ORIGINAL ARTICLE

The Effect of Energy Drink on Histomorphological Changes in Skeletal Muscles of Wistar Albino Rats

Fatima Rehman[1], Zia Ul Islam[2], Lubna Faisal[3], Ayesha Mehwish[4], Sadia Rehman[5], Maryam Faiz Qureshi[6]

ABSTRACT

Objective: To observe the histomorphological alterations induced by consumption of energy drinks on skeletal muscles of Albino rats.

Study Design: An experimental study.

Place and Duration of Study: The study was conducted by the Department of Anatomy in collaboration with the animal house of Liaquat national hospital and medical college (LNH&MC), Karachi from November 2nd to December 1st 2020.

Materials and Methods: For the study, 30 adult male Wistar Albino rats weighing between 140 and 200 grammes were allocated evenly into 3 groups. Group A served as control, kept on a regular laboratory diet. Group B served as a low dose treated group receiving energy drink at a dose of 7.5ml/day while group C received a high dose of drink i-e 15ml/day via gastric tube. All the animals were sacrificed following completion of the experimental period and were subjected to microscopic examination for histo-morphological study. Data was analyzed by using SPSS version 25.

Results: The Hematoxylin and Eosin (H&E) stained sections revealed significant structural and parenchymal damage in skeletal muscle tissue. The regular parallel arrangement of skeletal muscle fibers was lost with the disappearance of nuclei. Dilated and congested blood vessels were observed in the treated tissues. Infiltration of mononuclear cells was also observed suggesting the inflammatory changes in the tissues of animals treated with caffeinated beverages in the present study.

Conclusion: The consumption of energy drinks produces a significant histo-morphological alteration in the skeletal muscles of Wistar Albino rats.

Key Words: *Congested Blood Vessels, Energy Drink, Loss of Nuclei, Mononuclear Cells, Skeletal Muscles.*

Introduction

Energy drinks (ED) are caffeinated beverages, consumed widely by athletes and sports-persons especially of younger age groups.[1] The basic purpose of their utilization is to gain instant energy for enhancing physical activity and cognitive performance. Other than caffeine, ED also contains taurine, ginko biloba, multivitamins, and a high content of glucose.[2] These beverages were first introduced in the market in the era of 1960 but a rapid boom in their production and consumption was seen in 1987 when they launched widely in Europe.[3]

The caffeinated beverages are marketed especially to attract the younger age groups.[4] They consume it to increase the attention span, augment the muscle strength, and as a source of mental stimulant .[5] With regular usage, the individual usually develops some degree of tolerance and needs to consume it at an even further higher quantity to acquire the desired effects. Similarly, its abrupt withdrawal leads to features like irritability, headaches, nervousness, and drowsiness.[6]

Caffeine is one of the major components of energy drinks. About 80 mg of caffeine is present in a 250 ml volume of energy drink.[7] Caffeine has got effects on many organ systems of the body. It has a stimulating effect on the nervous system, increases gastrointestinal motility, produces diuresis, and causes tachycardia with palpitation. Moreover, high content of sugar in it can cause obesity and increases

[1,2,3]*Department of Anatomy*
Liaquat National Hospital and Medical College, Karachi
[4]*Department of Anatomy/Biochemistry*[5]
Baharia University of Health Sciences, Karachi
[6]*Department of Medical Sciences Education*
Western University California, USA

Correspondence:
Dr. Fatima Rehman
Assistant Professor Anatomy
Liaquat National Hospital and Medical College, Karachi
E-mail: fatimakureshi@hotmail.com

Received: August 02, 2023; Revised: December 11, 2023
Accepted: December 14, 2023

https://doi.org/10.57234/jiimc.december23.1779

the chances of type -2 diabetes if consumed excessively and for a longer period.[8] Literature has revealed that the use of ED prolongs the period of skeletal muscle contraction, increases endurance performance, and thus delays process of fatigability.[9] The musculoskeletal system is one of the major organ systems of the body, composed up of a bony framework with skeletal muscle attached to it.[10] The stimulant effect of caffeine has been shown to affect the voluntary skeletal tissues of the body. It increases the endurance while performing a physical activity by reducing the perceiving effect. This leads to sustained performance which can contribute to muscle fatigue and cramps.[11]

The risks of heavy consumption of ED among young individuals and especially athletes to improve their performance in various capacities have become a serious concern nowadays that can lead to significant health problems in the future. Still the damage to skeletal muscles at microscopic level caused by caffeinated beverages is yet to be discovered. The present study was therefore aimed to identify the histomorphological alterations induced by the intake of ED in the Wistar albino rats.

Materials and Methods

This laboratory-based experimental study was conducted in the department of Anatomy in alliance with the animal house of Liaquat National Hospital and Medical College (LNH&MC), Karachi from November 2nd to December 1st'2020. The ethical approval for the required experimentation was sought from the ethical committee of LNH&MC (App # 0529-2020-LNH-ERC).

In the current investigation, 30 mature male Wistar Albino rats weighing between 140 and 200 gramms were used. The institution's policies were followed while handling the animals. They were kept under close watch for a week prior to the commencement of the trial to gauge their health. They were given a laboratory animal diet and unlimited access to water ad libitum. General conditions and behavior of rats were noted throughout the study. The study utilised a popular energy drink that is sold in 250 ml cans in Pakistan. It was bought from a local supermarket of Karachi, Pakistan. A single serving of drink contained about 80mg of caffeine, 37g of sugar and 1000mg of Taurine, in addition to other constituents. The drink was administered orally via 22-guage plastic-made flexible feeding tube in addition to the normal feed of the animals.The rats were divided into three groups each having ten animals. Group A served as control group (CG). Group B served as Low-dose treated group (LDTG), received 7.5ml of ED[12], while group C served as High-dose treated group (HDTG), received 15 ml ED[12], which is the maximum tolerable dose for the experimental animals as mentioned in the literature[13] administered daily for four weeks.

All the animals were anesthetized and later dissected following the completion of the experimental period. The bodyweight of rats was measured before the start and at the end of the study by using the OHAUS pioneer model# PA214 (made in the USA). The process of dissection and sample collection was done in the dissection hall, Anatomy department, LNH&MC.

To see the effect of ED on the histo-morphological changes in skeletal muscles, the Achilles' tendons including the whole muscle belly were used in the current study. They were carefully dissected out bilaterally from lower limbs, rinsed with normal saline, and fixed in10% buffered formalin, and further processed to get paraffin blocks. Tissue sections of 4µm thickness were sliced with a microtome and stained with H& E to observe the morphology and cytoarchitecture of the muscle tissue. Each slide was observed under the light microscope (Olympus model # CX21FS1) at 100X and 400X magnifications at four non-overlapping randomly selected fields. All histopathological (HP) changes were analyzed by detailed microscopic examination which was graded as none, mild, moderate and severe. Based on the findings, if HP changes were noted in one field, they were labeled as mild, in two fields as moderate, and three or four fields as severe.[12]

Data were analyzed by using SPSS version 25. Paired T test was used to assess the comparison of initial and final body weight in different study groups. Fischer's exact T-test was applied to observe the comparison of histopathological changes within study groups P-value equal to or less than 0.05 was considered as statistically significant for all the variables of the current study. The statistical significance of microscopic observations of the data related to microscopic observations (HP chages in skeletal muscle) was sought by applying the Chi-

square test. Results were displayed in frequency tables.

Results

The results of the present study revealed that the mean value of the initial body weight control group was 168.75±8.53 while the mean value of final body weight was 173.50±4.93. There was no significant gain in body weight of the control group was recorded (P–value=0.095). The mean value of the initial body weight of low dose treated rats was found to be 161.50±10.85 while the final body weight's mean value was decreased to 152±11.05. Therefore the reduction in the bodyweight of rats was observed in low dose energy drink animals (p-value 0.001). This trend was also observed in the high dose treated group where the initial body weight's mean value was found to be 185.38±12.46 while the final body weight's mean value was 172.00±13.51 (p-value 0.012) (Table-I).

An in-depth qualitative analysis was performed on 4µm sections stained with Hematoxylin and Eosin (H&E) to look for the following pathological changes: vascular congestion, skeletal muscle disarray, and mononuclear infiltration. The tissues were graded as none, mild , moderate and severe on the basis of severity of the above mentioned histopathological parameters.

The longitudinal section of the control group showed the regular pattern of skeletal muscle fibers arranged in parallel bundles with peripherally arranged nuclei separated by perimyseal connective tissue. Small blood vessels and capillaries were present in connective tissue lining surrounding muscle fibers with no sign of engorgement (Figure-1). However, in low dose treated animals moderate (60%) degree of vascular congestion was observed with dilatation and engorgement of the vessels. Moderate (20%) to severe (40%) congestion was observed in high-dose treated animals with some sections demonstrating Rouleaux formation and few diffusely present hemosiderin-laden histiocytes in perimysial connective tissue (Table-II, Figure-2).

It was determined by data in our study, a generalized feature of skeletal muscle fiber disarray was observed in most of the slides of low and high-dose treated animals however no skeletal muscle disarray was observed in any animal of the control group. Mild disarray was observed in the low dose treated group (40%) while half (50%) of the total number of high dose treated animals displayed severe loss of regular alignment of muscle fibers (Figure-3) . Splitting of the muscle fibers was also observed which appeared as a separation in the individual muscle fiber giving a patchy distribution. The nuclei were also completely absent at some fields of the microscopic sections (Figure-2). Mono-nuclear cell infiltration was also observed in both low and high-dose treated animals. None of the animals in the control group exhibited any inflammatory infiltrate while the moderate influx of inflammatory cells was revealed in 40% of the low dose treated group. The animals treated with a high dose of caffeinated beverages showed moderate (40%) to severe (20%) invasion of mononuclear cells predominantly lymphocytes (Table-II).

Table I: Comparison of Initial and Final Body Weight in Different Study Groups

	Initial body weight	Final body weight	P-value
Group A (control)	168.75±8.53	173.50±4.93	0.095**
Group B (LDTG)	161.50±10.85	152±11.05	<0.001*
Group C (HDTG)	185.38±12.46	172.00±13.51	0.012*

Paired t-test is applied.
*Significant at p-value ≤0.05
**Insignificant at p-value >0.05

Table II: Comparison of Histopathological Changes within Study Groups (A, B And C)

Histo-pathological Changes	Group A	Group B (LDTG)	Group C (HDTG)	P-value
Vascular Congestion				0.031*
None	10(100%)	2(20%)	1(10%)	
Mild	0(0%)	2(20%)	1(10%)	
Moderate	0(0%)	6(60%)	2(20%)	
Severe	0(0%)	0(0%)	4(40%)	
Skeletal Muscle Disarray				0.002*
None	10(100%)	4(40%)	0(0%)	
Mild	0(0%)	4(40%)	2(20%)	
Moderate	0(0%)	2(20%)	3(30%)	
Severe	0(0%)	0(0%)	5(50%)	
Mono nuclear infiltration				0.152**
None	10(100)	3(30%)	0(0%)	
Mild	0(0%)	3(30%)	4(40%)	
Moderate	0(0%)	4(40%)	4(40%)	
Severe	0(0%)	0(0%)	2(20%)	

Fisher Exact t-test is applied.
*Significant at p-value ≤0.05
**Insignificant at p-value >0.05

https://doi.org/10.57234/jiimc.december23.1779

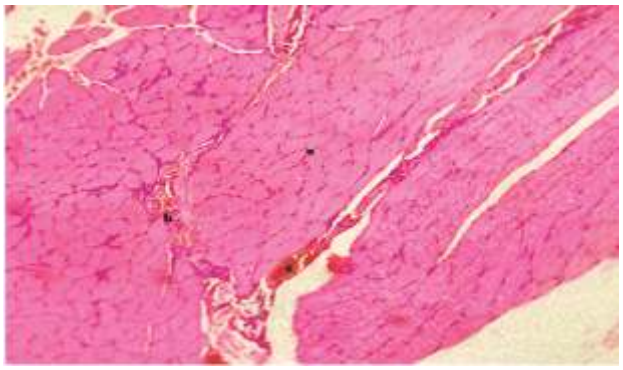

Figure 1: Group A (CG) at100x magnification a:in this H&E-stained, transverse (left field) and longitudinal (right field) sections of the skeletal muscle, numerous muscle fibers in the form of fascicles are seen in which dark stained basophilic nuclei are found to be peripherally arranged.b:the perimysium (loose connective tissue surrounding the bundles of muscle fibers) is also visible at this magnification.c:small capillaries are running in-between the muscle fibers.

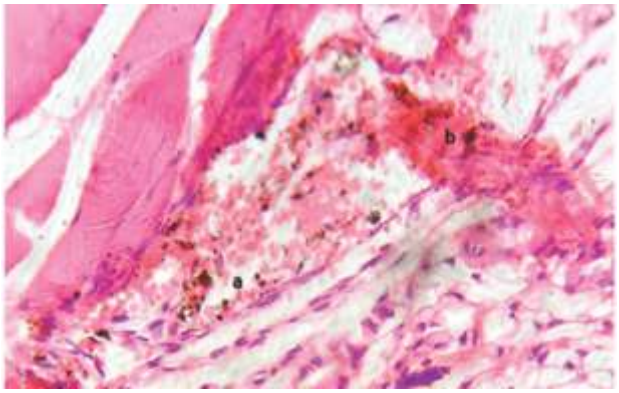

Figure 2: In Group C (HDTG) at 400 X magnification *a.* In this H&E stained-tissue, hemosiderin laden histiocytes with inflammatory infiltrates are seen *b.* an engorged and dilated blood vessel can also be be seen (left field)

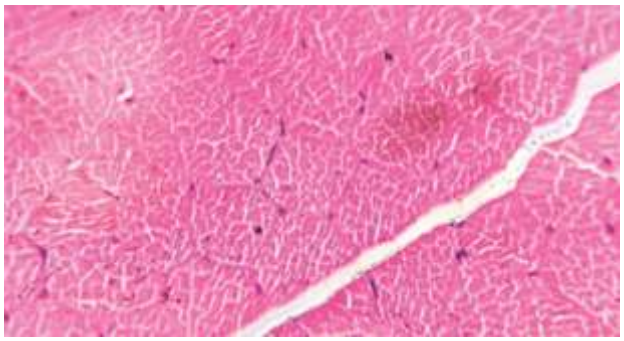

Figure 3: In Group B (LDTG) at 100 X magnification a. In this H&E stained-tissue section, muscle fiber disarray is noted. b: A loss of orientation and splitting in the regular parallel arrangement of skeletal muscle fibers were also observed. The fibers had a patchy distribution with the loss of cross-striations and peripheral nuclei.

Discussion

The present study was aimed to investigate the histo-morphological changes induced by energy drink consumption of Wistar Albino rats. Both heads of gastrocnemius along with soleus were identified and dissected from their point of origin till the insertion of the Achilles' tendon and were observed under the light microscope after processing of tissue.

A report titled “additional information on energy drink published by European Scientific Committee on food” in 2003[14] described the substantial reduction in body weight of experimental mice when they treat with energy drink (Red bull) to evaluate the oral toxicity of the drink. These findings are in agreement with the present study where a significant reduction in body weight was also observed in energy drink-treated animals. This outcome can be explained based on poor generalized well-being of the animals, impaired glucose tolerance, and thermogenic effect of caffeine.[15]

Skeletal muscle is a vital tissue needed for the maintenance of physical independence and quality of life across the lifespan. The preservation of normal cytoarchitecture of skeletal muscles is vital as its loss can contribute to the loss of functional capacity of an individual.[16]

At present, the consumption of caffeinated beverages among youngsters is increasing to boost up their stamina and attain vitality. Long-term consumption of energy drinks significantly decreases the fat content in the skeletal muscles stored in the form of triacylglycerols leading to a decrease in strength, metabolic rate, and aerobic capacity and thus, in functional capacity[17], which may affect the young generation producing musculoskeletal disorders at an early age.

The microscopic observations in the present study were found to be more remarkable in animals treated with higher doses of caffeinated beverages as compared to low dose treated groups. Skeletal muscle disarray, mononuclear infiltration, and vasodilatation were served as a part of the examination. A loss of orientation and splitting in the regular parallel arrangement of skeletal muscle fibers were observed. The fibers had a patchy distribution with the loss of cross-striations and peripheral nuclei. These findings were concomitant

with the observations made by Alotaibi et al[18], who treated the skeletal muscles of rats with varying doses of caffeine and nicotine and found structural damage in the treated tissues as loss of striations, absence of nuclei with degenerative changes. These alterations could be a result of an adaptive response of the affected tissue due to a lack of oxygen and nutrient supply in response to inflammation.

Oxidative stress is a major attributing factor in producing the signs of inflammation. The generation of reactive oxygen species (ROS) induces a pro-oxidant environment which results in cellular dysfunction and tissue injury.[19] Scientific data has proved that consuming energy drinks leads to the generation of ROS in the body and thus results in pathological events.[20] Damage to the muscle tissue leads to leakage of inflammatory mediators in the parenchyma of tissue. The current study also revealed the presence of mononuclear infiltrates predominantly lymphocytes scattered in myofibrils of the treated tissues demonstrating the inflammatory changes.

Hyperemia is one of the earliest features of inflammation characterized by vasodilatation and congestion of vessels.[21] This outcome was also observed in the present study where a dose-dependent increase in the vascular congestion. These results are in agreement with the study conducted by Abd-El-Halim et al[22] when they observed the effect of acrylamide on tongue musculature of Albino rats and observed congested blood vessels with the fragmentation of myofibers. These findings can be an attribute of an inflammatory rection resulting in collagen deposition and vascular re-epithelialization.

Based on the findings of the present work, it can be stated that exposure to caffeinated beverages can lead to damage to skeletal muscle tissues, therefore its intake needs to be regulated. The present study was one of its kind in the fact that very limited work has yet been done on the effect of caffeinated drinks on skeletal muscles. Large scale studies should be planned in future in order to devise precautionary strategies against the harmful effects of these drinks.

Conclusion

In conclusion, data of the present study indicated the significant histo-morphological alterations were present on the microscopic examination of skeletal muscle tissue as a result of consuming the energy drinks. Caffeine was the most blamed element and aided by other ingredients of the drinks having a stimulating effect.

Future Recommendations

Further researches on a larger scale using different muscular tissues of the body should be encouraged to make a comparative analysis. Investigations regarding the optimal dosage of consumption of these beverages as well as the reversibility of the effects would also be essentially addressed on a large scale study in future.

Conflict of Interest

No conflict of interest has been declared by any author in the current study.

Funding Source

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Soós R, Gyebrovszki Á, Tóth Á, Jeges S, Wilhelm M. Effects of Caffeine and Caffeinated Beverages in Children, Adolescents and Young Adults: Short Review. Int J Environ Res Public Health. 2021;18(23):12389. doi: 10.3390/ijerph182312389.
2. Hladun O, Papaseit E, Martín S, Barriocanal AM, Poyatos L, Farré M, Pérez-Mañá C. Interaction of Energy Drinks with Prescription Medication and Drugs of Abuse. *Pharmaceutics*. 2021; 13(10):1532. doi: 10.3390/pharmaceutics13101532.
3. Cruz-Muñoz V, Urquizu-Rovira M, Valls-Ibañez V, Manresa-Domínguez J-M, Ruiz-Blanco G, Urquizu-Rovira M et al. Consumption of soft, sports, and energy drinks in adolescents. The BEENIS study. An Pediatr (Barc). 2020;93:242–250. https://doi.org/10.1016/j.anpede.2020.01.010.
4. Miller KE, Dermen KH, Lucke JF. Caffeinated energy drink use by U.S. adolescents aged 13-17: A national profile. Psychol Addict Behav. 2018;32(6):647-659. doi: 10.1037/adb0000389.
5. Khan AA, Ali T, Imran M, Ali SA and Khan M. Understanding of health risks by the use of energy drinks among millennial generation of Karachi, Pakistan. Vol. 5, No. (3) 2017, Pg. 247-256. DOI : https://dx.doi.org/10.12944/CRNFSJ.5.3.09.
6. Borlu A, Oral B, Gunay O. Consumption of energy drinks among Turkish University students and its health hazards. Pak J Med Sci. 2019;35(2):537-542. doi: 10.12669/pjms.35.2.638.
7. Benson S, Tiplady B, Scholey A. Attentional and working memory performance following alcohol and energy drink: A randomized, double-blind, placebo-controlled, factorial design laboratory study. PLoS ONE.2019; 14(1):1-17. DOI:

10.1371/journal.pone.0209239.
8. Chandio ZA, Siddiqua A, Khaskheli MI, Waghani A, and Metlo WA. Review effect of caffeine overdose. RADS J Biol Res Appl Sci. 2020;11(2): 154-158. DOI: https://doi.org/10.37962/jbas.v11i2.266.
9. Erdmann J, Wiciński M, Wódkiewicz E, et al. Effects of Energy Drink Consumption on Physical Performance and Potential Danger of Inordinate Usage. Nutrients. 2021; 13(8):2506. doi: 10.3390/nu13082506.
10. Kerkman JN, Daffertshofer A, Gollo L L, Breakspear M, Boonstra TW. Network structure of the human musculoskeletal system shapes neural interactions on multiple time scales. Sci. Adv. 2018; 4(6):1-11. DOI: 10.1126/sciadv.aat0497.
11. Barcelos PR , Lima FD, Carvalho NR, Bresciani G, Royes LF. Caffeine effects on systemic metabolism, oxidative-inflammatory pathways, and exercise performance. Nutrition Research.2020; 80:1-17./doi.org/10.1016/j.nutres.2020.05.005.
12. Rehman, F., Islam, Z., Hameed, U., & Rehman, S. The effect of energy drink on the pancreas of wistar albino rats – a microscopic study. PAFMJ. 2020; 70(2):524-28. https://www.pafmj.org/index.php/PAFMJ/article/view/4223.
13. Al Sammak M, Qassim AH, Hamdi OR. Histological Changes of Stomach and Intestine Induced by Energy Drink (Tiger) in Adult Male Rats. Open Access Maced J Med Sci. 2021 Sep. 8;9(A):735-40. DOI: https://doi.org/10.3889/oamjms.2021.6617 https://ec.europa.eu/food/system/files/2020-12/sci-com_scf_out169_en.pdf .
14. Harpaz E, Tamir S, Weinstein A & Weinstein Y. The effect of caffeine on energy balance. Journal of Basic and Clinical Physiology and Pharmacology, 2017;28(1): 1-10. DOI: 10.1515/jbcpp-2016-0090 .
15. Alamilla RA, Paulussen KJM, Askow AT, Burd NA,. Chapter 3 - Dietary approaches to maintaining muscle mass. Sarcopenia. Elsevier.2021;81-107. https://doi.org/10.1016/B978-0-12-822146-4.00003-X.
6. Chu H, Zhou W, Wang S. Effect of Sports Energy Drink on Fat Metabolism and Weight Loss of College Students. Journal of Food Quality, 2022; 1-8 . https://doi.org/10.1155/2022/3978964.
17. Alotaibi F, Abounasef SK, Fikry H. Effects of Combined Nicotine and Caffeine on the Rat Skeletal Muscles: A Histological and Immunohistochemical Study. J Microsc Ultrastruct. 2019;7(4):147-152. doi: 10.4103/JMAU.JMAU_1_19.
18. Behl T, Makkar R, Sehgal A, Singh S, Sharma N, Zengin G, et al. Current Trends in Neurodegeneration: Cross Talks between Oxidative Stress, Cell Death, and Inflammation. *International Journal of Molecular Sciences.* 2021; 22(14):7432. doi: 10.3390/ijms22147432.
19. Valle MTC, Couto-Pereira NS, Lampert C, Arcego DM, Toniazzo AP, Limberger RP, et al.Energy drinks and their component modulate attention, memory, and antioxidant defenses in rats. Eur J Nutr 2018;57: 2501–2511. DOI: 10.1007/s00394-017-1522-z.
20. Valerio de Mello Braga LL, Simão G, Schiebel CS, Caroline dos Santos Maia A, Turin de Oliveira NM, Barbosa da Luz B, Corso CR, et al. Rodent models for anticancer toxicity studies: Contributions to drug development and future perspectives. Drug Discovery Today. 2023; https://doi.org/10.1016/j.drudis.2023.103626.
21. Abd el-halim NY, Sofii MM, Mohamed EAM, and Nasralla MM. Effect of Acrylamide on the Tongue Musculature of Adult Male Albino Rat and the Possible Protective Role of Vitamin E: Light and Electron Microscopic Study. Med. J. Cairo Univ. 2019; 87(3): 1933-1944. DOI:10.21608/MJCU.2019.54050.

CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors have declared no specific grant for this research from any funding agency in public, commercial or nonprofit sector.

DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.