Original Article

Dietary boron intake does not change the ischemic tolerance and preconditioning response in isoproterenol-induced myocardial injury in healthy rats

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Aim: Boron is important for many enzymes, most of which also have a role in the ischemic tolerance of the myocardium. We aimed to evaluate the influence of dietary boron intake on ischemic tolerance in a rat model.

Materials and methods: Rats were divided into 3 groups according to the ischemia protocol: Sham (S) (n = 20 total), ischemia only (Isc-only) (n = 40 total), and remote ischemic preconditioning + ischemia (RIPC+Isc) (n = 40 total) groups. These 3 groups were further divided into 4 according to the amount of daily dietary boron (<1 mg/kg, 2 mg/kg, 13 mg/kg, and 100 mg/kg). After 4 weeks of this diet, the rats were operated on, and baseline hemodynamic data and postoperative blood serum were obtained.

Results: Troponin I (Tn I) levels were lower in the RIPC+Isc group than in the Isc-only group (P < 0.01), whereas Tn I levels were not different among the dietary groups (P = 0.725). The Tn I levels were higher in the RIPC+Isc and in the Isc-only group than in the S group (P < 0.001 and P < 0.001, respectively).

Conclusion: Boron in diets did not influence the extent of myocardial injury in the groups. Furthermore, dietary boron does not deteriorate the response of the most potent endogenous mechanism of the heart against ischemia.

Key words: Boron, ischemia, ischemic preconditioning, cardioprotection, murine model, myocardial injury

Introduction

Throughout the world, ischemic heart disease is the leading cause of mortality and morbidity (1). Much effort is being spent on understanding and improving the mechanisms that enhance the ischemic tolerance of the heart. In this particular study, we hypothesized that boron may be a part of the endogenous protective responses of the ischemic myocardium, due to the fact that boron, a widely accessible trace element, is important in the function of many different enzymes, such as steroid hormones, oxidoreductases, and hydroxylases, most of which also have a role in the ischemic tolerance and the ischemic preconditioning response of the myocardium (2). However, there are no data on the effects of boron on ischemic myocardium, neither in humans nor in animal models. The aim of this study is to evaluate the influence of dietary boron intake on

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ischemia and ischemic preconditioning response in an experimental rat model. After an ischemic episode, a temporary loss of contractile function occurs, a process referred to as 'stunning' (3). Repetitive periods of ischemia on stunning myocardium lead to a prolonged postischemic left ventricular (LV) dysfunction known as hibernation (4). Hibernation is actually an endogenous protective mechanism, which is thought to occur via altered flow/function relation, metabolic adaptation, alteration of Ca^{2+} homeostasis, changes in mechanisms of cell death, or autophagy (5). Because quality of life and mortality are closely related to the amount of functional myocardium after ischemia, anything that enhances the ischemic tolerance of the heart is of great clinical importance (6). Ischemic preconditioning, a term used to define the diminished degree of vulnerability of the heart to prolonged ischemic episodes after brief, repetitive periods of ischemia, is by far the most powerful endogenous protective mechanism against ischemic insult (3). Boron has been accepted as a trace element for plants since 1923 (7). The importance of modulator effects of boron on animal systems was discovered in the late 1980s (8). Despite its being known as a cofactor of 26 different enzymes regulating energy metabolism, steroid hormones, immune system, coagulation system, hydroxylation, and oxidation–antioxidation reactions (2), very little is known about the influence of boron on cardiovascular system hemodynamics. Isoproterenol (ISO), a synthetic catecholamine and beta-adrenergic agonist, is a well-known agent and is widely used to induce myocardial injury in experimental models (9,10). For the first time in medical literature, we aimed to study the effects of dietary boron on the extent of injury and the remote ischemic preconditioning response in an ISO-induced myocardial injury model.

Materials and methods

Study design

The present study was a prospective, controlled animal laboratory study.

Animals

Male Sprague–Dawley rats, 14–16 weeks of age (250–500 g), were used for the experiments. The experiments were conducted in parallel to the ethical norms of the local ethics committee. All animals were fed with a special basal diet and distilled water, and a certain amount of supplementary boron was given with orogastric gavage, according to the experimental group. The animals were kept in standard laboratory conditions under natural light and dark cycles. They were free to access water and food during the experimental period.

Study protocol

Male Sprague–Dawley rats, 14–16 weeks of age (n = 100), were divided into 3 groups according to the ischemia protocol: Sham (S) (n = 20 total), ischemia only (Isc-only) (n = 40 total), and remote ischemic preconditioning + ischemia (RIPC+Isc) (n = 40 total) groups. These 3 groups were further divided into 4 groups according to the amount of boron supplied in their daily diet (<1 mg/kg, 2 mg/kg, 13 mg/kg, 100 mg/kg) (S group: n = 20 total, 5 for each diet group; Isc-only group: n = 40 total, 10 for each diet group; RIPC+Isc: n = 40, 10 for each diet group). The "boron < 1 mg/kg" group was given saline, and the other groups (boron = 2 mg/kg, 13 mg/kg, and 100 mg/kg) were given additional supplementary boron by orogastric gavage. After 4 weeks of the diet, the experiments were conducted. Fourteen rats died before the experiments started. Ten of these 14 rats were from the 100 mg/kg boron group, 3 rats belonged to the 13 mg/kg group, and 1 was from the group receiving <1 mg/kg boron. We weighed all the animals regularly and observed weight loss, fatigue, and decreased appetite in some of the animals receiving 100 mg/kg boron. We think that this observation, which was consistent with the symptoms seen in boron toxicity such as decreased appetite, weight loss, and the deaths in the 100 mg/kg boron group, might be associated with excess boron intake. For the animals that died, we performed autopsies and we did not detect any remarkable findings.

Following the administration of anesthesia using ketamine–xylazine, animals were placed on heating pads. The core temperature was measured using a rectal probe and was maintained at 37 °C. The right carotid artery was cannulated, and a Millar microtip pressure–volume conductance catheter was placed into the left ventricular apex in order to take hemodynamic data. After hemodynamic stabilization, the S group was vehicle-operated; in the Isc-only group, 150 mg/kg ISO, administered

subcutaneously (s.c.), was used to induce myocardial ischemia, and in the RIPC+Isc group, after 3 sets of 5 min of ischemia/reperfusion via femoral artery to induce RIPC, 150 mg/kg ISO was administered s.c. In all groups, hemodynamic data were collected at baseline. Four hours after ISO administration, blood samples were obtained and animals were euthanatized. During experiments, 15 rats had a mean arterial pressure (MAP) of <70 mmHg for more than 10 min or developed sustained ventricular tachycardia (VT)/ventricular fibrillation (VF) and, therefore, were excluded from the study.

Drugs and chemicals

Rats were fed with a special basal diet and distilled water with reduced boron content (<1 mg/kg) (Bil-Tek Ltd., Ankara, Turkey) (Table 1). The boron analysis of the basal diet was performed at the METU and TÜBİTAK MAM laboratories. Supplementary boron was provided by Hacettepe University (Ankara, Turkey) as boric acid. ISO was purchased from Sigma Chemicals (Germany).

Hemodynamic measurements

By the aid of the data gathered with the Millar microtip pressure–volume conductance catheter, heart rate (HR), LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), MAP, maximum pressure development (dP/dtmax) and minimum pressure development (dP/dtmin), ejection fraction (EF), stroke volume (SV), end-diastolic volume (EDV), end-systolic volume (ESV), and stroke work (SW) were calculated with LabChart 7.2 software (AD Instruments, UK) for the comparison of baseline values among groups. To correct for cardiac mass volume, saline calibration was done and parallel conductance volume (Vp) was calculated. Cuvette calibration was performed to turn the raw signal acquired by the conductance catheter into absolute volume.

Table 1. Chemical composition of basal diet (Bil-Tek Ltd., Turkey).

Analysis	%	Vitamins	/kg	Minerals	/kg
Dry matter	93.00	Vitamin A	75,000 IU	Manganese	400 mg
Crude protein	45.00	Vitamin D$_3$	20,000 IU	Iron	300 mg
Crude cellulose	3.00	Vitamin E	500 mg	Zinc	300 mg
Crude ash	10.00	Vitamin K$_3$	12.5 mg	Copper	25 mg
Crude lipid	2.00	Vitamin B$_1$	12.5 mg	Iodine	5 mg
Methionine	2.00	Vitamin B$_2$	35 mg	Cobalt	1 mg
Lysine	3.50	Niacin	300 mg	Selenium	0.75 mg
Calcium	2.50	Cal. D-pantothenate	60 mg		
Phosphorus	2.00	Vitamin B$_6$	20 mg		
Cystine	0.50	Vitamin B$_{12}$	100 mg		
Potassium	1.60	Folic acid	5 mg		
Linoleic acid	1.00	D-biotin	0.5 mg		
Tryptophan	0.50	Choline chloride			
Sodium	0.10				
Meth+Cystine	2.50				
Arginine	3.0				
Threonine	1.60				
Metabolic energy (kcal/kg): 2750					

Biochemical assays

For the detection of the extent of myocardial necrosis, troponin I (Tn I) levels were measured with a Centaur XL autoanalyzer (Siemens, USA). The measurements were expressed as ng/mL for Tn I (normal range: 0.00–0.99 ng/mL; interassay and total % covariance values were 2.1 and 2.9, respectively).

Statistical analysis

After normality tests were conducted, results were expressed as median ± interquartile range (IQR) for all groups. Statistical analysis was performed with SPSS 17.0 (SPSS Inc., USA). For the hemodynamic parameters and Tn I results, comparison between the groups was done using the Kruskal–Wallis test. Further analysis of the difference between 2 groups was done using the Mann–Whitney U test with Bonferroni correction. P < 0.05 was accepted as significant for comparison between all groups, and P < 0.016 was accepted as significant for comparison between 2 groups.

Results

Analysis of the data gathered with the Millar microtip pressure-volume conductance catheter revealed that there were no significant differences among groups in terms of LV systolic indices (Table 2). The Tn I levels were significantly lower in the RIPC+Isc group than in the Isc-only group (23.3 ± 8.2 vs. 28.8 ± 9.3; P < 0.01) (Figure 1), whereas Tn I levels were not different among the diet groups (24.4 ± 27.8 vs. 21.6 ± 25.9 vs. 26.7 ± 24.5 vs. 22.9 ± 14.2; P = 0.725) (Figure 2 and Table 3). The Tn I levels were significantly higher in the experimental groups than in the S group (23.3 ± 8.2 vs. 0.64 ± 0.6, P < 0.001; 28.8 ± 9.3 vs. 0.64 ± 0.6, P < 0.001) (Figure 1).

Discussion

According to our results, for the first time in the medical literature, it is shown that the amount of dietary boron does not affect the baseline LV systolic indices or the extent of myocardial injury

Table 2. Baseline hemodynamic values of dietary groups.

	< 1 mg/kg (n = 19)	2 mg/kg (n = 18)	13 mg/kg (n = 20)	100 mg/kg (n = 14)
HR, bpm	377 ± 19	395 ± 21	374 ± 38	387 ± 24
MAP, mmHg	100 ± 12	99 ± 15	97 ± 15	93 ± 11
LVSP, mmHg	122 ± 15	131 ± 18	118 ± 19	127 ± 7
LVEDP, mmHg	11 ± 5	10 ± 5	12 ± 4	8 ± 6
EF, %	55 ± 7	59 ± 7	52 ± 7	54 ± 7
LV dP/dtmax, mmHg/s	9149 ± 1141	8850 ± 1006	10,115 ± 1240	8495 ± 1855
LV dP/dtmin, mmHg/s	9467 ± 1309	9752 ± 1383	8900 ± 1543	9504 ± 1416
EDV, μL	308 ± 41	270 ± 46	287 ± 45	303 ± 31
ESV, μL	221 ± 42	211 ± 38	244 ± 19	233 ± 25
SV, μL	138 ± 8	139 ± 12	129 ± 15	133 ± 15
SW, mmHg × μL	12,221 ± 1109	10,841 ± 1798	13,022 ± 1671	11,394 ± 1881

dP/dtmax, maximum pressure development; dP/dtmin, minimum pressure development; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HR, heart rate; LVSP, maximal LV pressure; LVEDP, LV end-diastolic pressure; MAP, mean arterial pressure; SV, stroke volume; SW, stroke work.

All values are expressed as median ± IQR. Comparison between all groups was done with the Kruskal–Wallis test and there were no significant differences between groups in any of the parameters (P > 0.05 for all).
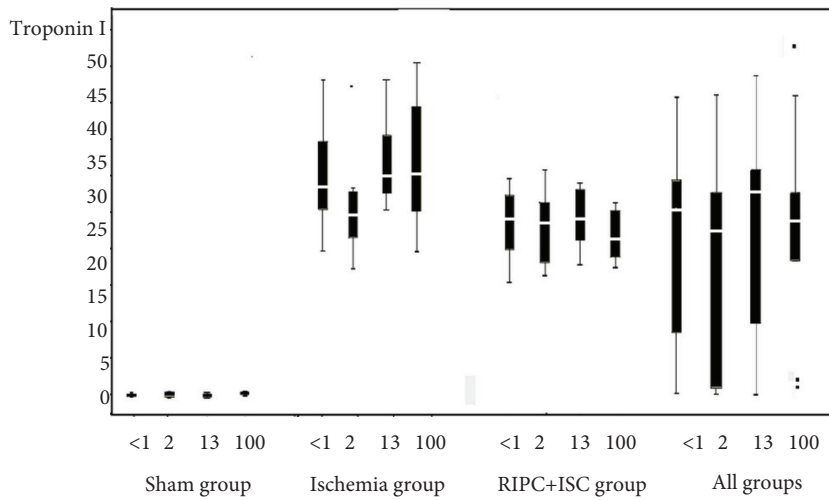
Figure 1. The mean troponin I levels between groups according to ischemia protocol. Values are given as median ± IQR. IQR: interquartile range, RIPC: remote ischemic preconditioning, Tn I: troponin I. There are no significant differences between dietary boron groups (P = 0.725).
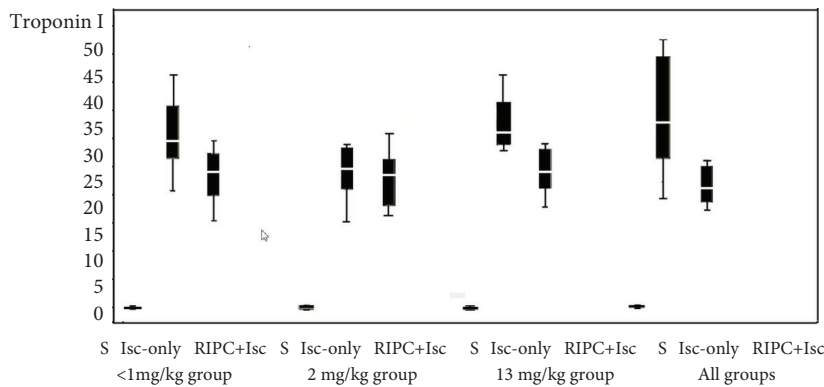

Figure 2. The mean troponin I levels between groups according to dietary boron groups. Values are given as median ± IQR. IQR: interquartile range, RIPC: remote ischemic preconditioning, Tn I: troponin I. The sham group was significantly different from the Isc-only and RIPC+Isc groups (P < 0.001), and the differences between the Isc-only and RIPC+Isc groups were found to be significant (P < 0.001).

after myocardial insult, and that the mitigation of myocardial injury provided by RIPC in the early phase is protected and not deteriorated by the amount of dietary boron.

Cardiovascular disease is the leading cause of death throughout the world. In the setting of acute coronary syndromes, the primary goal is to restore the abrupt cessation of blood flow in order to salvage ischemic tissue before it is irreversibly injured, which in turn leads to improved residual ventricular function and clinical outcomes (6). However, reperfusion itself causes cell death, a process referred to as reperfusion injury (6). In clinical practice, the unpredictable nature of the onset of ischemia limits the usage of experimental interventions to those shown to be effective against ischemia. Most studies aim to discover the answer to the ultimate question of how the resistance of heart tissue can

Table 3. Effect of dietary boron on serum troponin I levels after isoproterenol-induced myocardial injury in rats.

Groups		Troponin I	P
<1 mg/kg	Sham (n = 5)	0.62 ± 0.47	
	Isc-only (n = 7)	28.7 ± 8.3	
	RIPC+Isc (n = 7)	24.4 ± 8.4	
	Total (n = 19)	24.4 ± 27.8	0.752
2 mg/kg	Sham (n = 5)	0.73 ± 0.37	
	Isc-only (n = 7)	25.5 ± 6.4	
	RIPC+Isc (n = 6)	23.8 ± 9.8	
	Total (n = 18)	21.6 ± 25.9	0.752
13 mg/kg	Sham (n = 5)	0.52 ± 0.6	
	Isc-only (n = 7)	31.1 ± 8.9	
	RIPC+Isc (n = 8)	24.5 ± 7.9	
	Total (n = 20)	26.7 ± 24.5	0.752
100 mg/kg	Sham (n = 3)	0.87 ± 0.32	
	Isc-only (n = 5)	31.1 ± 17.3	
	RIPC+Isc (n = 6)	21.6 ± 7.0	
	Total (n = 14)	22.9 ± 14.2	0.752

Each value is median ± IQR in each group, and all group comparisons were done with the Kruskal–Wallis test. There were no significant differences between the dietary boron groups (P = 0.725).

be improved in ischemia and reperfusion injury. In 1986, a preliminary work showed that brief episodes of ischemia and reperfusion make the heart more resistant to ischemia, a process referred to as ischemic preconditioning (IPC) (3). This paradigm has been studied by many researchers in order to understand the mechanisms that improve ischemic tolerance (11–13). In other words, this paradigm implied that myocardial protection against ischemia is possible and that these mechanisms may be translated into clinical practice. The paradigm of IPC was extended to such a degree that brief ischemia in an organ that is distant or remote from the heart, such as the limbs, intestine, kidney, or skeletal muscle, also protects the heart against ischemia. This phenomenon is known as RIPC (14,15). There are many models for RIPC induction applied in different species with different RIPC protocols and RIPC sites. Kharbanda et al. produced hind-limb ischemia with 5-min cycles of ischemia and reperfusion in pigs (14). In another report, Kristiansen et al. produced RIPC via 5-min cycles of ischemia and reperfusion using limbs as the RIPC site in rats (16). In our particular study, we chose the femoral artery as the RIPC site and 5-min cycles of ischemia and reperfusion as the RIPC protocol. Furthermore, cycles of intermittent limb ischemia provide an acceptable method for cardioprotection, which, when used, was shown to be effective with reduced cardiac injury markers in cardiac surgery and in coronary angioplasty, as well (17–19). Tn I is closely related with the severity of myocardial injury (20). Because it is a sensitive marker of myocardial injury, we used Tn I to detect the change in the extent of myocardial injury with different experimental

protocols. In our experimental setting, we used ISO to induce myocardial ischemia and the formation of myocardial infarction, such as lesions, which is widely used in toxicology studies (9,10). In the rat, at high doses, ISO stimulates beta-1 and beta-2 receptors, resulting in an abnormally high heart rate and reduced blood pressure. As a result of an oxygen demand–supply mismatch, anoxia/hypoxia occurs in cardiac tissue (10). These changes are closely related to elevation of serum cardiac Tn, which shows the extent of myocardial injury (20). The elevations in the levels of serum Tn become evident within 4 h of drug administration, and levels of cardiac Tn show a gradual decline from 4 to 24 h after dosing (21). Thus, we obtained hemodynamic data and tissue samples at 4 h after ISO administration. IPC has been shown to have 2 protection phases; the "early" IPC or "first window" protects the heart for 1 or 2 h and then wanes, and the "late" IPC or "second window of protection" appears 24 h after the first IPC protocol and lasts for 24–48 h (22,23). Obtaining the hemodynamic data and tissue samples in the very first hours of ischemia seems rational to evaluate the first phase responses of IPC. On the other hand, this is a limitation of our study, because what we found reveals only information on the "early" phase of IPC, not the "late" IPC. Moreover, the evaluation of "late" IPC response with this protocol would be inappropriate, because there are some data present on ketamine deteriorating the late IPC response (24,25).

Boron was accepted in 1923 as an essential element for all vascular plants (7). However, the importance of boron for humans and animals seems to have been overlooked until the 1980s (8). Being the fifth element and the only nonmetal in the Group IIIA elements, boron contains both metal and nonmetal characteristics (26). Boron is commonly found in nature in the form of borates, is accessible to all plants and animals, is absorbed 100% across the mucous membranes by humans and animals, and is excreted mostly in the urine (2,27–29). The primary sources are fruits and vegetables, whereas animal sources provide lower amounts of boron (30). The assessment of boron intake is difficult because there is no national database showing the amount of boron present in foods and personal care products (31). Because boron is widely accessible, deficiency is not common; however, boron deficiency may cause abnormal

bone growth or impaired growth and exacerbate the deficiency symptoms of vitamin D_3, whereas boron toxicity shares similarities to pellagra symptoms (8,26,32). In the case of chronic toxicity, poor appetite, nausea, weight loss, and decreased seminal volume and sexual activity are seen (32). Although adult doses of 18 to 20 mg of boron have been shown to be fatal, death from boron toxicity is unusual (32). The World Health Organization suggests a daily intake of 2 mg of boron, whereas the minimum daily dosage reported for the "lowest-observed-adverse-effect level" (LOAEL) is 13–13.6 mg/kg (33,34). Therefore, in our experiments, we gave the rats 2 mg/kg and 13 mg/kg of boron daily to observe the influence of a physiologic dose of boron on ischemic heart tissue. Boron is able to interact with important biological substances, including polysaccharides, pyridoxine, riboflavin, dehydroascorbic acid, and pyridine nucleotides (28). Hunt reported that boron influences the activity of at least 26 different enzymes seen in animal, plant, cultured, and chemical reaction systems (2). Boron plays a role in regulating enzymatic activity in pathways involved in energy substrate metabolism, insulin release, nucleic acid synthesis, and the immune system (2). Serine proteases and oxidoreductases require pyridine or flavin nucleotides (nicotinamide adenine dinucleotide [NAD+], nicotinamide adenine dinucleotide phosphate [NADP], and flavin adenine dinucleotide [FAD]), and boron reversibly inhibits their activities by competing for NAD or FAD (2). Boron also inhibits glycolytic enzyme activity in vitro and modifies insulin release by altering the metabolism of NADPH. Boron may also lower the level of oxidative damage, which is accomplished by decreasing the production of NADPH and the activity of delta-glutamyl transpeptidase (2). Although the influences of boron in steroid hormone metabolism, calcium metabolism, bone development, and energy metabolism are known to a degree, there is no report regarding the effects of boron on ischemic heart tissue. Contractile dysfunction after ischemia is thought to be related to changes in metabolic pathways, alteration of calcium homeostasis and energy metabolism, and changes in cell death mechanisms, all of which are somehow related to boron, as mentioned above (5). With this particular study, we aimed to examine the possible effects of boron on ischemic heart tissue for the first time in medical literature. According to

our results, the amount of dietary boron does not affect the baseline LV systolic indices or the extent of myocardial injury after the myocardial insult. The mitigation of myocardial injury provided by RIPC in the early phase is protected and not deteriorated by the amount of dietary boron. However, there is no significant difference between ischemia and IPC groups in terms of dietary amount of boron. Our diet was not lacking in calcium or magnesium, and in the boron-deficient group the amount of boron supplied was around 1 mg/kg. One possible reason for this may be that, as emphasized in the literature, the effects of boron become more pronounced where there is a lack of calcium or magnesium. Another possibility is that, because boron is a trace element, very small amounts in the diet may mask the effects of absolute boron deficiency on early IPC response. It should also be kept in mind that the pronounced effects of boron were shown in disease conditions such as diabetes, postmenopause, and rachitism models (35). The effects of boron on IPC response in disease conditions need to be investigated.

In this study, we aimed to examine the possible effects of boron on ischemic heart tissue for the first time in the medical literature. According to

our results, the amount of dietary boron does not affect the baseline LV systolic indices or the extent of myocardial injury after the myocardial insult. The mitigation of myocardial injury provided by RIPC in the early phase is protected and not deteriorated by the amount of dietary boron.

As mentioned above, our diet contained around 1 mg/kg boron and was not lacking in calcium and magnesium, an occurrence that may mask the effects of absolute boron deficiency. There are data available regarding the process of ketamine deteriorating the late IPC response, but this cannot be considered as a limitation, because we evaluated the "early" IPC response; however, evaluating only the "early" IPC response may be a limitation of the present study, because in order to comprehend the IPC response mechanisms, "late" IPC responses should be evaluated, as well. Lastly, if more subjects had been included in the subgroups, our results would have been more precise and appropriate.

Acknowledgment

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