Tocotrienol-Rich Fraction Modulates Cardiac Metabolic Profile Changes in Isoprenaline-Induced Myocardial Infarction Rats
(Fraksi Kaya Tokotrienol Memodulasi Perubahan Profil Metabolisma Jantung pada Tikus Infarksi Miokardium Aruhan Isoprenalin)

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ABSTRACT
In myocardial infarction (MI), the occurrence of energy depletion, oxidative stress, and decreased amino acids metabolism alter tissue metabolites. Evidence has shown that tocotrienol-rich fraction (TRF) prevents myocardial injury in MI. However, the protective mechanism at the metabolite level is unknown. Male Sprague-Dawley rats were grouped into control, isoprenaline (ISO)-induced MI (MI), healthy rats receiving 200 mg/kg TRF (200TRF), and MI rats receiving 200 mg/kg TRF (200TRF+MI) groups. TRF was administered via oral gavage daily for 12 weeks followed by intraperitoneal ISO injection (85 mg/kg) for two consecutive days at a 24-hour interval to induce MI. High-performance liquid chromatography was performed to analyze serum α-tocopherol and tocotrienol concentration whereas ultra-high-performance liquid chromatography-mass spectrometry was used for the untargeted metabolomic study. Serum α-tocopherol but not tocotrienol was increased in the 200TRF (p=0.121) and 200TRF+MI (p<0.05) following TRF supplementation. Multivariate analysis by Orthogonal Projections to Latent Structures Discriminant Analysis showed high predictability of the group comparison models for MI vs control and 200TRF+MI vs MI (cross-validation: Q^2>0.7, R^2Y>0.8, p<0.05). A total of 84 and 37 metabolites [when covariance of p≥0.05 (magnitud) and p(corr)≥0.5 (reliability)] were significantly different in the myocardial homogenates of MI vs control and 200TRF+MI vs MI, respectively. MI rats had reduced S-adenosylmethionine and L-cystathionine that might worsen MI by disturbing glutathione metabolism; decreased phosphoribosyl-pyrophosphate and purine salvage process that might impair DNA synthesis, and elevated glucose-6-phosphate suggesting enhanced anaerobic glycolysis possibly for rapid production of energy. Conversely, TRF supplementation reversed the impaired metabolic pathways caused by MI.

Keywords: Isoprenaline; liquid chromatography-mass spectrometry; metabolomics; myocardial infarction; tocotrienol-rich fraction

ABSTRAK
Dalam infarksi miokardium (MI), penurunan tenaga, tekanan oksidatif, serta pengurangan metabolisme asid amino mengubah metabolit dalam tisu. Buktiku terkini menunjukkan bahwa fraksi kaya tokotrienol (TRF) mencegah kecederaan miokardium dalam MI. Namun, mekanisme perlindungannya pada tahap metabolit masih tidak diketahui. Tikus Sprague-Dawley jantan dibagi kepada kumpulan kawalan, MI aruhan isoprenalin (ISO) (MI), tikus sihat yang menerima 200 mg/kg TRF (200TRF), dan MI yang menerima 200 mg/kg TRF (200TRF+MI). TRF diberikan melalui gavaj oral setiap hari selama 12 minggu diikuti suntikan ISO secara intraperitoneum (85 mg/kg) dua hari berturut-turut berselang 24 jam untuk mengaruh MI. Kromatografi ceccair herprestasi tinggi digunakan untuk menganalisis kepekatan 24 metabolit untuk mengaruh MI. Kromatografi ceccair herprestasi ultra-tinggi-spektrometri jisim digunakan untuk kajian metabolomik tanpa sasaran. Serum α-tokoferol tetapi tidak tokotrienol telah meningkat bagi kumpulan 200TRF (p=0.121) dan 200TRF+MI (p<0.05) setelah disisipan TRF. Analisis multivariat oleh Unjuran Ortogra kepada Analisis Diskriminasi Struktur Pendam menunjukkan kebolehraman yang tinggi bagi model perbandingan antara kumpulan MI vs kawalan dan 200TRF+MI vs MI (pengesahan silang: Q^2>0.7, R^2Y>0.8, p<0.05). Sebanyak 84 dan 37 metabolit [apabila kovarians p≥0.05 (magnitud) dan p(corr)≥0.5 (kebolehpercayaan)] berbeza secara signifikan dalam homogenat miokardium masing-masing bagi kumpulan MI vs kawalan dan 200TRF+MI vs MI. Tikus MI mengalami penurunan S-adenosilmetionina dan L-cystationina yang mungkin meningkatkan keterusan MI melalui gangguan metabolisme glutation; pengurangan fosforibosil-pirofosfat dan proses penyelamatan purina yang mungkin menjejaskan sintesis DNA, serta peningkatan glukosa-6-fosfat bagi proses glikolisis anaerob yang mungkin bertujuan menghasilkan tenaga secara pantas. Sebahkanya, suplementasi TRF menghalang penjejaskan pada laluan metabolisme yang dicetukus oleh MI.

Kata kunci: Fraksi kaya tokotrienol; infarksi miokardium; isoprenalin; kromatografi ceccair-spektrometri jisim; metabolomik
INTRODUCTION

Myocardial infarction (MI) is defined as clinical evidence of myocardial necrosis due to reduced oxygen supply to the heart (Thygesen et al. 2012). It is a part of a disease spectrum known as ischemic heart disease or coronary heart disease that causes 12.3% of total global deaths, translated to about 7.2 million deaths annually (Finegold et al. 2013; Sanchis-Gomar et al. 2016; World Health Organization 2017). Patients suffering from MI are becoming increasingly younger especially in some developing countries where it affects people aged 55-59 years, in contrast to males aged 63-68 years and females aged 73-79 years in the developed countries (Institute of Medicine 2010; Seong & John 2016). Being one of the commonest causes of death combined with its high mortality and morbidity rate, the disease impacts not only the individuals who suffer from it, but also to the nation in terms of the overall productivity as well as financial burden in order to provide medical centers with adequate resources for the disease treatment (Che-Muzaini & Norsa'adah 2017; Lee et al. 2017).

High morbidity and mortality rates associated with MI has prompted extensive studies globally. To understand various aspects of the disease including identifying potential new treatment, several experimental animal models have been developed, including induction method via isopropanol (ISO) administration through parenteral route (Murugesan et al. 2011; Panda et al. 2017). This is one of the most easily accessible, validated and commonly used technique to induce MI in rats. Also known as isoproterenol, ISO is a sympathomimetic agent that acts exclusively on β-adrenergic receptors with very little action on α-adrenergic receptors. Given the high β-adrenergic receptor expression in myocardial tissue (MIMS 2019), ISO administration produced significant effects on the heart. ISO causes positive chronotropism and inotropism which increase the rate as well as the force of cardiac contraction. However, it has no effect on α-receptors resulting in coronary arterial vasodilation that leads to hypotension in the coronary vascular bed. Cumulatively, ISO increases cardiac output and reduces diastolic as well as mean arterial pressure resulting in oxygen supply and demand mismatch leading to hypoxia (Biaggionio & Robertson 2014). Exposure to higher doses of ISO leads to ischemia with subsequent MI due to changes in various physiological processes in the myocardium including increased global adenosine triphosphate (ATP) synthesis, increased oxidative damage, inflammation, and activation of apoptotic pathways along with cell necrosis (Liu et al. 2013; Siddiqui et al. 2016).

Untargeted metabolomics is the study of global metabolic profile in a biological system whereby all the low molecular weight compounds or metabolites that are present and participate in biochemical reactions at a certain time point will be captured and quantified (Horgan & Kenny 2011). Alterations of the metabolome are the end-result of a series of changes that occur in the genomic, transcriptomic as well as proteomic levels. Metabolic profile can be influenced by diseases, lifestyle and dietary habit, along with other environmental factors such as exposure to pollutants and intake of medications (Clark-Matott et al. 2015; Huang et al. 2014; Riera-Borrull et al. 2017; Viana et al. 2016; Vlaanderen et al. 2017). Thus, studies that utilize metabolomic-based approach would provide insight regarding the overall molecular mechanisms of disease progression and how a therapeutic agent works at the metabolic level. Several authors have reported on the metabolic profile changes in MI by using biological samples from serum, plasma or myocardial tissue homogenate. By comparing MI rats with healthy controls, a number of altered biochemical pathways has been reported including β-oxidation of fatty acid, metabolism of amino acids such as glycine, serine, arginine and proline, and glycophospholipid metabolism, among many others (Liu et al. 2013).

Recently, there have been increasing interests toward natural products in combating MI either to be used as preventive strategies or as an adjunct to the current pharmacological therapies. Cardioprotective effects of vitamin E have been discovered and studied for many years in both pre-clinical and clinical fields that show its promising future in the management of MI (Zarkasi et al. 2019). Vitamin E is a general term used to describe naturally occurring compounds that share similar chemical structures and functions comprising several isomers of tocopherols and tocotrienols (Atia & Abdullah 2014). Many evidence suggests that tocotrienol is more powerful than tocopherol. For instance, the former provides a better anti-aging effect than the latter on senescent myoblasts (Khor et al. 2016). Furthermore, it possesses stronger anti-inflammatory and antioxidant properties compared to tocopherol which may be contributed by its higher rate of cellular uptake as well as more uniform distribution in the plasma membrane (Fairus et al. 2006; Maniam et al. 2008; Ng & Ko 2012; Serbinova et al. 1991). Tocotrienol is present in foods of plant origin and it is most abundant in rice bran, palm oil and annatto seed (Kannappan et al. 2012). Tocotrienol-rich fraction (TRF) can be obtained from serial processes of esterification, distillation, and concentration of its plant source (Top & Gapor 2005). It has been observed to reverse MI-related findings such as increased lactate dehydrogenase as well as aspartate and alanine aminotransferases in rat model attributing the effects toward its antioxidant property as well as its ability in stabilizing the proteasome (Bardhan et al. 2015; Das et al. 2008). Additionally, TRF at the dose of 200 mg/kg/day was reported to significantly decrease serum creatine kinase-myocardial band, which is a specific marker for myocardial injury, in ISO-induced MI rats (Zainalabidin et al. 2018). However, the cardioprotective mechanism of TRF on MI at the metabolic level is still unknown.

Therefore, the aim of this study was to determine the changes in metabolic profile caused by TRF on rats with ISO-induced MI.
TREATMENT OF ANIMALS AND COLLECTION OF SAMPLES

Male Sprague-Dawley rats (n=24) weighed 250-300 g were purchased from the Laboratory Animal Research Unit, Universiti Kebangsaan Malaysia (UKM) while ethical approval was obtained from the UKM Animal Ethics Committee (approval no. BI0K/PP/2018/ZAKIAH/23-JAN.-/893-JAN.-2018-DEC.-2018-NAR-CAT2). They were allowed to acclimatize for the first seven days prior to the commencement of the experiment following which they were randomly assigned into four groups (n=6 per group): control, MI, 200TRF, and 200TRF+MI. Rats from all groups were given normal chow diet with access to tap water ad libitum. Male Sprague-Dawley rats were sacrificed via cervical dislocation.

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Briefly, serum samples were added with butylated hydroxytoluene (Sigma-Aldrich, St. Louis, MI, USA) to reduce oxidation of the analyte of interest followed by addition of 100% ethanol (Merck, Darmstadt, Germany) for deproteinization. Liquid-liquid extraction was performed by vortexing the mixture followed by separation via centrifugation at 5,000 rpm for 15 min while maintaining the temperature at 18°C. The supernatant was transferred to a new tube, mixed with n-hexane (Merck) and vortexed, resulting in a formation of three different layers from which the uppermost layer was collected and vacuum dried for 40 min. The dried sample was reconstituted with n-hexane and filtered through 0.2 µm regenerated cellulose membrane syringe filter (Minisart RC4, Sartorius Stedim Biotech, Göttingen, Germany) to remove impurities before it was injected into the high-performance liquid chromatography (HPLC) system for analysis.

HPLC System

All solvents used for determination of serum α-tocopherol and tocotrienol were HPLC-grade. Normal phase liquid chromatography was performed on a Shimadzu LC-10AT VP HPLC system controlled by Shimadzu Class-VP (version 6.0) software (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on Luna® Silica (2) column, 250 mm length × 4.6 mm diameter, particle size 5 µm (Phenomenex, Torrance, CA, USA) with the column oven temperature maintained at 30°C while the injection volume was 20 µL. The mobile phase consisted of a mixture between n-hexane and isopropanol (Merck) at 99:1 ratio with a flow rate of 1.5 mL/min under 38 kgf/cm² of pressure. Analyte detection was performed by fluorescence detector when the excitation and emission wavelengths were set at 294 and 330 nm, respectively. Results obtained for each sample were compared to a standard curve for the determination of serum α-tocopherol and tocotrienol concentration (Jaafar et al. 2018).

METABOLOMATIC ANALYSIS FROM HEART HOMOGENATE

Myocardial Tissue Sample Preparation

Myocardial tissue sample preparation was performed according to Marney et al. (2013) with some modifications. Briefly, the myocardial sample of the left ventricle was frozen with liquid nitrogen and crushed. For every 10 mg of heart tissue, 500 µL of chilled 80% methanol (Fischer Scientific, Hampton, NH, USA) was added followed by homogenization with a sonicator at 30 amp while on ice. Once homogenized, the sample was mixed with 300 µL chilled chloroform and centrifuged at 20,000 × g at 4°C for 10 min. The supernatant was collected and vacuum dried. For every 10 mg of heart tissue used, 200 µL of 20% methanol was added to reconstitute the dried sample. The mixture was filtered to remove impurities before it was injected into the ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) system for analysis.

MEASUREMENT OF SERUM α-TOCOPHEROL AND TOCOTRIENOL CONCENTRATION

Sample preparation was performed according to Khan et al. (2010) with some modification. Briefly, serum samples were added with butylated hydroxytoluene (Sigma-Aldrich, St. Louis, MI, USA) to reduce oxidation of the analyte of interest followed by addition of 100% ethanol (Merck, Darmstadt, Germany) for deproteinization. Liquid-liquid extraction was performed by vortexing the mixture followed by separation via centrifugation at 5,000 rpm for 15 min while maintaining the temperature at 18°C. The supernatant was transferred to a new tube, mixed with n-hexane (Merck) and vortexed, resulting in a formation of three different layers from which the uppermost layer was collected and vacuum dried for 40 min. The dried sample was reconstituted with n-hexane and filtered through 0.2 µm regenerated cellulose membrane syringe filter (Minisart RC4, Sartorius Stedim Biotech, Göttingen, Germany) to remove impurities before it was injected into the high-performance liquid chromatography (HPLC) system for analysis.
**UHPLC-Orbitrap MS System**

All solvents for metabolomic analysis were MS-grade. Liquid chromatography was performed on UHPLC Dionex UltiMate™ 3000 Rapid Separation System coupled with Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fischer Scientific) containing heated electrospray ionization probe (HESI-II). The system was controlled by Chromeleon Xpress and MS Thermo Xcalibur™ (version 4.0) system data. Chromatographic separation was performed by using reversed phase Syncros™ C18 column, 100 mm length × 2.1 mm diameter, particle size 1.7 µm (Thermo Scientific) maintained at 55°C with an injection volume of 2 µL (n=3 samples with 3 technical replicates for each sample).

Samples were randomly injected into the system while maintaining automatic sampler temperature at 10°C. The flow rate of the solvents was set at 0.45 mL/min using Optima® ultrapure water (Fischer Scientific) as mobile phase A and acetonitrile (Fischer Scientific) as mobile phase B where both contained 0.1% (v/v) formic acid (Thermo Fischer Scientific). Elution gradient program for 15 min was set as followed: 0 min, 0.5% B; 5.5 min, 50% B; 6-12 min, 98% B; 13-15 min, 0.5% B. Positive and negative ionization modes were performed under these conditions: Sheath gas flow rate at 50 arbitrary unit (AU), auxiliary gas flow rate at 18 AU, sweep gas flow rate at 0 AU, capillary temperature at 320°C and spray voltage of 3.5 kV for positive mode or 3.0 kV for negative mode. MS spectra were obtained using full MS/data-dependent MS² (TopN) (dd-MS2) by which MS² was triggered when high-intensity ion was discovered in full MS scan. Full MS scan parameters include: Default charge state of 1, resolution of 60,000, automatic gain control (AGC) of 1e6, maximum injection time (IT) of 120 ms, and a scan range between 100-1,000 m/z.

Parameters for dd-MS2 scan were as followed: resolution of 15,000, AGC target of 5e4, maximum IT set at 50 ms, loop count of 5, TopN of 5, isolation window of 1.5 m/z, and stepped normalized collision energy at 20, 40 and 60. Settings for the dd include: Minimum AGC target of 8e2, intensity threshold at 1.6e4, exclude isotope ‘on’, and dynamic exclusion at 10 s. The MS system was calibrated by using Pierce LTQ ESI Positive Ion and Pierce LTQ ESI Negative Ion Calibration Solutions (Thermo Scientific). For quality control (QC), an aliquot of equal volume (2 µL) from each sample was pooled and mixed in a separate vial labeled as QC sample to assess the reproducibility and reliability of the LC-MS/MS system. The pooled QC sample was injected thrice prior to analysis of the individual sample and was repeated after every sixth sample injection.

**Data Pre-processing**

The data file ‘.RAW’ was processed with Thermo Scientific Compound Discoverer™ 2.0 (CD 2.0) software using untargeted workflow. This workflow used ‘Detect Unknown Compounds’ node to find chromatographic peaks for unknown compounds [molecular weight (m/w) × retention time (RT)] and ‘Predict Composition’ node to determine possible compositions of the elements that formed the compounds. Additionally, the workflow performed searches in mzCloud and ChemSpider databases to annotate unknown compounds that have been detected by the LC-MS system (Figure 1). This pre-processed data was then exported to the ‘.xlsx’ file for subsequent statistical analysis.

**FIGURE 1.** Workflow for data pre-processing in Thermo Scientific Compound Discoverer™ 2.0 (CD 2.0) whereby ‘Untargeted Metabolomic Analysis with Statistics’ approach was used followed by database search in mzCloud and ChemSpider to annotate the detected unknown compounds.
Multivariate Analysis
The peak areas for the detected metabolites were analyzed using the ‘Statistical Analysis’ module in MetaboAnalyst 4.0 online software (Chong et al. 2018). The data was pre-processed by subjecting to normalization processes that include quantile normalization to make the data distribution for different groups more identical, cube root transformation to adjust the data distribution toward normal distribution, and mean centering to eliminate offset data and focus on differences rather than similarities for each variable.

Unsupervised Principal Component Analysis (PCA) and supervised Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) were used as multivariate analytical methods. In OPLS-DA, the covariance between X and Y had been maximized to identify which metabolites were responsible for separation between different rat groups. Furthermore, permutation test was done to assess the statistical significance of group separations with p-value set at ≤0.05. Differentially expressed metabolites (DEMs) were then selected if their covariance of \( p_c \geq 0.05 \) (magnitude) and \( p_{corr} \geq 0.5 \) (reliability) on S plot of OPLS-DA (Jung et al. 2010; Suvagandha et al. 2014).

Metabolites Annotation
DEMs were annotated by cross-referencing the Human Metabolome Database (HMDB) (http://www.hmdb.ca/) and Metlin Library database (https://metlin.scripps.edu/) with mass accuracy set at 5 ppm (Durani et al. 2017). MS/MS spectra of putative metabolites were also compared with the online mzCloud database linked to CD 2.0 software or on its website (https://www.mzcloud.org) before they were used for further analysis. Additionally, fold change analysis was also performed by comparing ‘MI vs control’ and ‘200TRF+MI vs MI’ to determine the fold changes for these metabolites.

Pathway Analysis
Annotated DEMs were subsequently analyzed by using the ‘Pathway Analysis’ module in the MetaboAnalyst 4.0 software to obtain a clearer picture of the disturbed biochemical pathways in ISO-induced MI rats and the metabolic pathways changes following TRF supplementation in MI. For the mapping process, the metabolite library for Rattus norvegicus had been selected. The algorithm for pathway analysis was performed by applying the ‘Fischer exact test’ for over-representational analysis while ‘relative-betweenness centrality’ was selected for the analysis of the pathway topology. Metabolic pathways with impact-values > 0.1 were considered to be relevant (Durani et al. 2017).

Statistical Analysis
Data for serum α-tocopherol and tocotrienol was analyzed by using IBM Statistical Package for the Social Sciences (SPSS®) (version 23.0). Since the data distribution was skewed, central tendency and dispersion were expressed in median and interquartile range (IQR) while differences among groups were assessed by using non-parametric techniques.

Results and Discussion
EFFECTS OF TRF ON SERUM α-TOCOPHEROL AND TOCOTRIENOL CONCENTRATION
Measurement of serum α-tocopherol and tocotrienol concentrations was performed at the beginning and at the end of the study period. There was no difference for serum α-tocopherol among all rat groups. Supplementation with TRF was found to increase the serum α-tocopherol levels for 200TRF and 200TRF+MI rats at week 12 compared to the same group at baseline (Figure 2(A)). Additionally, serum α-tocopherol for the 200TRF+MI group was significantly higher than MI rats with respective median (IQR) of 25.42 μg/mL (20.7-40.5 μg/mL) and 14.17 μg/mL (12.6-17.6 μg/mL) \( (p<0.05) \). Supplementation of TRF produced a similar elevation of serum α-tocopherol in the 200TRF compared to control rats by 91.1% although the change was statistically insignificant \( (p=0.121) \). On the other hand, serum tocotrienol was similar at baseline for all rat groups. Supplementation with TRF for 12 weeks did not cause any change in the serum tocotrienol concentrations for 200TRF and 200TRF+MI compared to the same groups.

![Figure 2](image-url)  
**Figure 2.** Changes in the serum α-tocopherol (A) and tocotrienol (B) concentrations (μg/mL) at baseline and week 12. Values are shown in median, error bars represent interquartile range (IQR). *Significantly differed from the same group at baseline with \( p<0.05 \). †Significantly differed from the MI group at week 12 with \( p<0.05 \). Abbreviations: MI (ISO-induced MI rats), 200TRF (healthy rats receiving 200 mg/kg/day TRF); 200TRF+MI (ISO-induced rats receiving 200 mg/kg/day TRF)
at baseline. Similarly, serum tocotrienol levels did not differ between 200TRF and control as well as between 200TRF + MI and MI groups at the end of the study period (Figure 2(B)).

These findings could be explained by the differences in biological processes involving transport and bioavailability of vitamin E within the blood circulation. Firstly, supplementation of vitamin E maximally increases the blood level of tocopherol and tocotrienol at a different time point. Administration of TRF orally in human subjects caused plasma α-tocotrienol to reach peak concentration earlier than α-tocopherol, recorded at about 4 h and 6 h, respectively. Additionally, post-prandial elevation of plasma α-tocotrienol was still significantly lower than α-tocopherol with a mean concentration of 4.79 ± 1.2 µg/mL for the former and 13.46 ± 1.68 µg/mL for the latter (Fairus et al. 2006). Thus, findings on serum α-tocopherol and tocotrienol in this study might be affected by the differences in the bioavailability for both vitamin E vitamers.

Secondly, cellular uptake is higher for tocotrienol as compared to tocopherol. In a study using Jurkat cell line E6-1, α-tocotrienol reportedly entered the cell 70 times faster than α-tocopherol (Saito et al. 2004). As a result, tocotrienol might be subjected to higher clearance from blood circulation. Therefore, no differences were observed in the plasma tocotrienol concentration in rats receiving TRF supplementation compared to non-supplemented groups in the current study.

Thirdly, different protein carriers have different affinity for vitamin E vitamers and might affect its blood concentration. Majority of dietary vitamin E is transported by lipoproteins to reach peripheral tissues. However, the observed amount of tocopherols were higher than tocotrienols in chylomicron, low-density lipoprotein as well as high-density lipoprotein which stood at approximately 21%, 58% and 45%, respectively (Fairus et al. 2012). In addition, there is a special protein carrier for tocopherol transport in the blood circulation, especially for α and γ-isomers. It is an albumin protein family known as afamin (Dieplinger & Dieplinger 2015). Simulation study performed on the predicted tertiary structure of afamin found that the protein had 18 different binding sites for tocopherol alone (Voegele et al. 2002).

The explained mechanisms might lead to higher blood tocopherol level as compared to tocotrienol. Subsequently, any changes in relation to its concentration could be easily and clearly detected. Further study is needed to measure the level of vitamin E in the cardiac tissue for a better understanding of the relationship between bioavailability and efficacy of the cardioprotective effect of TRF.

| TABLE 1. Permutation test and cross-validation results for Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) |
| Groups | ESI (+) | Q2   | p-Value Q2 | R2Y   | p-Value R2Y |
| MI vs control | + | 0.752 | <0.0005* | 0.891 | 0.0265* |
| 200TRF vs control | - | 0.891 | <0.0005* | 0.998 | 0.0005* |
| 200TRF+MI vs MI | + | 0.230 | 0.0430* | 0.385 | 0.3680 |
| - | 0.353 | 0.0160* | 0.491 | 0.2055 |
| + | 0.819 | <0.0005* | 0.951 | 0.0005* |
| 0.731 | 0.0010* | 0.993 | 0.0065* |

*Statistically significant when p<0.05 in permutation test. Abbreviation: ESI (electrospray ionization). Cross-validation: Q2 = quality assessment, R2Y = degree of fit to the data

| TABLE 2. The number of differentially expressed metabolites (DEMs) between different group comparisons |
| Groups | DEMs | Total | Annotated | Unannotated |
| MI vs control | ESI (+) | 150 | 84 | 106 |
| 200TRF+MI vs MI | ESI (-) | 40 | 37 | 48 |

ESI = electrospray ionization
| m\w     | Putative Metabolites                      | Identifiers     | ESI | FC   |
|---------|------------------------------------------|-----------------|-----|------|
| 129.0428 | 5-Oxoproline                             | HMDB00060262    | +   | ↑ 14.26 |
| 163.12104| Acetylcysteine                           | ^511            | +   | ↑ 2.06 |
| 280.10608| Phenylalanyl-aspartate                    | HMDB0028991     | +   | ↑ 1.98 |
| 168.08247| Uric acid                                | HMDB0000289     | +   | ↑ 1.69 |
| 393.86701| 2-(14,15-Epoxyeicosatrienoyl) glycerol   | ^4758           | +   | ↓ 1.46 |
| 252.00116| 2'-Deoxyinosine                          | ^1187           | +   | ↓ 1.30 |
| 459.35185| 5-Methyltetrahydrofolic acid             | ^262            | +   | ↓ 1.24 |
| 297.08974| 5'-Methylthiodenosine                    | HMDB0001173     | +   | ↓ 1.41 |
| 340.27968| α-D-Glucose-1,6-bisphosphate             | ^1711           | +   | ↓ 1.75 |
| 335.31899| β-Nicotinamide mononucleotide            | ^871            | +   | ↓ 1.20 |
| 260.13719| γ-Glutamyl-L-isoleucine                  | *62019          | +   | ↓ 1.16 |
| 299.07701| D-4'-Phosphopantothenate                 | HMDB0001016     | +   | ↓ 1.53 |
| 429.90703| D-α-Tocopherol                           | ^328            | +   | ↓ 2.01 |
| 159.12609| DL-2-Aminoocanoic acid                   | HMDB0000991     | +   | ↓ 1.98 |
| 219.99107| L-5-Hydroxytryptophan                    | ^254            | +   | ↓ 1.38 |
| 204.11107| L-Acetylcarnitine                        | ^879            | +   | ↓ 1.07 |
| 186.11880| L-Alanyl-L-proline                       | ^461            | +   | ↓ 1.35 |
| 240.13492| L-Anserine                               | ^1308           | +   | ↓ 1.69 |
| 267.97498| L-Homocysteine                           | ^476            | +   | ↓ 1.42 |
| 149.05120| L-Methionine                             | HMDB0000696     | +   | ↓ 1.78 |
| 117.07926| L-Valine                                 | HMDB0000883     | +   | ↓ 1.27 |
| 115.06368| L-Proline                                | HMDB0000162     | +   | ↓ 1.40 |
| 309.10605| N-Acetylneuraminic acid                  | ^1249           | +   | ↓ 1.46 |
| 217.13166| O-Propanoylcarmitine                     | HMDB0062514     | +   | ↓ 2.08 |
| 181.07400| o-Tyrosine                               | HMDB0006050     | +   | ↓ 1.50 |
| 206.04355| (R)-Lipoic acid                          | HMDB0001451     | +   | ↓ 1.05 |
| 399.37128| S-Adenosylmethionine                     | ^896            | +   | ↓ 1.20 |
| 425.35075| Acetylcarmitine C18:1                    | HMDB0005065     | +   | ↓ 1.26 |
| 135.06165| Adenine                                  | 296             | +   | ↓ 1.54 |
| 267.09692| Adenosine                                | 297             | +   | ↓ 2.00 |
| 226.10675| Carnosine                                | HMDB0000033     | +   | ↓ 1.01 |
| 483.46558| Cer(d18:0/12:0)                           | HMDB0011758     | +   | ↓ 1.22 |
| 511.49656| Cer(d18:0/14:0)                           | HMDB0011759     | +   | ↓ 1.22 |
| 539.52779| Cer(d18:0/16:0)                           | HMDB0011760     | +   | ↓ 1.24 |
| 567.55941| Cer(d18:0/18:0)                           | HMDB0011761     | +   | ↓ 1.21 |
| 595.59105| Cer(d18:0/20:0)                           | HMDB0011764     | +   | ↓ 1.29 |
| 555.52319| Cer(t18:0/16:0)                           | HMDB0010697     | +   | ↓ 1.22 |
| 243.08565| Cytidine                                 | HMDB0000089     | +   | ↓ 1.57 |
| 347.06327| Deoxyguanosine 5'-monophosphate          | HMDB0001044     | +   | ↓ 1.60 |
| 307.56758| Deoxyuridine monophosphate (dUMP)         | ^1196           | +   | ↓ 1.42 |
| 450.34634| Geranylgeranyl pyrophosphate             | ^1288           | +   | ↓ 1.37 |
| 188.11630| Glycyl-L-leucine                         | ^430            | +   | ↓ 1.51 |
| 283.09183| Guanosine                                | HMDB0000133     | +   | ↓ 2.27 |
|     | Metabolite                              | HMDB ID       | FC     | p-Value |
|-----|-----------------------------------------|---------------|--------|---------|
| 247.14209 | Hydroxybutyrylcarnitine                 | HMDB0013127   | +      | ↓ 4.23  |
| 507.57505 | Inosine triphosphate                    | ^1236         | +      | ↓ 1.06  |
| 228.14756 | Leucylproline                           | ^468          | +      | ↓ 2.26  |
| 453.28596 | Lyso phosphatidylethanolamine (16:0)    | HMDB0011473   | +      | ↓ 1.40  |
| 479.30161 | Lyso phosphatidylethanolamine (18:1)    | HMDB0011476   | +      | ↓ 2.24  |
| 529.31731 | Lyso phosphatidylethanolamine (22:4)    | HMDB0011493   | +      | ↓ 3.81  |
| 525.28588 | Lyso phosphatidylethanolamine (22:6)    | HMDB0011496   | +      | ↓ 1.35  |
| 121.91751 | Nicotinamide (nicotinamide)             | ^517          | +      | ↓ 1.31  |
| 122.58708 | Nicotinic acid                          | ^519          | +      | ↓ 1.26  |
| 132.02470 | Oxaloacetic acid                        | ^1353         | +      | ↓ 1.90  |
| 793.56342 | PE(20:1(11Z)/20:4(8Z,11Z,14Z,17Z))      | ^60664        | +      | ↓ 3.34  |
| 437.29129 | PE(P-16:0e/0:0)                          | HMDB0011152   | +      | ↓ 1.21  |
| 193.09526 | Phenylacetylglycine                     | ^540          | +      | ↓ 6.82  |
| 164.04758 | Phenylpyruvic acid                      | ^1389         | +      | ↓ 1.47  |
| 390.35369 | Phosphoribosyl-pyrophosphate            | ^264          | +      | ↓ 1.08  |
| 225.94431 | Porphobilinogen                         | ^1330         | +      | ↓ 2.71  |
| 143.09489 | Proline betaine                         | HMDB0004827   | +      | ↓ 2.05  |
| 218.12684 | Propionylcarnitine                      | ^884          | +      | ↓ 1.26  |
| 383.10805 | Succinyladenosine                       | HMDB0000912   | +      | ↓ 1.04  |
| 241.91824 | Thymidine                               | ^1230         | +      | ↓ 2.05  |
| 112.02762 | Uracil                                  | HMDB0000300   | +      | ↓ 1.37  |
| 281.04194 | 1-Methylenosine                         | ^105          | -      | ↑ 1.24  |
| 216.03884 | 2-C-Methyl-D-erythritol 4-phosphate     | ^64013        | -      | ↑ 2.38  |
| 133.03601 | L-Aspartic acid                         | ^462          | -      | ↑ 1.89  |
| 131.06803 | Creatine                                | ^357          | -      | ↑ 1.44  |
| 260.02867 | Glucose-6-phosphate                     | HMDB0001401   | -      | ↑ 3.90  |
| 169.04910 | Norepinephrine                          | ^318          | -      | ↑ 2.04  |
| 246.04947 | Phosphatidyleglycerol                   | ^186          | -      | ↑ 1.82  |
| 226.08094 | Porphobilinogen                         | ^1330         | -      | ↑ 1.82  |
| 168.02694 | Uric acid                               | ^753          | -      | ↑ 1.80  |
| 233.90871 | 5-Methoxytryptophan                     | ^2613         | -      | ↑ 1.56  |
| 283.09054 | 8-Hydroxy-deoxyguanosine                | HMDB0003333   | -      | ↑ 1.82  |
| 249.96666 | γ-Glutamylcysteine                      | ^427          | -      | ↓ 1.99  |
| 222.01841 | L-Cystathionine                         | ^1829         | -      | ↓ 1.48  |
| 204.02472 | L-Tryptophan                            | ^1830         | -      | ↓ 1.25  |
| 189.06253 | N-Acetyl-L-glutamic acid                | ^1832         | -      | ↓ 1.78  |
| 218.05446 | N-Acetylsertotonin                      | ^313          | -      | ↓ 1.50  |
| 347.06214 | Adenosine monophosphate (AMP)           | HMDB0000045   | -      | ↓ 1.54  |
| 317.95390 | Coenzyme Q2                             | ^379          | -      | ↓ 1.45  |
| 148.07208 | Mevalonic acid                          | ^1338         | -      | ↓ 1.82  |
| 167.02385 | Quinolinic acid                         | ^1262         | -      | ↓ 1.53  |

Putative metabolites were annotated by cross-referencing the online databases including Human Metabolome Database (HMDB), Metlin (*) and mzCloud (^). Symbol: (↑) increased; (↓) decreased. Abbreviation: ESI (electrospray ionization); FC (fold change); m/w (molecular weight). The list was sorted according to ionization mode, up- or downregulation, followed by alphabetical order.
projections were not over-fit by OPLS-DA; while the permutation test for R²Y between 200TRF and control groups was insignificant (Table 1). For the former two group comparisons, Q² and R²Y values based on cross-validation were high (>70%), indicating that the projections had good predictability (Erita et al. 2017).

A total of 190 metabolites were differentially expressed between MI rats and control groups with 84 of them were annotated by cross-referencing to the online databases (Table 2). On the other hand, a total of 85 metabolites were differentially expressed between 200TRF+MI and MI groups with 37 of them were annotated. Since OPLS-DA did not produce a distinct separation between 200TRF and control groups, the number of DEMs, as well as metabolites annotation procedures, were not performed.

Table 3 shows the list of annotated DEMs in between MI and control groups. Out of 84 metabolites, 64 were detected in positive ion mode while another 20 metabolites were found in negative ion mode.

Table 4 lists the annotated DEMs in between 200TRF+MI and MI groups. From 37 annotated metabolites, 30 and 7 metabolites were detected in positive and negative modes, respectively.

Pathway analysis based on annotated DEMs showed that MI and TRF supplementation affected several metabolic pathways in the rats’ myocardium. Comparing between MI and control as well as 200TRF+MI and MI groups, pathways that are relevant to both group comparisons include cysteine and methionine metabolism, nicotinate and nicotinamide metabolism, pentose phosphate pathway, as well as starch and sucrose metabolism (Figure 4).

Since MI induced by ISO administration produced marked myocyte loss as well as increased fibrosis within the subendocardium of rat’s left ventricular free wall (Brooks & Conrad 2009), this part of the heart was selected for metabolomic analysis in the current study. Metabolomic analysis utilizing UHPLC-Orbitrap MS followed by multivariate analysis using the OPLS-DA technique could differentiate and identify metabolic changes in the myocardial tissue of control, MI and 200TRF+MI rats. Interestingly, non-supplemented MI resulted in alterations of different sets of biochemical processes than TRF-supplemented MI rats. This indicates that TRF might protect against MI from biochemical aspects dissimilar than during the disease development itself. Nevertheless, four metabolic pathways were observed to be affected in both MI vs control and 200TRF+MI vs MI group comparisons.

Cysteine and Methionine Metabolism
Methionine is an essential amino acid for humans with a recommended daily intake of 15 mg/kg/day (Kurpad et al. 2003). Metabolism of methionine would produce intermediates such as S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), homocysteine and L-cystathionine (Poloni et al. 2015). The enzyme cystathionine-β-synthase (CBS) is responsible for the synthesis of L-cystathionine from homocysteine (Koutmos et al. 2010). Furthermore, trans-sulfuration process of L-cystathionine by cystathionine-γ-lyase will transform it into cysteine, an initial substrate in glutathione (GSH) synthesis (Kabil et al. 2011).

Deficiency of CBS causes hyperhomocysteinemia, which is an established risk factor for cardiovascular disease that includes pre-mature MI (Ukachukuw et al. 2012). An earlier study reported that CBS could be allosterically activated by SAM which also stabilized the enzyme and improved cell viability under oxidative stress condition (Prudova et al. 2006). In the current study, since the reduction of L-cystathionine as well as SAM occurred in the myocardial tissue of MI rats by 1.48 and 1.20 times compared to control, respectively, it gave an impression that destabilization of CBS might have occurred in MI. Using metabolomics approach in rat MI model induced by coronary artery ligation, Prudova et al. (2006) reported that myocardial SAM concentration decreased gradually in a time-dependent manner and downregulated significantly at day 10 post-MI (Nam et al. 2017), which was consistent with our findings.

TRF might protect myocardial tissue against MI by reversing these changes. In the current study, MI rats receiving TRF supplementation for 12 weeks had a significant elevation of L-cystathionine as well as SAM by 1.97 and 1.95 times compared to MI group, respectively. These changes reflected that TRF might be able to activate CBS by increasing the expression of SAM, its allosteric activator. Subsequently, this would enhance conversion of homocysteine to L-cystathionine as well as drive glutathione production via cysteine synthesis. Indeed, the relation between vitamin E and SAM had been demonstrated in earlier work. When mouse primary hepatocyte was exposed to ethanol, the resulting increased oxidative stress led to a marked decrease of SAM/SAH ratio by 53% while treatment with vitamin E significantly prevented this alteration (Gyamfi & Wan 2006).

Therefore, inhibition of cysteine and methionine metabolism in MI might have a direct impact on the antioxidative system via GSH synthesis due to decreased SAM production that was successfully reversed by TRF supplementation.

Nicotinate and Nicotinamide Metabolism
MI is strongly related to increased production of reactive oxygen species including hydrogen peroxide (H₂O₂) that leads to oxidative stress (Bae et al. 2016). The process of neutralizing H₂O₂ into water molecule requires concerted efforts by glutathione and co-factor nicotinamide adenine dinucleotide phosphate (NADP) (Arai et al. 2017; Berkhohlz et al. 2008). NADP is generated from nicotinate and nicotinamide metabolism. In this biochemical process, nicotinic acid and nicotinamide would be converted to nicotinamide adenine dinucleotide (NAD) via the Preiss-Handler pathway and salvage pathway, respectively (Singhal & Cheng 2019). This would be followed by
| m/w     | Putative Metabolites                                      | Identifiers     | ESI | FC    |
|---------|---------------------------------------------------------|-----------------|-----|-------|
| 260.13719 | γ-Glutamyl-L-isoleucine                                   | HMDB0011170     | +   | ↑ 1.65|
| 155.03487 | L-Histidine                                              |                 |     | ↑ 1.59|
| 399.37128 | S-Adenosylmethionine                                     |                 |     | ↑ 1.95|
| 267.09692 | Adenosine                                                | HMDB0000050     | +   | ↑ 1.53|
| 511.49656 | Cer(d18:0/14:0)                                           | HMDB0011759     | +   | ↑ 1.97|
| 539.52779 | Cer(d18:0/16:0)                                           | HMDB0011760     | +   | ↑ 2.96|
| 543.33311 | Lysophosphatidylcholine(20:4)                            | HMDB0010395     | +   | ↑ 7.90|
| 437.29129 | PE(P-16:0e/0:0)                                           | HMDB0011152     | +   | ↑ 11.28|
| 390.35369 | Phosphoribosyl-pyrophosphate                             |                 |     | ↑ 1.91|
| 218.12684 | Propionylcarnitine                                       |                 | +   | ↑ 1.41|
| 393.86701 | 2-(14,15-Epoxyeicosatrienoyl) glycerol                   |                 |     | ↓ 2.70|
| 214.06244 | 2-Deoxyribose-5-phosphate                                |                 | +   | ↓ 3.17|
| 145.11044 | 3-Dehydroxy carnitine                                     | HMDB0006831     | +   | ↓ 3.38|
| 219.99107 | L-5-Hydroxytryptophan                                    |                 | +   | ↓ 1.41|
| 240.13492 | L-Anserine                                               |                 | +   | ↓ 1.53|
| 158.06931 | L-Dihydropyronotic acid                                  |                 | +   | ↓ 2.56|
| 309.10605 | N-Acetylneuraminic acid                                  | HMDB0000230     | +   | ↓ 1.56|
| 217.97662 | N-Acetylserotonin                                         |                 | +   | ↓ 1.48|
| 206.04355 | (R)-Lipoic acid                                          | HMDB0001451     | +   | ↓ 1.30|
| 260.02988 | Glucose-6-phosphate                                      | HMDB0001401     | +   | ↓ 2.85|
| 111.08001 | Histamine                                                | HMDB0000870     | +   | ↓ 1.50|
| 109.06441 | Hypotaurine                                              |                 | +   | ↓ 1.62|
| 228.14756 | L-UCProleline                                            | HMDB0011175     | +   | ↓ 2.74|
| 121.91751 | Nicotinamide (niacinamide)                               |                 |     | ↓ 6.89|
| 122.58708 | Nicotinic acid                                            |                 | +   | ↓ 3.07|
| 506.26443 | Phosphatidylglycerol                                     | *80008          | +   | ↓ 4.44|
| 143.09489 | Proline betaine                                          | HMDB0004827     | +   | ↓ 2.12|
| 561.79007 | Protoporphyrin IX                                        |                 | +   | ↓ 3.17|
| 299.28268 | Sphingosine                                              | HMDB0000252     | +   | ↓ 1.90|
| 427.36636 | Stearoylcarnitine                                        | HMDB000848      | +   | ↓ 2.05|
| 249.96666 | γ-Glutamylcysteine                                       |                 |     | ↑ 1.34|
| 222.01841 | L-Cystathionine                                          |                 |     | ↑ 1.97|
| 189.06253 | N-Acetyl-L-glutamic acid                                 |                 |     | ↑ 2.27|
| 347.06214 | Adenosine monophosphate (AMP)                            | HMDB0000045     | -   | ↓ 1.48|
| 244.06850 | Uridine                                                  | HMDB0000296     | -   | ↓ 1.61|
| 131.06803 | Creatine                                                 |                 | +   | ↓ 1.42|
| 226.08094 | Porphobilinogen                                          |                 | +   | ↓ 1.70|

Putative metabolites were annotated by cross-referencing the online databases including Human Metabolome Database (HMDB), Metlin (‘*’) and mzCloud (‘^’). Symbol: (↑) increased; (↓) decreased. Abbreviation: ESI (electrospray ionization); FC (fold change); m/w (molecular weight). The list was sorted according to ionization mode, up- or downregulation, followed by alphabetical order.
FIGURE 3. Score plot for Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA). (A-B) ESI (+) and (-) for MI vs control; (C-D) ESI (+) and (-) for 200TRF vs control; (E-F) ESI (+) and (-) for 200TRF+MI vs MI. Each group consisted of three biological replicates with three technical replicates for every biological sample. Each point represents one replicate.
phosphorylation of NAD to NADP by NAD kinase (Love et al. 2015).

In the current study, reduction of nicotinic acid and nicotinamide (or niacinamide) occurred by 1.26 and 1.31 times, respectively, in MI compared to control. Incidentally, γ-glutamylcysteine molecule, an intermediate in GSH synthesis (Wu et al. 2004), was also found to be decreased by 1.99 times indicating a disturbance in new GSH molecule production. From these findings, we could infer that myocardium might rely on pre-existing GSH in combating oxidative stress to form oxidized glutathione (GSSG), thus lowering the GSH itself. In fact, previous study reported that myocardial GSH concentration was reduced by approximately 40% during MI due to conversion from GSH to GSSG (Li et al. 2009). Reduced nicotinic acid and nicotinamide in heart homogenate of MI rats in the current study could be explained as a physiological response to limit oxidative damage where these molecules might be channeled into NAD and NADP syntheses to keep the pre-existing glutathione in its reduced state.

Supplementation of MI rats with 200 mg/kg/day TRF for 12 weeks resulted in further reduction of nicotinic acid and nicotinamide by 3.07 and 6.89 times compared to MI rats, respectively. However, they were accompanied by 1.34 times increased of γ-glutamylcysteine expression in MI rats receiving vitamin E supplementation at the dose of 1,500 nmol/g to 65.3 ± 5.0 nmol/g (p<0.05) as compared to non-supplemented MI rats (Hill et al. 2005).

**Pentose Phosphate Pathway**

Myocardial tissue damage during MI is contributed by apoptosis and necrosis that lead to nuclear and mitochondrial deoxyribonucleic acid (DNA) release as well as increased purine metabolism (Casey et al. 2007; Fauconnier et al. 2011). This process had been substantiated in the previous study whereby plasma DNA concentration derived from both nucleus and mitochondria elevated significantly in acute MI patients compared to control group (Wang et al. 2015). As a result, serum metabolites involving purine degradation were also increased significantly (Al-Shamiri et al. 2009). Since there is a flux of damaged DNA and metabolites of purine degradation into the bloodstream from the infarcted heart, their concentrations naturally would decrease in the tissue itself. Certainly, metabolic profile in the current study found that myocardial adenosine and adenosine monophosphate (AMP) were decreased by 2.00 and 1.54 times, respectively, in MI rats compared to control.

Pentose phosphate pathway is closely related to purine metabolism. The molecule phosphoribosyl-pyrophosphate (PRPP), which represented the pathway, is vital in purine salvage process (McCarty et al. 2018). Availability of PRPP closely affected the rate of adenosine salvage in rat cardiomyocytes (Dow et al. 1987). Adenosine kinase could transform adenosine directly to AMP. Additionally, adenosine could be first converted to adenine before adenosine phosphoribosyltransferase enzyme catalyzed an AMP-producing reaction that requires ribose phosphate moiety from PRPP (Buckoreell et al. 2011; Frenguelli et al. 2019). Hence, in the current study, reduction of adenosine and AMP accompanied by reduced PRPP by 1.08 times indicated that there was a reduced purine salvage process due to impaired pentose phosphate pathway in MI.

Conversely, MI rats receiving TRF supplementation had a significant reversal of these changes than untreated MI group with an elevation of 1.53, 1.48 and 1.91 times each for adenosine, AMP and PRPP. These findings suggested that the cardioprotective mechanism of TRF might be related to the activation of the purine salvage pathway.

![Diagram](Image)
pathway in order to regenerate new substrates for DNA synthesis. Salvage pathway requires lower energy as compared to de novo purine synthesis (Moffatt & Ashihara 2002). Cardiomyocytes had been reportedly suffered from reduced energy production in a hypoxic environment (Qiu et al. 2018). As such, this energy-saving process is very crucial in MI.

**Starch and Sucrose Metabolism**

During prolonged hypoxia, there is a sudden decrease in oxygen supply resulting in cardiac ischemia. The lack of oxygen causes mitochondria to function sub-optimally and disrupts the tricarboxylic acid (TCA) cycle, long-chain fatty acids β-oxidation and electron transport through the electron transport chain resulting in rapid shortages of NADH, FADH2, oxaloacetate, and malate dehydrogenases) and electron transfer through the electron transport chain, disrupting the tricarboxylic acid cycle (isocitrate, α-ketoglutarate, succinate, fumarate, and malate dehydrogenases) and ATP synthesis (mitochondrial F0F1 ATP synthase) in aged mouse brain (Park et al. 2008). Perhaps, a similar suggested that blood glucose concentration might be elevated as well. A cross-sectional study in human subjects found that the degree of MI severity was closely related to higher blood glucose readings at the time of hospital admission regardless of whether the patients had prior diabetes mellitus or not. MI patients with moderately high (7.8-11.0 mmol/L) and severely high (≥11.1 mmol/L) blood glucose had higher mortality risk than their euglycemic counterparts (Zhao et al. 2017). Furthermore, a study by Ali et al. (2016) utilizing gas chromatography-mass spectrometry and proton nuclear magnetic resonance techniques found that serum α- and β-glucose levels in acute MI patients were significantly raised, with p-value <0.0001 for each observation.

On the contrary, myocardial glucose-6-phosphate was significantly reduced by 2.85 times in 200TRF+MI compared to MI rats. From this finding, we could deduce that TRF supplementation might revert the anaerobic glycolysis back to aerobic process as well as improve overall glucose utilization within the myocardial tissue. Earlier study demonstrated that supplementation of α- and γ-tocopherol mixture significantly increased transcription of genes related to glycolysis (aldolase, enolase, and glucose phosphate isomerase), TCA cycle (isocitrate, succinate, and malate dehydrogenases) and ATP synthesis (mitochondrial F0F1 complexes of ATP synthase) in aged mouse brain (Park et al. 2008). Perhaps, a similar
mechanism might have occurred in the cardiac tissue of 200TRF+MI rats whereby TRF might improve mitochondrial functions which directly enhanced aerobic glycolysis, TCA cycle as well as energy production in the form of ATP synthesis.

The roles of these metabolic pathways on the pathogenesis of MI and cardioprotective mechanisms of TRF worth further confirmatory studies to provide new insights for prevention and treatment of MI. Figure 5 summarizes the metabolic profile and biochemical pathway changes that were involved in MI and the effects of TRF supplementation.

CONCLUSION

Supplementation of TRF increased serum α-tocopherol but not serum tocotrienol possibly due to the differences in the bioavailability of vitamin E’s vitamers. In ISO-induced MI rats, TRF reversed the changes of metabolic pathways involving cysteine and methionine metabolism, nicotinate and nicotinamide metabolism, pentose phosphate pathway as well as starch and sucrose metabolism that were altered by MI. Ultimately, the changes brought by TRF might positively affect myocardial antioxidantive system via new GSH production, activate adenosine salvage pathway possibly for DNA synthesis, and enhance glucose utilization through aerobic glycolysis.

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