cuff machine on your left arm. The cardiovascular measurements be recorded in the following time intervals: 10 minutes; 30 minutes; 60 minutes; 90 minutes and 120 minutes after consumption.
- Whilst in the relaxed, sitting position, you will be given the opportunity to fill out two questionnaires related to the study. You're also encouraged to bring additional work or something to read
- Food and drinks may not be consumed during the experimental procedure. You are, however, encouraged to bring lunch/snack for AFTER the trial is over.

5. <u>Potential risks of experimental protocol:</u>

Adverse events or physical discomfort from the consumption of energy drinks may include headaches, sleepiness, jitters, nausea, heart palpitations, dizziness, sweating, an upset stomach or diarrhoea

6. <u>Emergency protocol:</u>

The study will be conducted in a clinical setting under the supervision of a certified nurse at the UNISA Science Campus (Florida) - ground floor of the NB Pityana building

Should you experience any physical discomfort, you must IMMEDIATELY report to the research team member assisting you. The experiment will be ceased, and you'll receive medical assistance from the nurse.

7. <u>Data collection and Safe keeping:</u>

All the data will be collected on site during the experiment through data sheets and questionnaires. All documents will be stored safely in a file which is kept by the researcher.

The hard copies will be safely stored by the researcher in a locked cabinet in Roodepoort, Gauteng, for a maximum of 5 years. In addition, all documentation will be scanned, and the digital copies will be stored on Cloud storage on Google Drive under the researcher's personal account. Datasets will be managed by the supervisors and only distributed to individuals involved with the study to be used for the duration of their research project.

8. <u>Participant reimbursement for travel costs</u>

As a participant of the study, you will receive a reimbursement of R250 for travel costs. Because the study requires you to be present for two non-consecutive days, you will receive R100.00 on the first appointment then the balance of R150.00 on the final appointment.

9. Voluntary recruitment

Participation of the study is STRICTLY on a voluntary basis and will commence AFTER you sign a consent form. You have the opportunity to ask any question to any research team member throughout the duration to which you're a part of the study

10. Confidentiality

Your participation in the study will handled with most confidence at all times, including in the final publication. Your contact details, however, will be required in your consent form so we can send you reminder notifications for your appointments, as well as to update you on the findings of the research (via a link sent to your email).

None of your personal details will be documented or reflected in the study. This will be achieved by using numbering/coding as pseudonyms to represent your individual contribution to the study. i.e all your questionnaires and data sheets will only reflect your participation number. No name, surname or student number will be requested.

11. Withdrawal from study

As a participant, you have the opportunity to withdraw yourself and/or your data from the study at any moment before the submission of questionnaires, without negative consequence.

Appendix B

Participant Screening Checklist

For Office Use Only: Kindly Mark the relevant boxes with an 'X'

Characteristics	**Yes**	**No**
Ethnicity: Black/African		
Age: 18 to 29 years		
Sex: Male		
Are you currently enrolled as a UNISA student?*		
If answered "NO" to any of the questions above, thank the person for their time and do not invite to partake in the study **If answered "YES" to all the questions above, continue with the following questions**		
Diagnosed with a chronic disease (such as hypertension, diabetes, epilepsy)		
Allergies to energy drinks or its contents		
Diagnosed with conditions that are affected by caffeine such as arrhythmia, heart attack and stroke		
If answered "YES" to any of the second set of questions above, thank the person for their time and do not invite to partake in the study **If answered "NO" to the second set of questions, invite the person to partake in the study.**		

*Note that due to a low enrolment rate during the COVID lock-down period, recruitment was expanded to other university students residing in Gauteng.

Appendix C

PARTICIPANT INFORMATION SHEET

Ethics clearance reference number: 2019/CAES_HREC/187
Research permission reference number:

<date>

Title: The Effects of Acute Energy Drink Consumption on the Cardiovascular System of University Students

Dear Prospective Participant

My name is Ayanda Nompumelelo Nyalela and I am doing research with Dr. Caitlynd Myburgh and Dr Elize Symington (lecturers in the Department of Life and Consumer Sciences) towards a Master's of Science (MSc) in Life Sciences at the University of South Africa. We are inviting you to participate in a study titled 'The Effects of Acute Energy Drink Consumption on the Cardiovascular System of University Students'.

WHAT IS THE PURPOSE OF THE STUDY?

I am conducting this research to find out the effects of acute energy drink (ED) exposure on the cardiovascular system of generally healthy, black, male university students in the context of potential risk for hypertension development.

WHY AM I BEING INVITED TO PARTICIPATE?

Epidemiological evidence often highlights that black ethnic groups are most likely to have a higher prevalence of hypertension in comparison to other ethnical groups. Not only is hypertension prevalence becoming a greater problem in South Africa, but research clearly highlights both the presence of early vascular aging as well as an increased risk for hypertension and cardiovascular diseases in black people. Despite these alarming trends, there's a growing concern regarding ED consumption increasing significantly, particularly in young adults who are faced with stressors such as academic performance at university level or those engaging in athletics. Even though there are claims of beneficial effects of EDs, excessive amounts can cause caffeine intoxication which is known to cause adverse

physiological effects affecting mainly the neurological and cardiovascular systems. These include heart palpitations, hypertension, diuresis, over-stimulation of the central nervous system, vomiting, nausea, hypokalaemia, metabolic acidosis and convulsions. Thus, our study is focused on young, black, males to determine the association between the hypertension prevalence development along with the increased consumption of energy drinks by young adults.

As a participant, you were chosen by the researcher/research team member using the consecutive sampling method for recruitment on a voluntary basis at the UNISA Science campus in Florida (Gauteng). Your initial invitation to the study was based on the following factors which qualify you as a suitable participant: your gender, ethnic group, age and being a Unisa student. Your contact details will not be obtained until after you provide written consent to participate in study. For this experiment, we aim to obtain 30 participants for our sample group.

WHAT IS THE NATURE OF MY PARTICIPATION IN THIS STUDY?

The study involves questionnaires pertaining to your use and general knowledge on energy drinks, as well as other factors related to your cardiovascular health such as exercise, use of medication and smoking habits.
In addition, the study involves an experimental procedure where we will take a blood pressure measurement before and a few times after consuming either an energy drink or carbonated fruit juice. For the blood pressure measurement your left arm will be used to conduct cardiovascular measurements for blood pressure (mmHg) and heart rate (bpm) while in a seated position using a blood pressure cuff machine by a member of the research team. The baseline cardiovascular measurements (0 minutes) will be conducted then you will be given a period of 10 minutes to consume one of the two different beverages: a 500ml carbonated juice (placebo) or a 500ml energy drink (intervention). You will be randomly assigned a beverage in a blind manner, in no particular order. Following the administration of the beverage, the research team member will continue to take cardiovascular measurements for a total period of 120 minutes, in intervals of 30 minutes. The total experiment will last a duration of 140 minutes, which includes an additional 10 minutes for questionnaires.

CAN I WITHDRAW FROM THIS STUDY EVEN AFTER HAVING AGREED TO PARTICIPATE?

Participating in this study is voluntary and you are under no obligation to consent to participation. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a written consent form. You are free to withdraw at any time before submissions of any questionnaires and without giving a reason. All questionnaires are anonymized and will be captured and used for the deductions of the study.

WHAT ARE THE POTENTIAL BENEFITS OF TAKING PART IN THIS STUDY?

After completion of the study, your results will be shared with you and discussed. These types of measurements are typically conducted at clinics, many times at a cost, however, you will receive this at no financial cost. In addition, our hopes will be to further educate you on the risks that common use of EDs pose on physiological function and thus, provide you with tools to which will encourage you making better choices that will benefit your health and others.

As a participant, you will also contribute to the knowledge of the biological effects of energy drinks on the cardiovascular system of young, apparently healthy male university students. This may benefit the society in terms of regulations regarding energy drinks. Furthermore, your contribution will assist in the study's aim to extend and validate knowledge on the pathologies caused by ED administration to the cardiovascular system.

ARE THERE ANY NEGATIVE CONSEQUENCES FOR ME IF I PARTICIPATE IN THE RESEARCH PROJECT?

While these energy drinks are unrestricted and freely available in your local supermarket, there is a small chance that you may experience slight discomfort in the form of headaches, restlessness, jitters, nausea, heart palpitations, dizziness, sweating, an upset stomach or diarrhea.

The study will be conducted in a clinical setting under the supervision of a certified nurse at the UNISA Science Campus (Florida) clinic. Should you experience any physical discomfort, you must IMMEDIATELY report to the research team member assisting you. You'll receive medical assistance from the nurse.

WILL THE INFORMATION THAT I CONVEY TO THE RESEARCHER AND MY IDENTITY BE KEPT CONFIDENTIAL?

Yes. Your name will not be recorded anywhere, and no one will be able to connect you to the answers you give. We will collect your contact details in order to contact you for the second date of your participation, however this will not be entered into the dataset but kept separate in a file. You will be allocated a participant number and you will be referred to in this way in the data, any publications, or other research reporting methods such as conference proceedings.

All your questionnaires and data sheets for the experiment will reflect only your participant number and will only be accessible to the researcher and the team. All research team members will handle documentation with confidentiality by adhering to the conditions reflected in the signed *confidentiality agreement* forms. Your answers may be reviewed by people responsible for making sure that research is done properly, including the transcriber, external coder, and members of the Research Ethics Review Committee. Otherwise, records that identify you will be available only to people working on the study, unless you give permission for other people to see the records.

Therefore, In the case of the study being published (research reports, journal articles, or conference proceedings), the data will maintain anonymity by only reflecting your participant number, ensuing that no reports of the study can be associated or traced back to you.

HOW WILL THE RESEARCHER(S) PROTECT THE SECURITY OF DATA?

Hard copies of your answers will be stored by the researcher for a period of five years in a locked cupboard/filing cabinet located in Roodepoort, Gauteng, for future research or academic purposes. In addition, the electronic information will be stored for five years on a password protected computer that belongs ONLY to the researchers involved, as well as the Cloud space on the researcher's Google drive account. Future use of the stored data will be subject to further Research Ethics Review and approval if applicable. All hard copies of questionnaires and cardiovascular measurements for participants will, after a period of five years, be shredded and disposed. Digital copies will be permanently deleted both, on the Google Drive and the exclusive folder on the laptop of the researcher.

WILL I RECEIVE PAYMENT OR ANY INCENTIVES FOR PARTICIPATING IN THIS STUDY?

As a participant of the study, you will receive a reimbursement of R250 for travel costs. Because the study requires you to be present for two non-consecutive days, you will receive R100.00 at the first appointment then the balance of R150.00 at the final appointment.

HAS THE STUDY RECEIVED ETHICS APPROVAL

This study has received written approval from the Research Ethics Review Committee of the College of Agriculture and Environmental Sciences, Unisa. A copy of the approval letter can be obtained from the researcher if you so wish.

HOW WILL I BE INFORMED OF THE FINDINGS/RESULTS OF THE RESEARCH?

If you would like to be informed of the final research findings or should you require any further information or want to contact the researcher about any aspect of this study, please contact Ayanda Nompumelelo Nyalela on 0794757764 or email 54233550@mylife.unisa.ac.za.

Should you have concerns about the way in which the research has been conducted, you may contact my supervisor, Dr. Caitlynd Myburgh on 011 471 2819 or email: vzylc1@unisa.ac.za. Alternatively, you may contact my co-supervisor, Dr. Elize Symington on 011 471 3438 or email: syminea@unisa.ac.za. Contact the research ethics chairperson of the CAES General Ethics Review Committee, Prof EL Kempen on 011-471-2241 or kempeel@unisa.ac.za if you have any ethical concerns.

Thank you for taking time to read this information sheet and for participating in this study.
Thank you.

Ayanda Nompumelelo Nyalela

CONSENT TO PARTICIPATE IN THIS STUDY

I, _____ (participant name), confirm that the person asking my consent to take part in this research has told me about the nature, procedure, potential benefits and anticipated inconvenience of participation.

I have read (or had explained to me) and understood the study as explained in the information sheet.

I have had sufficient opportunity to ask questions and am prepared to participate in the study.

I understand that my participation is voluntary and that I am free to withdraw at any time without penalty (if applicable).

I am aware that the findings of this study will be processed into a research report, journal publications and/or conference proceedings, but that my participation will be kept confidential unless otherwise specified.

I agree to the recording of the <insert specific data collection method>.

I have received a signed copy of the informed consent agreement.

Participant Name & Surname…… (please print)

Participant Signature……..Date……

Researcher's Name & Surname……(please print)

Researcher's signature……..Date……

Appendix D

Data sheets for anthropometric and cardiovascular measurements

Anthropometric Measurements - Data sheet 3			
Participant nr.			
weight (kg)	**Height (m)**	**BMI (kg/m²)**	**waist circumference (cm)**

Cardiovascular Measurements - Data spreadsheet 1 (Exp.1)								
participant nr.								
Blood Pressure measurements (mmHg) - diastolic (DBP) and systolic (SBP)								
Time (minutes):	**DBP Reading 1**	**DBP Reading 2 (after 2minutes)**	**Average DBP**	**SBP Reading 1**	**SBP Reading 2 (after 2minutes)**	**Average SBP**	**pulse pressure (reading 1)**	**pulse pressure (reading 2)**
0								
10								
30								
60								
90								
120								
Beverage Administered:								

Cardiovascular Measurements - Data spreadsheet 1 (Exp.2)								
participant nr.								
Blood Pressure measurements (mmHg) - diastolic (DBP) and systolic (SBP)								
Time (minutes):	**DBP Reading 1**	**DBP Reading 2 (after 2minutes)**	**Average DBP**	**SBP Reading 1**	**SBP Reading 2 (after 2minutes)**	**Average SBP**	**pulse pressure (reading 1)**	**pulse pressure (reading 2)**
0								
10								
30								
60								
90								
120								
Beverage Administered:								

Appendix E

Socio-demographic questionnaire

Instructions:

Kindly complete all the questions by marking the appropriate block with an 'X' or by filling in your answer on the line provided.

For office use only

Participant nr.

1. How old are you?

   _____ years

2. What qualification are you currently registered for? State in the space provided below.

   _____

3. In which year of your studies are you currently registered for? Mark the relevant box with an 'X'

Year 1	
Year 2	
Year 3	
Year 4	
>year 4	

4. Have you ate or drank anything (except water) in the last 10 hours?

Yes	No

5. Have you participated in any strenuous exercise in the last 48 hours?

Yes	No

6. Do you have any allergies? If yes, please state them on the space provided below.

Yes	No

_____

7. Do you smoke?

Yes	No

7.1 If yes, please state how many cigarettes you've smoked in the last 24hours in the space provided below:

_____

8. Have you consumed any over the counter medication in the past 10 hours? This includes pain tablets, headache pills, etc.

Yes	No

8.1 If yes, please state the name (or type) of medication below:

_____

9. Are you currently using any prescribed medication?

Yes	No

10. Have you been diagnosed with any cardiovascular disease?

Yes	No

10.1 If yes, please indicate your diagnosis in the space provided:

_____

11. Will you kindly fill in your contact number and/or email address in the space provided below:

**this information is required strictly for the purpose to contact you as a reminder for your test sessions, as well as to give you updates on the research once completed. Details will be handled with confidentiality.

Contact number: _____

Email address: _____

Thank you for taking the time to fill in this questionnaire

Appendix F

The Use and Knowledge of Caffeine

Instructions

Mark the appropriate blocks with an X or write your answers on the line provided.
To change your answers, draw a line across the X.

For office use only

Participant nr:

1. Do you ever consume any caffeine-containing products? E.g. coffee, tea, Coca-Cola®, Bioplus®, Stay Awake®, Red Bull®, Play®, Monster®etc.

1	Yes	0	No	V1

If **YES** continue with Question 2.
If **NO** go straight to Question 7.

2. How frequently do you use **any** caffeine-containing products?
Only choose **one** option.

1	More than one serving in a day, every day of the week	V2
2	7 or more servings in a week on a weekly basis	
3	Less than 7 servings in a week on a weekly basis	
4	Sporadically e.g. occasionally before tests/exams, stay awake in class	

3. For which purpose(s) do you use caffeine?
You may choose more than one option.

1	Academic purposes e.g. to extend study time, stay awake in class	V3
2	Increase vigilance for driving e.g. long-distance driving	
3	Sport e.g. to enhance performance	
4	Social e.g. drinking coffee socially	
5	To recover from heavy alcohol consumption e.g. hangovers	
6	I like the taste e.g. enjoy the taste of coffee/tea/energy drinks	
7	Other, specify: _____	

Only answer Question 4 and Question 5 if you answered option (1) in Question 3.
If you do NOT use caffeine for academic purposes, go straight to Question 6.

4. Which of the following caffeine containing product(s) do you use for **academic purposes**?
You may choose more than one option.

1	Coffee (caffeinated)	V4
2	Tea / Iced tea (excluding Rooibos)	
3	Cool drinks e.g. Coca-Cola®, Mountain Dew®	
4	Energy tonics e.g. Bioplus®, Stay Awake®, Superboost®	
5	Energy drinks e.g. Red Bull®, Monster®	
6	Other, specify: _____	
7	Other, specify: _____	

5. How frequently do you use **any** caffeine-containing products for **academic purposes only**? Only choose **one** option.

1	More than one serving in a day, every day of the week	V5
2	7 or more servings in a week on a weekly basis	
3	Less than 7 servings in a week on a weekly basis	
4	Sporadically e.g. occasionally before tests/exams, stay awake in class	

Only mark the options you think are correct in Questions 6, 7 and 8.

6. Which of the following are benefits of caffeine consumption?

1	Caffeine has no health benefits	7	Increases risk for heart disease	V6
2	Increases alertness	8	Increases short-term memory	
3	Reduces risk for type 2 diabetes	9	Increases long-term memory	
4	Substitute for sleep	10	Prevents impotency	
5	Increases alcohol tolerance	11	Prevents Alzheimer's disease	
6	Reduces risk for Parkinson's Disease	12	Slows down metabolism	

7. Which of the following are side effects of caffeine?

1	Caffeine has no side effects	6	Slow beating of the heart	V7
2	Dry eyes	7	Increases beating of the heart	
3	Hot flushes	8	Acne	
4	Increases force of the heart	9	Increases stomach acids	
5	Impotence	10	Increases breathing rate	

8. Which of the following are withdrawal symptoms of caffeine?

1	There are no withdrawal symptoms	6	Constipation	V8
2	Headache	7	Irritability	

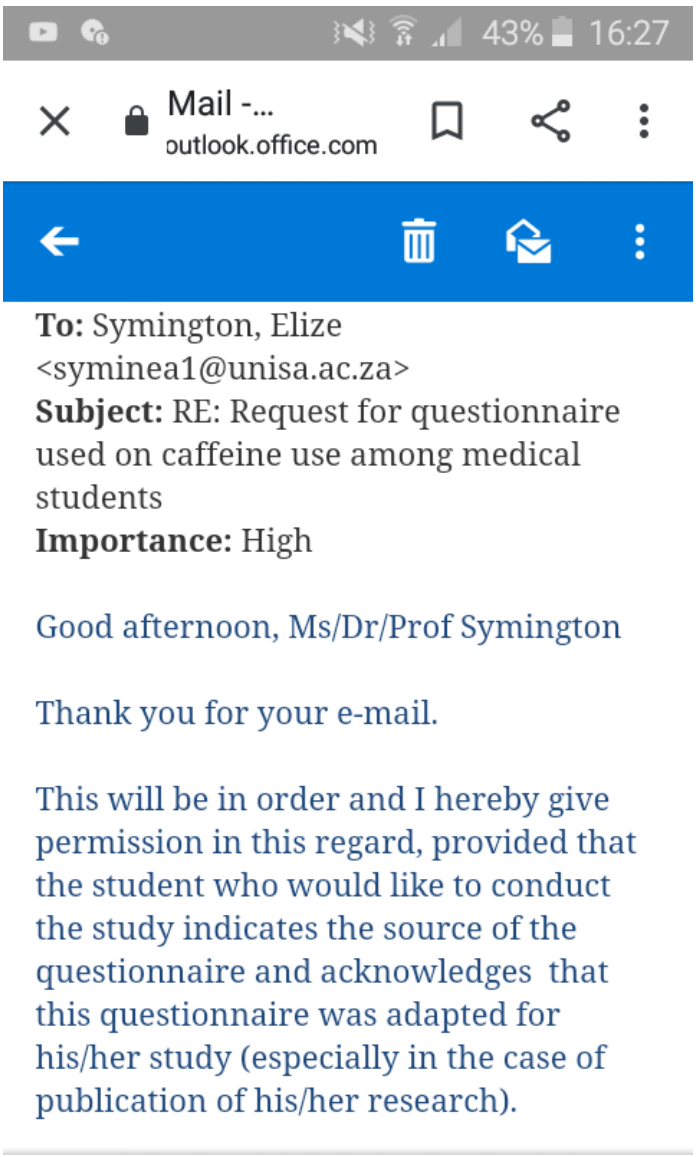
3	Fatigue	8	Decreased alertness
4	Nasal congestion	9	Drowsiness
5	Forgetfulness	10	Aggression

Thank you for completing the questionnaire.

Adapted and redesigned by Dr EA Symington (UNISA)
with permission from Dr. Carol Larson (UFS), March 2019

Appendix G

Proof of Permission to use adopted questionnaire



43% 16:27

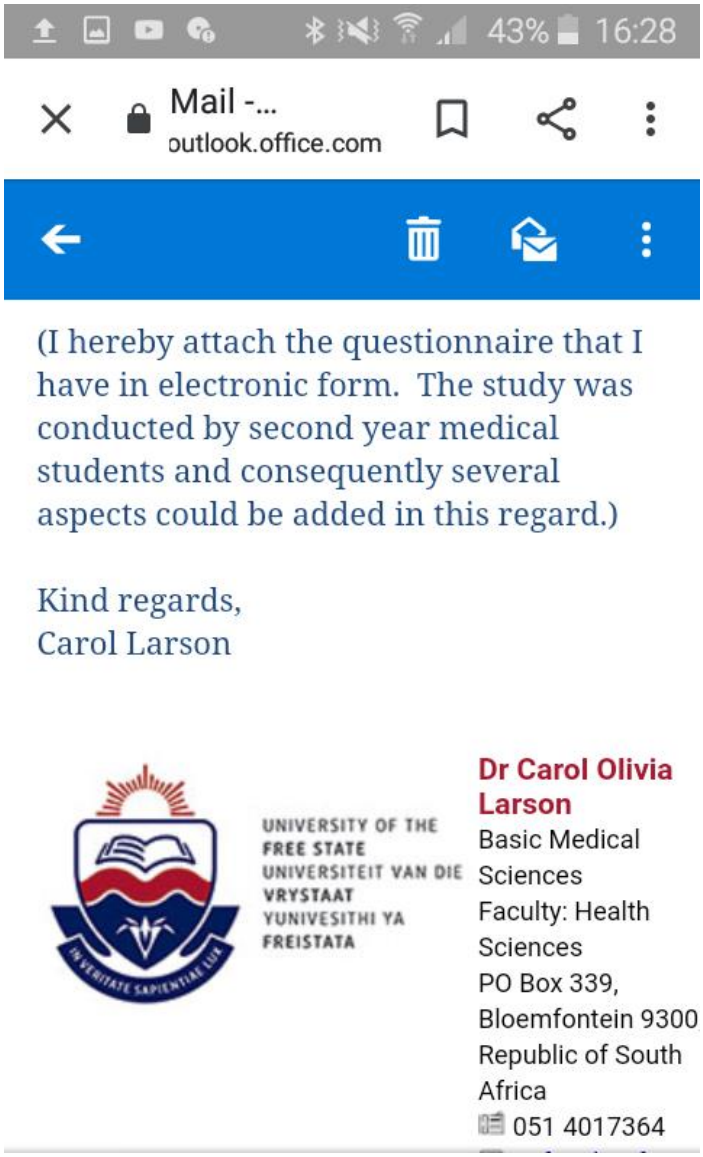
Mail -...
outlook.office.com

To: Symington, Elize <syminea1@unisa.ac.za>
Subject: RE: Request for questionnaire used on caffeine use among medical students
Importance: High

Good afternoon, Ms/Dr/Prof Symington

Thank you for your e-mail.

This will be in order and I hereby give permission in this regard, provided that the student who would like to conduct the study indicates the source of the questionnaire and acknowledges that this questionnaire was adapted for his/her study (especially in the case of publication of his/her research).



43% 16:28

Mail -...
outlook.office.com

(I hereby attach the questionnaire that I have in electronic form. The study was conducted by second year medical students and consequently several aspects could be added in this regard.)

Kind regards,
Carol Larson

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

Dr Carol Olivia Larson
Basic Medical Sciences
Faculty: Health Sciences
PO Box 339,
Bloemfontein 9300
Republic of South Africa
051 4017364

Appendix H

Permission Request Letter for UNISA clinic and nursing staff

Ayanda Nompumelelo Nyalela

UNISA MSc student and researcher

Student number: 54233550

Email 54233550@mylife.unisa.ac.za /Tel. 079 475 7764

Date:

Permission letter for conducting research at the University of South Africa (UNISA) Science campus-clinic and nursing staff assistance

With reference to previous conversations between the student, supervisors (Dr. C Myburgh and Dr. EA Symington), and the UNISA nursing staff, we kindly request your permission to conduct our research project in the UNISA Science campus health clinic, located on the ground floor of the NB Pityana building, for the following proposed dates:

- 23 March – 16 May, 2020 (intake 1)
- 8 June – 7 August, 2020 (intake 2)

The aim of our study is to determine the effects of acute energy drink (ED) exposure on the cardiovascular system of generally healthy, black, male university students in the context of potential risk of hypertension development. We will achieve this by evaluating changes in heart rate (bpm) and arterial blood pressure (systolic and diastolic, mmHg) before and 10, 30, 60, 90 and 120 minutes after the administration of EDs; comparing the effect of ED consumption on heart rate (bpm) and arterial blood pressure (systolic and diastolic, mmHg) to a placebo and; determining if any relationships exist between ED consumption and cardiovascular variables such as heart rate and blood pressure.

Using a quantitative, experimental research design, with a randomized, controlled, cross-over approach, black male students aged between 18-29 years will be recruited from the UNISA Science campus (n≥30). The sample group will undergo screening in the form of questionnaires, where data on dietary intake and medical history will be assessed. This will be followed by obtaining anthropometric measurements. The experimental procedure requires participants to ingest 500ml of an energy drink - *Monster, Mucho-Loco Flavour* (intervention) or a diluted (ratio of 1:3) carbonated fruit juice - *Magalies – Peach and Mango* with carbonated water (placebo), following a fasting period of 10 hours. Cardiovascular measurements will be

recorded by the research team using a blood pressure cuff for a total duration of 120 minutes per participant, in intervals of 30 minutes. A washout period of at least 6 days following the first assessment will be granted, where participants may proceed with normal day-to-day activities. After the washout period, participants are required to return for the second assessment period where the alternative beverage will be administered, and the same standard procedure of experiment will be followed.

Although energy drinks are unrestricted and freely available in South African local supermarkets, there is a small chance that participants may experience slight discomfort in the form of headaches, restlessness, jitters, nausea, heart palpitations, dizziness, sweating, an upset stomach or diarrhoea. Thus, the health clinic and supervision of the nursing staff are a necessity to ensure the safety of all participants, should any adverse events occur. We ask for permission for the following:

1. To set up our experiment in the UNISA clinic facility
2. The supervision of the nursing staff on an on-call basis
3. To have access to medical attention and facilities, should any adverse events occur in participants or research team

On behalf of the research team (fieldworkers), we are herewith requesting permission to conduct the research project in your department for the abovementioned reasons.

Ayanda Nompumelelo Nyalela, MSc student

Signature: _____ Date: _____

Dr. C Myburgh, supervisor

Signature: _____ Date: _____

Dr. EA Symington, co-supervisor

Signature: _____ Date: _____

UNISA nurse

Signature: _____ Date: _____

Appendix I

The College of Agriculture and Environmental Sciences (CAES) requires all fieldworkers to sign a confidentially agreement when executing certain specified fieldworker activities related to a research project that has been cleared by the CAES Human Research Ethics Committee.

FIELDWORKER CONFIDENTIALITY AGREEMENT

I, _____the undersigned who has been approached to act as a fieldworker in the following project:

The Effects of Acute Energy Drink Consumption on the Cardiovascular System of University Students

agree to assist the researcher with the following activities/duties:

1. Undergo the necessary training offered by the research team in preparation for recruitment, informed consent and data collection
2. The recruitment of potential participants on the UNISA Science campus through distributions of research pamphlets
3. To facilitate in setting up the experiment at the UNISA clinical facility
4. To facilitate in professional data collection by taking anthropometric and cardiovascular measurements using the experimental tools at given time intervals
5. To facilitate in the capturing, collection and submission of all paper work and data

I will, during my activities/duties as aforementioned, come into possession of certain confidential information.

This will certify that, during the execution of my duties/activities as fieldworker:

1. *I will treat all information related to the project, data I have collected obtained from participants/sources in writing or verbally, discussions between the researcher and fieldworker and any information shared with me regarding the identification of the participants/respondents in the strictest of confidence and will not reveal that information to any third party without prior written consent from the researcher.*
2. *I will not do or allow anything to be done which might compromise the interest of the researcher or the University of South Africa or any of the proposers in respect of any intellectual property rights flowing from the confidential information.*
3. *I will not use the information stipulated in paragraph 1 for any reason other than for the purpose I agreed upon.*

4. *I will not act as a fieldworker where a conflict of interest exists. Should there be doubt about an apparent conflict of interest, I will advise the researcher who will then indicate whether participation in the fieldwork process is permissible or not.*

THIS DONE AND SIGNED AT _____(place) on _____(date)

SIGNATURE _____