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Original article

Attenuation of low-grade chronic inflammation by phytonutrients: A computational systems biology analysis

V.A. Shiva Ayyadurai [a, *], Prabhakar Deonikar [a], Raveendhara R. Bannuru [b]

[a] Systems Biology Group, CytoSolve Research Division, CytoSolve, Inc., Cambridge, MA, USA
[b] Center for Treatment Comparison and Integrative Analysis (CTCIA), Division of Rheumatology, Tufts Medical Center, Boston, MA, USA

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SUMMARY

Background: Low-grade chronic inflammation (LGCI) is a strong and independent risk factor for many chronic diseases, like cardiovascular, musculoskeletal, metabolic, and neurological conditions. Dietary intervention studies have reported evidence for the role of plant-derived flavonoids in modulation of LGCI. This research explores the efficacy of Fruit/Berry/Vegetable (FBV) juice powder on LGCI.
Methods: The study employs computational systems biology: 1) to identify biomolecular mechanisms of LGCI; 2) to identify the bioactive compounds of FBV juice powder and their specific effects on mechanisms of LGCI; and, 3) to predict the quantitative effects of those bioactive compounds on LGCI.
Results: Four molecular pathways that are affected by the compounds of FBV include: 1) TNF-α production; 2) CCL2 production; 3) IL-1β production; and 4) reactive oxygen species production. The bioactive compounds including luteolin, epicatechin, epigallocatechin gallate, lycopene, quercetin, vitamin A, vitamin C and vitamin E in FBV significantly lowered TNF-α production, CCL2 production, IL-1β production, and reactive oxygen species production.
Conclusion: FBV provides a combination of active ingredients that synergistically affect multiple modalities of low grade chronic inflammation to help improve blood circulation and energy levels, and lower muscle soreness.

1. Introduction

Low-grade chronic inflammation (LGCI) is defined by the persistent presence of elevated levels of circulating cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNFα), and interleukin-1 beta (IL-1β) that promote disease progression [1]. LGCI is a strong and independent risk factor for many chronic diseases, like cardiovascular, musculoskeletal, metabolic, and neurological conditions [2–4].

LGCI drives the pathogenesis of osteoarthritis by accelerating catabolic responses in chondrocytes, inflammation of the synovial membrane, and promoting pain in the joint [5]. Inflammatory mediators including pro-inflammatory cytokines, complement proteins, toll-like receptors, etc. are also implicated in pathogenesis of several age-related neurodegenerative diseases such as dementia and Alzheimer's disease [6–8]. In addition, LGCI in metabolically active tissues such as liver, pancreas, and adipose tissue leads to metabolic disorders such as obesity, insulin resistance and consequently diabetes mellitus, and fatty liver disease [9,10].

Diet is a significant contributing factor that modulates systemic LGCI [4,11]. Various compounds of the diet including dietary fats, dietary carbohydrates, and micronutrients affect LGCI [12,13]. Saturated fatty acids from dietary fats have been shown to promote pro-inflammatory cytokine-induced metabolic stress that leads to pathologies such as type II diabetes mellitus and obesity [14]. On the other hand, polyunsaturated omega 3 fatty acid derived metabolites have been shown to counteract the pro-inflammatory state [15]. Glycemic load from dietary carbohydrates plays a significant role in the pathogenesis of type II diabetes mellitus and cardiovascular disease via LGCI and oxidative stress [16]. Diet consisting of high fiber content has been shown to effectively reduce pro-inflammatory biomarkers [17] whereas diet with low fiber content led to a pro-inflammatory environment [18].

* Corresponding author. Systems Biology Group, CytoSolve Research Division, 701 Concord Avenue, Cambridge, MA, 02138, USA.
E-mail address: vashiva@cytosolve.com (V.A.S. Ayyadurai).

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Plant-derived micronutrients such as flavonoids have been ascribed anti-oxidant and anti-inflammatory properties [4,19]. Dietary intervention studies have reported evidence for the role of plant-derived flavonoids in modulation of pro-inflammatory cytokines such as TNF-α and C reactive protein (CRP) [20–23]. Including phytonutrient rich fruit juices in diets of athletes was shown to improve athletic performance [24]. Fruits, berries and vegetables are significant sources of phytonutrients, and their effect has been extensively studied in the clinic [25]. A series of randomized controlled trials have shown that dietary supplementation of dehydrated fruit, berries and vegetables (FBV) juice concentrates increased the bioavailability of plant-based flavonoids [26–28], carotenoids and vitamins [25]. The supplementation of FBV juice powder also lowered the biomarkers of systemic LGCI such as: 1) DNA damage in peripheral blood lymphocytes [29]; 2) monocyte chemotactic protein-1 (MCP-1); 3) macrophage inflammatory protein 1-β (MIP-1β); 4) regulated-on-activation-normal-T-cell-expressed-and-secreted (RANTES) [30]; 5) TNF-α [31]; and, 6) reactive oxygen species (ROS) [32]. These studies demonstrate an accumulating evidence to support the role of plant based flavonoids from FBV juice powder on the mitigation of LGCI.

1.1. Research aim

While the clinical data provides empirical evidence of the value of FBV juice powder, the mechanisms of action of phytonutrients in FBV juice powder on LGCI are not well understood. Such understanding demands the need to uncover complex molecular systems that conventional *in vitro* and *in vivo* methods find difficult to elicit. Emerging modern bioinformatics and computational systems biology methodologies, performed in silico - meaning computationally, provide the opportunity to explore such complex systems. The study herein employs a computational systems biology framework to: 1) identify potential molecular mechanisms involved in LGCI affected by bioactive compounds in FBV juice powder; 2) identify the critical bioactive compounds in FBV likely affecting LGCI; and, 3) quantitatively predict the synergistic effect of such bioactive compounds in attenuating LGCI.

1.2. CytoSolve: a framework for computational systems biology

CytoSolve is a well-established computational systems biology framework of technology and processes that provides the capability to derive molecular mechanisms of action; to create quantitative and predictive models of those mechanisms; and, to employ the resultant models to simulate complex biomolecular phenomena [33–37]. In neurovascular studies, the CytoSolve framework elicited and derived a multi-layered engineering molecular systems architecture integrating the anatomy of the neuro-vascular unit, molecular mechanisms, and disease to demonstrate the commonality of multiple neurovascular diseases as communication dysfunctions in common molecular signaling sub-systems and compounds [36]. In oncology, CytoSolve's capability has been employed for the in silico modeling of pancreatic cancer to identify and optimize a multi-combination therapeutic that was subsequently allowed for clinical trials by the United States Food and Drug Administration [38], has been used to identify the molecular systems architecture of interactome in acute myeloid leukemia (AML) microenvironment [39], and has been independently recognized by leading cancer researchers as a platform for developing multi-combination therapies [35]. In cardiovascular research, CytoSolve has been used to accurately model the release of nitric oxide (NO) production in endothelial cells subjected to shear stress [37]. In the area of plant biology, CytoSolve enabled the quantitative molecular systems understanding of C1 metabolism - a critical system of molecular pathways inherent to all plants, fungi and bacteria - to understand the systemic effects oxidative stress and genetic modification on C1 metabolism in soy [40–43]. Recently, CytoSolve was used to discover and model the mechanisms of immunomodulatory effect of bioactive compound in green tea on organ transplant tolerance [44].

In this present study, the CytoSolve framework is used to derive the molecular mechanisms of LGCI; identify the key bioactive compounds in FBV juice powder relevant to LGCI; and, mathematically model the LGCI biochemical pathways in order to quantify the individual and synergistic effects of bioactive compounds towards a molecular systems understanding of the clinical effect of FBV juice powder on LGCI.

2. Methods

This section describes the methodology used to identify the mechanisms of action of LGCI and to quantitatively predict the effects of the bioactive compounds of FBV on such mechanisms.

2.1. Workflow

There are six (6) steps to the methodology, as illustrated in Fig. 1a, and itemized below:

1) Organize and curate data from the scientific literature
2) Extract molecular pathway diagrams from the curated literature
3) Convert each molecular pathway diagram to a mathematical model
4) Integrate the ensemble of mathematical models to derive an integrated model of LGCI
5) Use the integrated model of LGCI to execute computer simulations
6) Validate the results from the computer simulations with clinical data

2.2. Organize and curate data from the scientific literature

The scientific literature is searched to identify journal papers that contain research on LGCI, molecular pathways of LGCI, bioactive compounds in FBV juice powder, and the effect of bioactive compounds in FBV juice powder on LGCI molecular pathways. Four (4) steps are necessary to organize and curate the journal papers, as itemized below:

1. Create a list of Medical Subject Headings (MeSH) keywords to optimize recall and precision of peer-reviewed articles
2. Search and retrieve the relevant peer-reviewed articles published between January 1980 to March 2018 from PubMed, Medline, and Google Scholar. These set of articles are stored as an “Initial Set” repository
3. Screen the titles and abstracts of articles in the Initial Set repository to identify most relevant articles based on our inclusion criteria. These set of articles are stored as the “Final Set” repository
4. Perform full-length review of peer-reviewed articles from the Final Set repository

Abstracts and unpublished literature were not sought as they have not been peer reviewed adequately to authenticate their results. The literature review inclusion criteria and categorization process are represented in Fig. 1b as per the PRISMA guidelines [45].

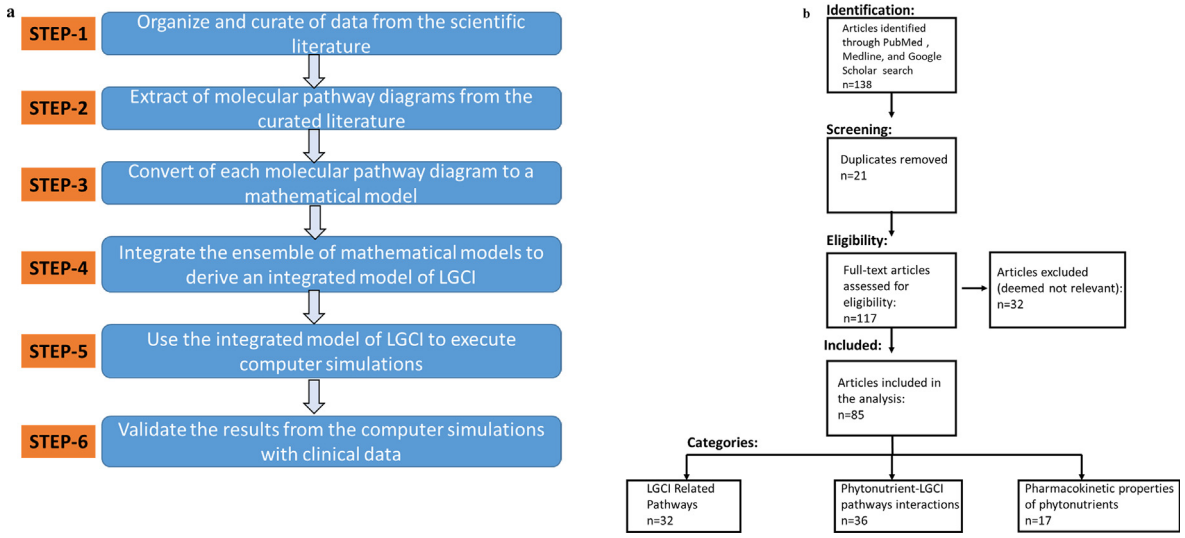

Fig. 1. a. Methodology Overview. The six steps involved in conducting this study are described. Steps 1 and 2 relate to performing systematic literature review to identify molecular pathways involved in low-grade chronic inflammation (LGCI) and the biochemical parameters required for computational modeling of LGCI pathways. Steps 3 to 6 relate to construction of individual LGCI models, integration of individual LGCI models, and simulations of LGCI models. Step 7 relates to validation of LGCI model with the clinical data. b. PRISMA flow diagram. The systematic literature review process included identifying the relevant literature from PubMed, Medline, and Google Scholar. The literature was then filtered to remove duplicate studies. Eligibility of articles for comprehensive review was determined using inclusion criteria detailed in the Method section.

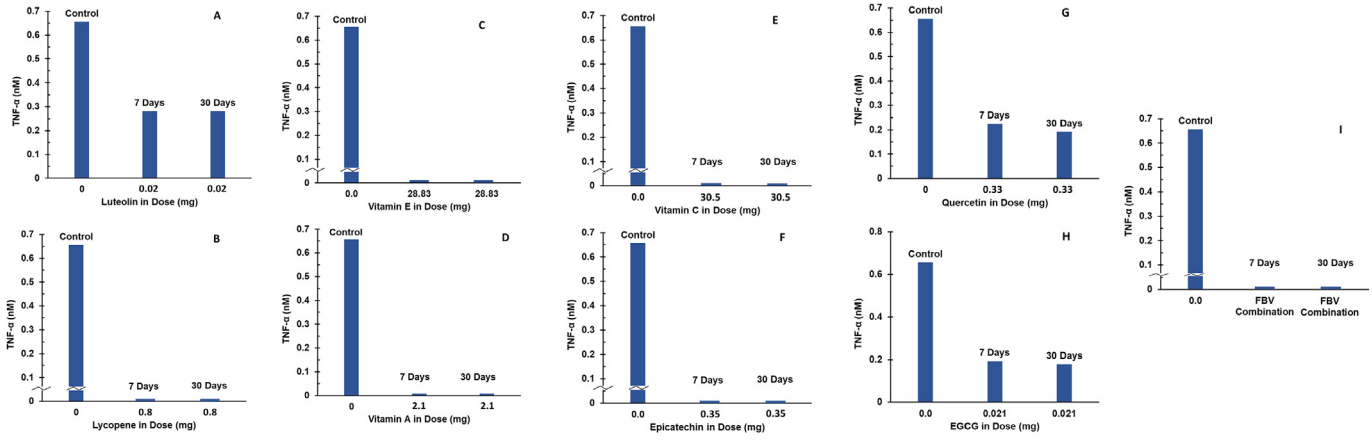

Fig. 2. Individual (panels A–H) and combination effect (panel I) of bioactive molecules from FBV juice powder on TNF-α production in adipocytes over simulations periods of 7 and 30 days. Luteolin (panel A), lycopene (panel B), vitamin E (panel C), vitamin A (panel D), vitamin C (panel E), epicatechin (panel F) and the combination of all bioactive molecules (panel I) reduced the levels of TNF-α over a period of 7 days. Increasing the duration of supplementation to 30 days did not lower the TNF-α levels any further. Supplementation of quercetin (panel G) and EGCG (panel H) lowered TNF-α levels with increased duration of supplementation. FBV – fruit berry and vegetables; TNF- α – Tumor necrosis factor – α; EGCG - epigallocatechin gallate.

The journal articles included in the Final Set were subjected to full-length review and were classified into three groups:

Group 1) Articles on LGCI pathways;
Group 2) Articles on FBV bioactive compounds interacting with LGCI pathways; and,
Group 3) Articles on pharmacokinetic properties of FBV bioactive compounds.

2.3. *Extract molecular pathway diagrams from the curated literature*

Journal articles in Group 1 are reviewed to gather data relevant to molecular pathways of LGCI. The steps to extract and represent molecular pathways diagrammatically are itemized below:

1. Identify and extract:
   a. chemical species involved in LGCI
   b. types of cells involved in LGCI
   c. cellular components (e.g. cytosol, mitochondria, nucleus, etc.) where the chemical species are present in each cell type
2. Identify and diagrammatically represent biochemical interactions in LGCI
3. Interconnect biochemical reactions to create molecular pathway diagram in each cell type involved in LGCI

The list of chemical species and their initial concentrations, the biochemical reactions, and the kinetic parameters involved in the biochemical reactions are provided in sections B1 and B2 of Appendix B.

Journal articles in Group 2 are reviewed to gather data relevant to bioactive compounds of FBV. Following information is extracted:

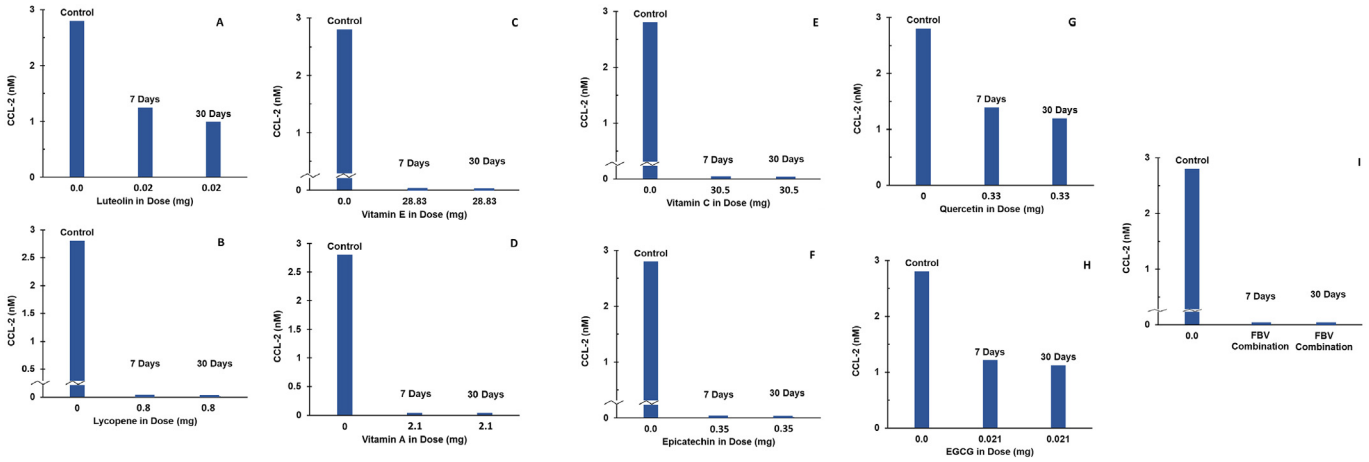

Fig. 3. Individual (panels A–H) and combination effect (panel I) of bioactive molecules from FBV juice powder on CCL2 production in adipocytes over simulations periods of 7 and 30 days. Luteolin (panel A), lycopene (panel B), vitamin E (panel C), vitamin A (panel D), vitamin C (panel E), epicatechin (panel F) and the combination of all bioactive molecules (panel I) reduced the levels of CCL2 over a period of 7 days. Increasing the duration of supplementation to 30 days did not lower the CCL2 levels any further. Supplementation of quercetin (panel G) and EGCG (panel H) lowered CCL2 levels with increased duration of supplementation. FBV – fruit berry and vegetables; CCL2 – C–C motif chemokine ligand 2; EGCG - epigallocatechin gallate.

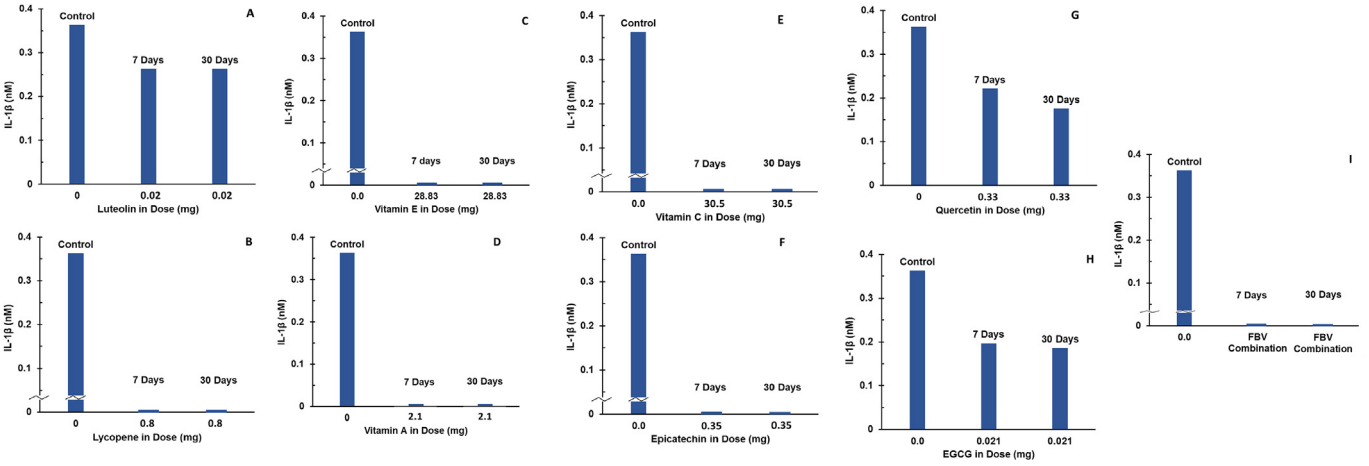

Fig. 4. Individual (panels A–H) and combination effect (panel I) of bioactive molecules from FBV juice powder on IL-1β production in adipocytes over simulations periods of 7 and 30 days. Luteolin (panel A), lycopene (panel B), vitamin E (panel C), vitamin A (panel D), vitamin C (panel E), epicatechin (panel F) and the combination of all bioactive molecules (panel I) reduced the levels of IL-1β over a period of 7 days. Increasing the duration of supplementation to 30 days did not lower the IL-1β levels any further. Supplementation of quercetin (panel G) and EGCG (panel H) lowered IL-1β levels with increased duration of supplementation. FBV – fruit berry and vegetables; IL-1β – Interleukin 1β; EGCG - epigallocatechin gallate.

1. Bioactive compounds of FBV juice powder
2. Concentration levels of bioactive compounds in FBV juice powder

Bioactive compounds and their concentration levels are provided in Table A3 of Appendix A.

Journal articles in Group 3 are reviewed to extract following information:

1. Pharmacokinetics of bioactive compounds of FBV
2. Reaction rate constants of biochemical reactions between bioactive compounds of FBV juice powder and their molecular targets in the LGCI molecular pathways

The kinetic parameters used in this study are derived using principles of Michaelis–Menten kinetics that are based on the steady-state approximation of the biochemical reactions [46,47]. This information is provided in section B1 and B2 of Appendix B.

2.4. Convert each molecular pathway diagram to a mathematical model

The steps to convert molecular pathway diagrams to mathematical models are itemized below:

1. Convert biochemical reactions involved in each of the molecular pathway into ordinary differential equations (mathematical expressions that describe the rate of change)
2. Represent each molecular pathway of LGCI as a system of ordinary differential equations
3. Encode the system of differential equations in a computer software source code format known as Systems Biology Markup

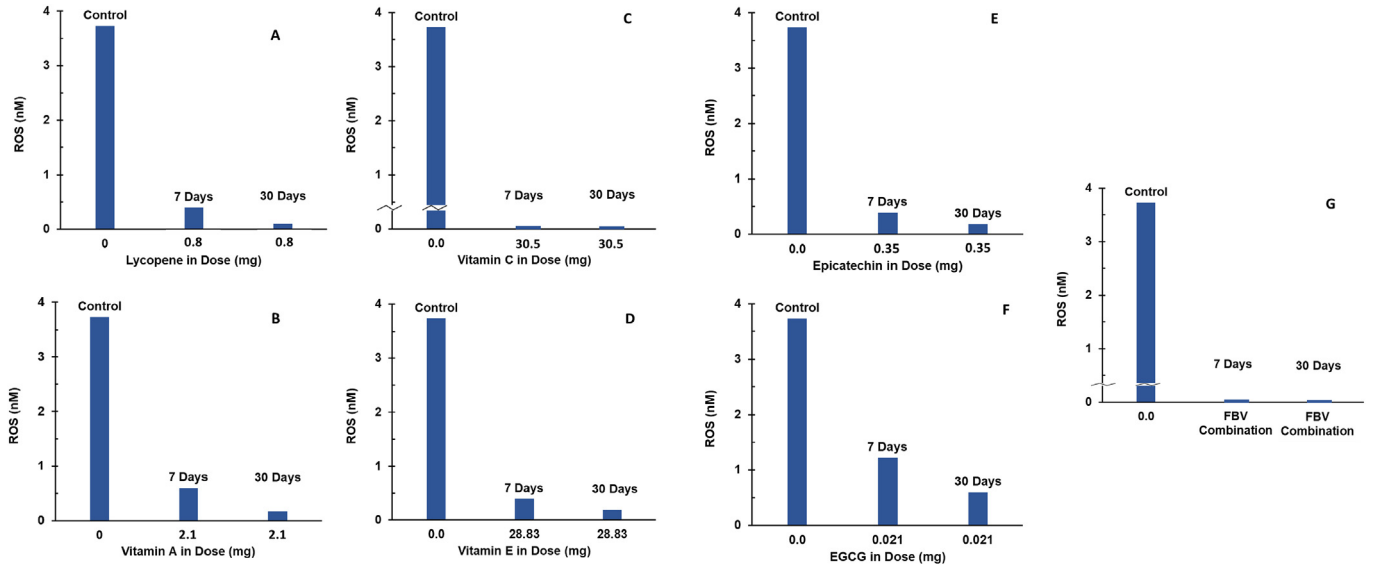

Fig. 5. Individual (panels A–F) and combination effect (panel G) of bioactive molecules from FBV juice powder on ROS production in endothelial cells over simulations periods of 7 and 30 days. Lycopene (panel A), vitamin A (panel B), vitamin C (panel C), vitamin E (panel D), epicatechin (panel E), EGCG (panel F) and the combination of all bioactive molecules (panel G) reduced the levels of ROS over a period of 7 days and increasing the duration of supplementation to 30 days lower the ROS levels even further. FBV – fruit berry and vegetables; ROS – reactive oxygen species; EGCG - epigallocatechin gallate.

Language (SBML) [48] to construct a mathematical model for a particular molecular pathway of LGCI
4. Store each model as a separate SBML file

2.5. Integrate the ensemble of mathematical models to derive an integrated model of LGCI

In order to create an integrative quantitative model of LGCI, it is necessary to mathematically couple the solutions across the ensemble of individual molecular pathway models. Such mathematical coupling is performed using the CytoSolve [34,44,49] computational engine, which is described in detail in Ayyadurai and Dewey, 2011 [33]. The computational architecture of CytoSolve enables the integration of plurality of molecular pathway models [33,49]. The CytoSolve architecture and the software layers that enable the coupling of multiple molecular pathway models to produce the integrative solution are described in detail in Ayyadurai et al., 2021 [44].

The steps to integrate the ensemble of mathematical models of LGCI are listed below:

1. Upload individual SBML files, constructed in Section 2.4, to CytoSolve engine
2. Update the initial conditions for the molecular species in all the mathematical models in the graphical user interface
3. Update simulation period was specified in the graphical user interface
4. Review and confirm molecular species and reaction duplicates across all the LGCI models in the graphical user interface
5. Commence integration of individual models of LGCI

2.6. Use the integrated model of LGCI to execute computer simulations

The integrated model of LGCI includes two major sub-systems: 1) Pro-inflammatory model; and, 2) Reactive oxygen species production model. The effect of individual bioactive compounds and combination of the bioactive compounds in FBV juice powder was assessed by estimating the cellular concentration levels of pro-inflammatory biomarkers - TNF-α, C-C Motif Chemokine Ligand 2 (CCL2), IL-1β -, and oxidative stress biomarker - ROS - in presence and absence of the FBV supplementation.

2.6.1. Computer simulations

The following computer simulations were performed:

1. Effect of individual and combination of bioactive compounds in FBV juice powder on TNF-α production
2. Effect of individual and combination of bioactive compounds in FBV juice powder on CCL2 production
3. Effect of individual and combination of bioactive compounds in FBV juice powder on IL-1β production
4. Effect of individual and combination of bioactive compounds in FBV juice powder on ROS production

For a computational system biology analysis wherein the simulation of biochemical reactions is being executed and the governing equations are well known, as is in this case, the error bounds are set prior to executing simulations [50,51]. Herein, the error bounds are set to 10^{-6} prior to the execution of simulations. This means that the solutions to the governing equations used in the simulation of specific biochemical reactions must be within these error bounds. Therefore, in silico – computational – results from such simulations will not have error bars, which customarily appear in results reported from *in vitro* and *in vivo* experimental studies [52].

2.6.2. Control conditions

Under control conditions, the bioactive compounds of FBV juice powder levels are set to zero. For the pro-inflammatory model, the adipocyte cell/pancreatic β cell was assumed to be under inflammatory conditions. Under such inflammatory conditions, IL-6 levels, which under normal physiological conditions are in the range of 0.00007–0.00014 nM [53,54], are found to increase to

0.00125 nM [55]. IL-6 upregulates TNF- α, CCL2, and IL-1β, which are the three pro-inflammatory LGCI biomarkers [56,57]. Using the initial condition of IL-6 = 0.00125 nM, the pro-inflammatory model was simulated over a period of 7 and 30 days to predict the concentrations of TNF- α, CCL2, and IL-1β at the end of the simulation period.

For the ROS production model, the endothelial cell was assumed to be in an inflammatory state, where NADPH activity – a source of ROS formation - is found to increase by approximately two-fold [58]. Using the initial condition of doubled NADPH activity under inflammatory conditions, the ROS production model was simulated over a period of 7 and 30 days to predict the ROS concentrations under control condition.

2.6.3. Steps for executing the computer simulations of the integrated model of LGCI

The following steps are performed to execute the computer simulations:

1. Input biochemical reactions for interaction between bioactive compounds of FBV juice powder and LGCI molecular pathways
2. Input the kinetic rate constants for each of the biochemical reaction
3. Input the initial concentrations for each of the molecular species in the biochemical reactions
4. Input the time period for the simulation of integrative models, and dose levels of bioactive compounds in FBV juice powder
5. Execute the integrative model of LGCI under control conditions
6. Execute the integrative model of LGCI in presence of bioactive compounds of FBV juice powder

The list of biochemical reactions, the rate equations, and the kinetic rate constants of the biochemical reactions, and initial concentrations for the molecular species involved in the biochemical reactions are listed in Appendix B. A dosage equivalent of six capsules of FBV juice powder, consistent with the label use instructions for the retail product, provided in Table A3 of Appendix A. Several previous clinical studies have used similar dose regimen of FBV juice powders studies [26,31,59]. The simulations were performed for a period of 7 days and 30 days. The bioactive compounds in FBV juice powder were administered at the beginning of the simulations, starting at t = 0s, and were maintained at same levels for the duration of the simulations.

2.6.4. Simulation outputs

The output from the above simulations includes the following:

1. Time-dependent concentration profile of TNF-α
2. Time-dependent concentrations CCL2
3. Time-dependent concentrations IL-1β
4. Time-dependent concentrations ROS

2.6.5. Analysis of simulation output

The steps for analyzing simulation output data are itemized below:

1. Export the raw data to Microsoft Excel
2. Extract the steady state levels of TNF-α, CCL2, IL-1β, and ROS
3. Plot state levels of TNF-α, CCL2, IL-1β, and ROS as a function of simulation time (7 days or 30 days) in presence and absence of individual bioactive compounds and combination of bioactive compounds in FBV juice powder

2.7. Validate the results from the computer simulations with clinical data

In a placebo-controlled randomized clinical study, Lamprecht et al. [31] showed that supplementation of FBV juice powder over a period of 8 weeks significant reduction in the levels of LGCI biomarker TNF-α. The steps to validate the integrative model of LGCI with these clinical findings are listed below:

1. Input biochemical reactions for interaction between bioactive compounds of FBV juice powder and LGCI molecular pathways
2. Input the kinetic rate constants for each of the biochemical reaction
3. Input the initial concentrations for each of the molecular species in the biochemical reactions
4. Input the time period 8 weeks (56 days) for the simulation of integrative models
5. Input dose levels of bioactive compounds in FBV juice powder same as those used in the Lamprecht et al. [31] clinical study
6. Execute the integrated model of LGCI

The steps for analyzing simulation output data and comparing them with the clinical data are itemized below:

1. Export the raw data to Microsoft Excel
2. Extract the steady state levels of TNF-α
3. Plot steady state levels of TNF-α in presence and absence of combination of bioactive compounds in FBV juice powder
4. Compare and plot the TNF-α simulation results with those from the Lamprecht et al. [31] clinical study

3. Results

A total of fifteen bioactive compounds from the FBV juice powder were tested on the integrated mathematical model of LGCI. The initial results (data not shown) indicated that, at the dose level used in this study, only eight of these fifteen active molecules including luteolin, epicatechin, epigallocatechin gallate (EGCG), lycopene, quercetin, vitamin A, vitamin C and vitamin E showed a measurable and significant effect on the four major molecular pathways of LGCI Hence, it was decided to focus on executing simulations of the effect of these eight bioactive compounds on the molecular pathways involved in LGCI. Figure 6 illustrates the effect of bioactive compounds in FBV juice powder on the LGCI molecular pathways. Biological effects of the bioactive compounds and their molecular targets are summarized in Table A2 in Appendix A.

3.1. Effect of FBV juice powder bioactive compounds on TNF-α production

Eight bioactive compounds in FBV juice powder including luteolin, lycopene, vitamin A, vitamin C, vitamin E, quercetin, epicatechin, and EGCG targeted the TNF-α production in adipocytes. The effect of individual bioactive compounds is shown in panels A–H, and results from the synergistic combination is shown in panel I of Fig. 2. TNF-α levels under control conditions are compared with those after administration of FBV powder, over 7 days and 30 days.

Under control conditions, the levels of TNF- α were estimated to be 0.65 nM. In presence of bioactive molecules from the FBV juice powder, the TNF-α concentrations decreased significantly, as shown in panels A–H of Fig. 2 for 7 and 30 days. All phytonutrients except luteolin, quercetin, and EGCG suppressed the TNF-α levels

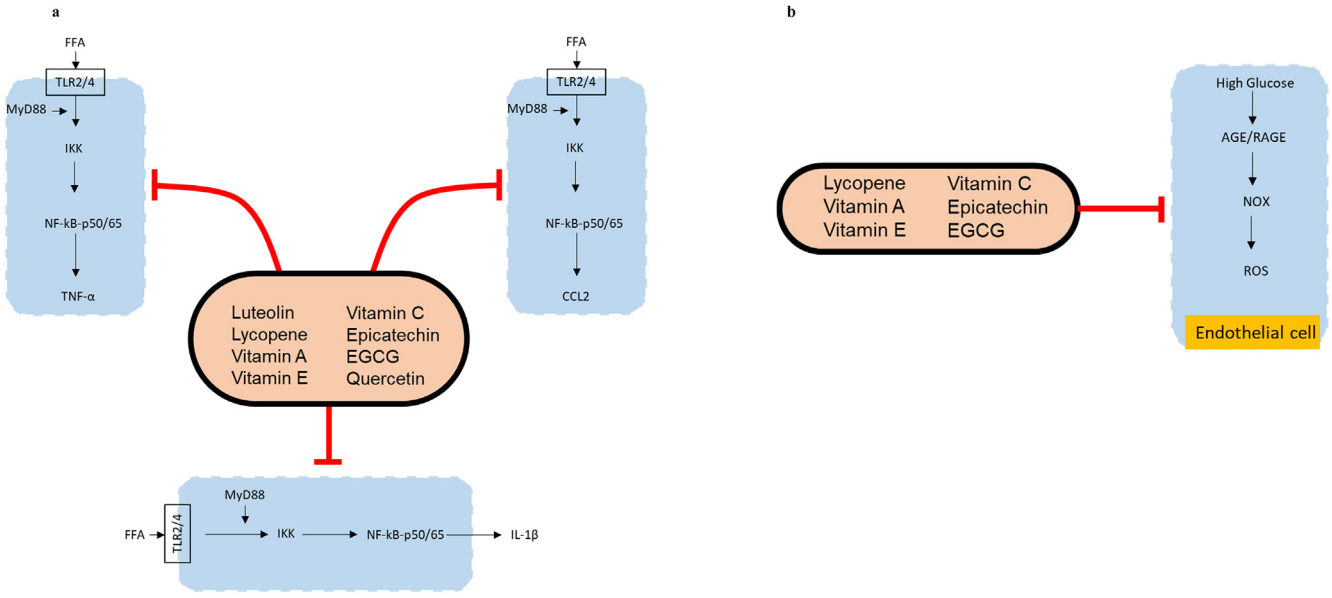

Fig. 6. Mechanisms of action of LGCI affected by the bioactive molecules in the FBV juice Powder. Oval with black outline contains the respective bioactive molecules. a) Eight (8) bioactive molecules in the FBV juice Powder affect three biomarkers of LGCI – TNF-α, CCL2 and IL-1β – in both adipocytes as well as pancreatic islet cells, and b) Six (6) bioactive molecules in the FBV juice Powder affect oxidative stress in endothelial cells. FBV – fruit berry and vegetables; TNF- α – Tumor necrosis factor – α; IL-1β – Interleukin 1β; CCL2 – C–C motif chemokine ligand 2; ROS – reactive oxygen species; EGCG - epigallocatechin gallate.

significantly; although the levels TNF-α were reduced significantly compared to the control in the presence of luteolin, quercetin, and EGCG. Effect of increasing the supplementation duration from 7 to 30 days did not further suppress the TNF-α levels in presence of all the phytonutrients except for quercetin, but maintained the same low levels. Combination of all the bioactive compounds had a synergistic non-linear effect and nearly eliminated the TNF-α production, as shown in panel I from Fig. 2. These results indicate that the FBV juice powder is effective in controlling LGCI by lowering the TNF-α levels significantly.

Lamprecht et al. [31] showed that supplementation of FBV juice powder over a period of 8 weeks in obese but otherwise healthy subjects led to a statistically significant reduction in the levels of LGCI biomarker TNF-α. Additional simulations over 8 weeks were performed to match the time period used in the Lamprecht et al. study and results are shown in Fig. 7. Both, the in silico results from our study and the clinical results from Lamprecht et al., showed consistent reduction in TNF-α levels with supplementation of FBV juice powder over a period of 8 weeks.

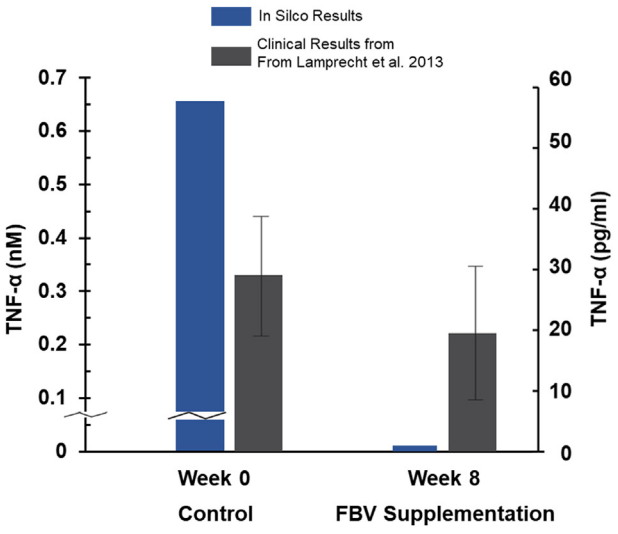

Fig. 7. Comparison between TNF-α levels predicted by the LGCI in silico model and measured in a clinical study (Lampreth et al., 2013) evaluating the effects of FBV juice powder. Blue bars represent the data from in silico results and dark grey bars represent data from the clinical study. TNF-α levels assessed before and after supplementation of FBV juice powder. Identical dosages (six capsules) of FBV juice powder were used over 56 days (eight weeks) for both in silico simulations the clinical study. Both, in silico and clinical data revealed that TNF-α levels decreased as a result of FBV juice powder supplementation. FBV – fruit berry and vegetables; TNF- α – Tumor necrosis factor – α; LGCI - low-grade chronic inflammation.

3.2. *Effect of FBV juice powder bioactive compounds on CCL2 production*

Eight bioactive compounds in FBV juice powder including luteolin, lycopene, vitamin A, vitamin C, vitamin E, quercetin, epicatechin, and EGCG targeted the CCL2 production in adipocytes. The effect of individual bioactive compounds is shown in panels A–H, and results from the synergistic combination is shown in panel I of Fig. 3. CCL2 levels under control conditions are compared with those after administration of FBV powder, over seven days.

Under control conditions, the levels of CCL2 were estimated to be 2.8 nM. In presence of bioactive molecules from the FBV juice powder, the CCL2 concentrations decreased significantly, as shown in panels A–H of Fig. 3. All phytonutrients except luteolin, quercetin, and EGCG suppressed the CCL2 levels significantly; although, the levels CCL2 were reduced significantly compared to the control in presence of luteolin, quercetin, and EGCG. Effect of increasing the supplementation duration from 7 to 30 days did not further suppress the CCL2 levels in presence of all the phytonutrients except for luteolin, quercetin, and EGCG, but maintained the same low levels. Combination of all the bioactive compounds had a synergistic non-linear effect and nearly eliminated the CCL2 production, as shown in panel I from Fig. 3. These results indicate that the FBV juice powder is effective in controlling LGCI by reducing the CCL2 production in adipocytes.

3.3. Effect of FBV juice powder bioactive compounds on IL-1β production

Eight bioactive compounds in FBV juice powder including luteolin, lycopene, vitamin A, vitamin C, vitamin E, quercetin, epicatechin, and EGCG targeted the IL-1β production in adipocytes. The effect of individual bioactive compounds is shown in panels A–H, and results from the synergistic combination is shown in panel I of Fig. 4. IL-1β levels under control conditions are compared with those after administration of FBV powder, over seven days.

Under control conditions, the levels of IL-1β were estimated to be 0.36 nM. In presence of bioactive molecules from the FBV juice powder, the IL-1β concentrations decreased significantly, as shown in panels A–H of Fig. 4. All phytonutrients except luteolin, quercetin, and EGCG suppressed the IL-1β levels significantly; although, the levels IL-1β were reduced significantly compared to the control in presence of luteolin, quercetin and EGCG. Effect of increasing the supplementation duration from 7 to 30 days did not further suppress the IL-1β levels in presence of all the phytonutrients except for luteolin, quercetin and EGCG, but maintained the same low levels. Combination of all the bioactive compounds had a synergistic non-linear effect and nearly eliminated the IL-1β production, as shown in panel I from Fig. 4. These results indicate that the FBV juice powder is effective in controlling LGCI by reducing the IL-1β production in adipocytes.

3.4. Effect of FBV juice powder bioactive compounds on ROS production

Six bioactive compounds in FBV juice powder including lycopene, vitamin A, vitamin C, vitamin E, epicatechin, and EGCG targeted the ROS production in endothelial cells. The effect of individual bioactive compounds is shown in panels A–F, and results from the synergistic combination is shown in panel G of Fig. 5. ROS levels under control conditions are compared with those after administration of FBV powder over seven days.

Under control conditions, the ROS levels were estimated to be 3.72 nM. In presence of bioactive molecules from the FBV juice powder, the ROS concentrations decreased significantly, as shown in panels A–F of Fig. 5. Effect of increasing the supplementation duration from 7 to 30 days further lowered the ROS levels in presence of all the phytonutrients except for vitamin C. It was further observed that no suppression of ROS occurred with increasing duration of supplementation, in presence of vitamin C. Combination of all the bioactive compounds had a synergistic non-linear effect and nearly eliminated the ROS production, as shown in panel G from Fig. 5. These results indicate that the FBV juice powder is effective in controlling LGCI by reducing the ROS production in endothelial cells.

4. Discussion

The results herein provide a significant opportunity to understand and develop interventions for LGCI related pathologies such as metabolic disorders and cardiovascular disease. The CytoSolve computational systems biology framework was used to understand how bioactive compounds in FBV juice powder may modulate LGCI. To our knowledge, this is the first study that has applied the computational systems biology approach to mathematically model the production of pro-inflammatory agents responsible for LGCI at a molecular systems level. Moreover, the resultant mathematical models were used to uncover mechanisms of action to attenuate those pro-inflammatory agents responsible for LGCI.

LGCI has emerged as one of the key factors in the emergence of cardiovascular disease, metabolic disorders such as diabetes, musculoskeletal pathologies such as osteoarthritis and several neuro-inflammatory diseases such as Alzheimer's Disease [2–4]. Endurance athletes are more at risk for developing LGCI-induced cardiovascular complications as they age [11,60,61]. Phytonutrients such as those found in FBV juice powder have been shown to lower LGCI and improve athletic performance [24,25]. Systems biology is emerging as a reliable tool to understand the underlying molecular mechanisms of action of how phytonutrients are able to promote health and reduce disease risk [62]. Four molecular mechanisms of action, involved in LGCI are identified. These mechanisms provide molecular targets for eight phytonutrients in the FBV juice powder. Our results indicate that after 7 days of supplementation, the biomarkers of LGCI such as TNF-α, CCL2, IL-1β and ROS were downregulated significantly, and continuing the FBV supplementation beyond ~4 weeks helped keep the LGCI biomarkers low.

Several clinical studies have shown that dietary supplementation of FBV juice concentrates lowered the biomarkers of systemic LGCI such as DNA damage in peripheral blood lymphocytes [29]; MCP-1 (CCL2), MIP-1β, and CCL5 [30]. These biomarkers correlate with improved blood circulation and energy levels [31], and lower muscle soreness.

In a placebo-controlled randomized clinical study, Lamprecht et al. showed that supplementation of FBV juice powder over a period of 8 weeks in obese but otherwise healthy subjects led to a statistically significant reduction in the levels of LGCI biomarker TNF-α [31]. Additional simulations over 8 weeks (56 days) were performed to match the time period used in the Lamprecht et al. study and results are shown in Fig. 7. Both, the in silico results from our study and the clinical results from Lamprecht et al., showed consistent reduction in TNF-α levels with supplementation of FBV juice powder over a period of 8 weeks. However, the level of reduction in TNF- α levels from in silico analysis differ from that seen in the clinical study. This difference can be explained by the fact that the in silico model calculated the value of TNF- α at the cell surface, whereas the clinical results are obtained from measuring the plasma concentrations of TNF- α. In addition, some of the model components' parameters were derived from experiments using different cell types, as well as different experimental conditions such as variations in culture conditions that adds to the uncertainty of the model predictions as the kinetics are affected by the experimental conditions and could explain the overestimation of downregulation of inflammatory biomarkers.

To our knowledge, this is a first study of its kind that identifies the core mechanisms of action behind the mitigation of LGCI by FBV juice powder observed in the clinic. In addition, the combinations of phytonutrients in the FBV juice powder were identified that synergistically reduced the levels of 1) LGCI biomarkers such as pro-inflammatory cytokines TNF-α, IL-1β, CCL2 (MCP-1) and, 2) ROS.

5. Strengths and limitations

5.1. Strengths

There are four key strengths of this study. First, to the best of our knowledge, this is the first computational model of LGCI that provides an understanding of the mechanistic and quantitative synergistic effects of bioactive compounds on LGCI.

Second, the method used in this study provides a scalable computational framework for modeling large-scale biological systems by dynamic integration of an ensemble of multiple molecular pathway models [33]. This method enables the development of large-scale models of complex biological systems that span multiple temporal and spatial scales as well as across diverse domains.

Rather than attempting to monolithically model systems of biochemical reactions, a distributed engineering systems approach – a relatively novel concept in systems biology– is employed that breaks a large scale biological system into an ensemble of smaller molecular pathway models that are computationally coupled. This approach makes the modeling of large-scale biological systems both tractable and scalable.

Third, the study employs the systems biology approach including bioinformatics and computational modeling, to curate and integrate molecular pathways, chemical species, and biochemical reactions to build a molecular systems architecture of LGCI, and a predictive and quantitative computational model of LGCI.

Fourth, the LGCI model results corroborate with clinical observations of the attenuation of LGCI pro-inflammatory agents such as TNF-α by bioactive components in FBV juice powders. Such corroboration provides us clinical evidence for confidence in the viability of the LGCI model developed in this study.

5.2. Limitations

There are three limitations of this study. First, although the framework developed in this study provides a detailed mechanistic understanding of LGCI that match well with published clinical data, some of the model components' parameters were derived from experiments using different cell types, as well as different experimental conditions such as variations in culture conditions that adds to the uncertainty of the model predictions [63]. Such issues of parameter estimation, however, are not unique to this study. They are common to a number of cellular mathematical models [63] and do warrant further experimental investigation and validation.

Secondly, while the current model corroborates clinical observations [31], *in vitro* or *in vivo* experimental studies can serve to further strengthen the conclusions from this study. Such experimental studies are planned in future work.

The LGCI model is currently based on four pathways: three pro-inflammatory pathways and ROS production pathway. The modular computational framework afforded by the study allows for ongoing expansion and integration of other relevant pathways. For example, the molecular pathway of ROS-induced TNF-α release by activation of ADAM17/TACE enzyme system [64] could be integrated to the expand the LGCI model and enhance its robustness.

6. Conclusions and future work

6.1. Conclusions

An integrative in silico – computational model - of LGCI is developed to predict the effect of phytonutrients in the FBV juice powder on four biomarkers of LGCI, namely, TNF-a, CCL2, IL-1β, and ROS. Eight phytonutrients in the FBV juice powder are identified whose synergistic combination lower all four biomarkers of LGCI, which is corroborated by clinical observations. All eight phytonutrients – luteolin, lycopene, vitamin A, vitamin E, vitamin C, epicatechin, EGCG, and quercetin – lower TNF-a, CCL2, and IL-1β, whereas only six of them – lycopene, vitamin A, vitamin E, vitamin C, epicatechin, and EGCG were efficient in lowering ROS.

The molecular systems architecture of LGCI developed from this effort offers systems understanding of complex molecular interactions occurring during a biological process or a disease such as LGCI. This architecture provides mechanistic understanding of anti-inflammatory effects of bioactive compounds in FBV juice powder that may improve LGCI.

6.2. Future work

The current LGCI model can be expanded by incorporating additional molecular pathways such as TNF-α release by activation of ADAM17/TACE enzyme system, since ROS can activate ADAM17/TACE enzyme through a MAPK cascade [64]. Such integration will likely improve the physiological relevance of the LGCI models.

Moreover, validation of the predictive effects of the bioactive compounds from FBV juice powder on LGCI models using either *in vitro* or *in vivo* experimental studies can further strengthen the conclusions from this study. *In vitro* experiments using adipocytes, pancreatic β cells, and endothelial cells could be performed to simulate the LGCI conditions. Comparison of biomarker levels – TNF-a, IL-1b, CCl2, and ROS – from such experimental studies and from the LGCI models is planned in future work.

This work may further be expanded to understand whether the FBV juice powder play a potential role in mitigation of imbalance in glucose metabolism, insulin resistance, osteoarthritis etc., as most of the pathways involved in LGCI also participate in these pathologies. Given the role of LGCI in muscle soreness and cardiovascular complications, the predictive models developed in this study may be used to develop personalized dietary supplementation.

Author contributions

S.A. and P.D.: Conceptualization, in silico simulations, data curation, figures generation, writing draft article. S.A., P.D. and R.B.: editing the draft article, reviewing the article.

Data availability

All relevant data are within the paper and its Supporting Information files.

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Declaration of competing interest

S.A. is the Founder and Chief Science Officer of CytoSolve, Inc. P.D. is a current employee of CytoSolve, Inc. R.B. has no conflicts or competing interests to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2022.03.010.

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