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Pomegranate juice consumption alleviates inflammation and cortisol levels in patients with opioid use disorders under medication for addiction treatment with methadone and buprenorphine: a randomized controlled trial

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Abstract

Background and aims: Patients with opioid use disorders (OUDs) under medication for addiction treatment (MAT) experience highly stressor events that enhance systemic inflammation, which is a severe symptom. Pomegranate, due to its bioactive compounds that exert potent antioxidant and anti-inflammatory properties, could putatively be a beneficial nutritional intervention for opioid addicted patients. Thus, the present study aimed to investigate the impact of pomegranate juice consumption on inflammatory response of patients under MAT.

Methods: In total, 58 patients under MAT were divided into the experimental group (n=40), comprising patients who consumed pomegranate juice (dosage: 250 ml/day, seven days/week, 120 days) and the control group including 18 patients who did not consume anything. These groups were further stratified based on MAT, namely methadone maintenance treatment (MMT) and buprenorphine maintenance treatment (BMT). Cortisol concentration and pro-inflammatory agents (IFN-γ, IL-6, IL-8, MCP-1) were measured in blood at Days 1 and 120.

Results: Cortisol levels and the concentrations of all measured cytokines were decreased in both MMT and BMT patients at Day 120 due to pomegranate juice consumption compared to the control. Furthermore, after consumption of the juice and at Day 120, cortisol and MCP-1 concentrations were decreased in a greater extent in patients under buprenorphine compared to those under methadone.

Conclusions: The obtained findings indicate that pomegranate juice consumption could be considered as an auxiliary intervention, in parallel with medication, towards rehabilitation of patients under MAT since it reduces inflammation, a severe biochemical and organismal symptom.

NCT05861544

Introduction

During the last decade, growing research evidence suggests that interactions between immune response and the central nervous system may play a crucial role in disease onset of patients with opioid use disorders (OUDs) [1]. Opioids act through blockage of mu-opioid receptors (MORs); thus, they prevent γ-aminobutyric acid (GABA) release, which in physiological conditions acts on dopaminergic neurons by inhibiting dopamine secretion [2, 3]. As a result, dopamine reuptake at the synaptic cleft in the reward system is inhibited. This biological response is strongly related to stressful events, hence activating hypothalamic-pituitary-adrenal (HPA) axis through abnormal immune-endocrine interaction [4, 5]. Experimental findings have shown that the use of illicit opioids such as heroine, due to activation of HPA axis and cortisol release, is related to generation of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), inteleukin-6 (IL-6), and inteleukin-1 beta (IL-1β) [6]. Of note, the long-acting opioids, namely methadone and buprenorphine, that are used as medication for addiction treatment (MAT) do not exert the same immunomodulatory effects [7, 8]. Research findings on patients under MAT have shown that both substances produce immune-endocrine responses in a dose-dependent manner by

elevating the concentration of cortisol and the release of pro-inflammatory cytokines, with monocyte chemoattractant protein-1 (MCP-1) and interleukin 8 (IL-8) being among them [9].

Methadone as methadone maintenance treatment (MMT) and buprenorphine as buprenorphine maintenance treatment (BMT) bind to MORs and are the main drugs used as treatment for OUDs. Both methadone and buprenorphine are considered the most effective MAT indicated by the decrease of illicit drug use they induce. Notwithstanding, because they are opioid drugs, they have been widely associated with neuroinflammation and oxidative stress compromising quality of life and drug seeking behavior of patients under OUDs [10–12]. Moreover, in the biochemical level, long-acting opioid agonists such as methadone have been related to toxic effects on brain cells. Moreover, they seem to repress neurogenesis by altering the mitochondrial outer membrane permeability leading to dramatic depletion of ATP subcellularly by impairing ATP synthesis [13]. Furthermore, higher levels of cytokine concentrations in MMT patients induce reactive oxygen species (ROS) generation, elevated cortisol levels due to HPA axis deregulation in both MMT and BMT patients and are accompanied by increased dropout rates from their therapy [9, 11, 14, 15]. Hence, concerns and considerations have emerged in the scientific community regarding the development of novel medication approaches based mostly on plant-derived products [16].

The notion of introducing plant compounds against addictive behaviors and towards rehabilitation is mainly based on their potent antioxidant and anti-inflammatory properties due to their composition in bioactive molecules, mainly polyphenols [17–20]. Plants such as ginseng, rosa damascena oil, nigella sativa, phoenix dactilyfera and pomegranate have been studied as auxiliary therapeutic interventions along with the standard medication treatment in patients with OUDs [21–27]. Specifically, pomegranate is a highly antioxidant and anti-inflammatory natural compound due to some of its constituents such as punicalagin, ellagic acid, anthocyanins and flavonoids, that are included in the great family of polyphenolics [25, 28]. Indeed, pomegranate juice exerts at least 20% more potent antioxidant activity compared to other commonly used plant-based beverages and products, namely red wine, and juices derived from grape, blueberry, black cherry, cranberry, orange (rich in ascorbic acid) and apple [23, 29]. Relevant preclinical studies have demonstrated that pomegranate reduces cell cytotoxicity and ROS production with concurrent decrease of the concentration of pro-inflammatory cytokines IL-1β, IL-6, INFγ, and TNFα in methadone-treated rats [30]. Furthermore, it has been demonstrated that pomegranate juice, when consumed by patients under MAT, improves blood oxidative stress that is inextricably linked to elevated generation of inflammatory cytokines [23].

The available evidence of the literature indicates that antioxidant compounds or products, due to their potent antioxidant properties, could putatively act advantageously against the health problems induced by chronic opioid use. This is a plausible biological action of such compounds or products given that oxidative stress and the resulting inflammation are common biochemical symptoms in patients with OUDs. Pomegranate juice is a rather neglected physical product towards this direction given that there is scarce evidence regarding its role in the inflammatory response of patients under MAT. Therefore, the present study, which is part of the evidence-based, multifactorial randomized controlled trial with the

acronym the NUTRIDOPE (NUTRItion-driven Detoxification of OPioid addicted patiEnts) study, aimed to investigate the role of pomegranate juice consumption on inflammation and cortisol levels of patients under MAT with methadone and buprenorphine.

MATERIALS AND METHODS

2.1. Participants

The participants of this investigation, which is part of a registered study in the international database ClinicalTrials.gov (Identifier: NCT05861544), were patients with OUDs under MAT with buprenorphine and methadone. In total 58 volunteers, according to statistical power calculation [31], were randomly divided into the experimental (n = 40) and the control (n = 18) groups, both of which were further stratified into two sub-groups according to MAT. Specifically, the experimental group comprised 19 patients under BMT and 21 under MMT, whereas 8 patients under BMT and 10 under MMT were included in the control group (Fig. 1). The randomization process was based on the unique patient code before entering the MAT programs and was performed by an independent person, who did not participate in the data collection and evaluation procedure. This individual had access to interim results and was responsible for terminating the trial in case there was a need for that, or any adverse effects had been raised before addressed to the appropriate expert. The volunteers were notified about the group they were allocated through a message in an opaque envelope, and they were informed concerning the benefits and the potential risks of the experiment. An anonymously written consensus form for trial participation and for data publication was signed by all participants before the study began. All personal data and information were confidential; they were kept in password protected computers and only the researchers had access to them. According to the inclusion criteria, patients aged over 20 years old with long term opioid use and suffering from physical and mental dependence due to use, were included in the investigation. Participants with severe psychopathology, current anti-inflammatory treatment and serious communicable diseases were excluded. Patients with relapse to other substances (i.e., opioids, methamphetamine, amphetamine, benzodiazepines, tetrahydrocannabinol and cocaine), monitored by weekly urine tests, were also excluded from the study (Fig. 1) [31].

2.2. Experimental procedure

A 100% natural pomegranate juice (variety: *wonderful*) was kindly donated by the company Rodi HELLAS SA (Pella, Greece) who systematically cultivates pomegranates. Pomegranate juice was consumed by the patients of the experimental group in a dosage scheme of 250 ml/day, seven days/week, for four months (i.e., 120 days). Their counterparts in the control group did not consume any similar beverage (i.e., there was not a placebo arm). The consumption of a similar pomegranate juice dosage seems to act beneficially on inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, metabolic and cardiovascular disorders and neurodegenerative diseases [32]. Pomegranate juice was consumed by the patients as an auxiliary nutritional intervention parallel to their medication (i.e., half an hour after methadone or buprenorphine administration). It has to be mentioned that no adverse effects

were observed, and the attrition rate was zero since all participants who agreed to take part in the investigation managed to complete it.

2.3. Medication for addiction treatment

Medication for addiction treatment comprised methadone hydrochloride solution (10 mg/ml) and buprenorphine/buprenorphine-naloxone pills (2 mg – 8 mg), based on the international guidelines [33]. Methadone mean dose was 60 mg/24 h, since, according to the literature, daily methadone doses between 40 and 100 mg are effective as maintenance treatment for patients with OUDs [34]. For buprenorphine, the mean dose was equal to 16 mg/24 h, as, based on the available data, this is a commonly used and efficient dose [35].

2.4. Measurement of blood biomarkers

Cortisol, interferon gamma (IFNγ), interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) were the biomarkers that were measured in the blood of the volunteers at two timepoints, that is at Days 1 and 120. Blood samples were drawn from a forearm vein and stored in clotting tubes. The blood was clotted for 30 min before centrifugation (10 min, 1000 g, 4°C). The supernatant (i.e., serum) was removed and used for the measurement of cortisol concentration (Cayman Cortisol Elisa Kit) and the aforementioned cytokines with a Milliplex Human Cytokine/Chemokine Magnetic Bead Panel according to the manufacturer's instructions and detected with a multiplex detection platform (Luminex® 200™ System). Regarding the measured biomarkers, IFNγ is a cytokine that recruits macrophages at the site of inflammation, whereas IL-6 is involved in the activation and proliferation of immune cells, including T cells, B cells, and macrophages promoting B-cell differentiation and orchestrating the organismal immune response to inflammation and infection [36, 37]. IL-8 is a chemokine that induces chemotaxis of neutrophils and other granulocytes to the inflammation site [38]. Finally, MCP-1 belongs to the family of chemokines and acts by recruiting monocytes, lymphocytes, and neutrophils at the inflammation site [39, 40]. Based on the literature, opioid use as a chronic stressful condition, potentially activates inflammatory response releasing central and peripheral inflammatory biomarkers including C-reactive protein (CRP), hormones (cortisol), growth factors, and cytokines [41, 42]. However, CRP can be elevated during the acute phase of an inflammatory process as a non-specific inflammatory biomarker, since both methadone and buprenorphine could maintain CRP in low levels [15]. Recent investigations have revealed that IFN-γ, IL-8, MCP-1 and cortisol are crucial biomarkers in immune-endocrine response in OUDs patients under MAT [9]. On Day 1, the demographic characteristics of the volunteers were also collected.

2.5. Ethical approval

Ethical Approval was given from the Institutional Review Board of the Organization Against Drugs (OKANA) (ref. number 44482-2/12/2020). All applied experimental procedures were in line with the European Union Guidelines reported in the 1964 Declaration of Helsinki as revised in 2013 and approved by the Institutional Review Board. A written informed consent for participation in the trial and data publication was provided by all volunteers before the start of the experimental procedure.

2.6. Statistical analysis

The results were analyzed using three-way [MAT (buprenorphine vs methadone), treatment (pomegranate juice vs nothing), time (Days 1 and 120)] ANOVA with repeated measures. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) (version 21.0 (SPSS Inc., Chicago, IL). The level of statistical significance was set at $p<0.05$.

Results

3.1. Demographic characteristics

The demographic characteristics of the volunteers are presented in Table 1. Regarding both the control and the experimental groups, the majority of the participants were men, their educational status was equal to secondary and high school, they had mostly the Greek nationality, they were lived in urban places and the minority of them had comorbidities. They were also middle-aged (i.e., 46 years old), they had been attending the MAT programs for the last 11 years, they started using illicit opioids between 18 and 20 years of age and the duration of substance use before entering a MAT program was between 13 and 16 years.

3.2. Cortisol concentration

Main effect of time ($p<0.001$) and interactions between treatment and MAT ($p=0.01$) as well as between treatment and time ($p=0.002$) were found (Figure 2). According to the pairwise comparisons, cortisol concentration was decreased due to pomegranate juice consumption in Day 120 in the BMT patients, compared to the control ($p<0.001$). Additionally, the BMT patients that consumed pomegranate juice had lower cortisol concentration in Day 120 compared to their MMT counterparts ($p=0.005$). Finally, pomegranate juice consumption decreased cortisol concentration in Day 120 compared to Day 1 both in patients under buprenorphine ($p<0.001$) and methadone ($p=0.05$).

3.3. Interleukin-8 (IL-8) concentration

Main effects of MAT ($p=0.04$) and time ($p=0.001$) and interaction between treatment and time ($p=0.003$) were observed (Figure 3). Based on the pairwise comparisons, IL-8 concentration was decreased due to pomegranate juice consumption in Day 120 in the BMT patients, compared to the control ($p=0.01$). Finally, pomegranate juice consumption reduced IL-8 concentration in Day 120 compared to Day 1 both in patients under buprenorphine ($p<0.001$) and methadone ($p=0.005$).

3.4. Interleukin-6 (IL-6) concentration

Main effects of MAT ($p=0.01$) and interactions between treatment and MAT ($p=0.05$), treatment and time ($p<0.001$), as well as treatment and MAT and time ($p<0.001$) were observed (Figure 4). In pairwise comparisons, IL-6 concentration was decreased due to pomegranate juice consumption in Day 120 both in the BMT ($p=0.001$) and MMT patients ($p<0.001$), compared to the control. Furthermore, it was found

increased in Day 120 compared to Day 1 both in patients under buprenorphine ($p<0.001$) and methadone ($p=0.001$) of the control group. On the contrary, IL-6 concentration was decreased in Day 120 compared to Day 1 both in patients under methadone ($p<0.001$) and buprenorphine ($p=0.002$) after pomegranate juice consumption.

3.5. Monocyte chemoattractant protein-6 (MCP-1) concentration

Main effects of treatment ($p=0.04$) and time ($p=0.01$) and interactions between treatment and MAT ($p=0.03$) as well as treatment and time ($p=0.02$) were found (Figure 5). The pairwise comparisons showed that MCP-1 concentration was decreased due to pomegranate juice consumption in Day 120 in the BMT patients, compared to the control ($p<0.001$). Additionally, the BMT patients that consumed pomegranate juice had lower MCP-1 concentration on Day 120 compared to their MMT counterparts ($p=0.01$). Finally, MCP-1 concentration was decreased on Day 120 compared to Day 1 in patients under methadone ($p=0.004$) due to pomegranate juice consumption.

3.6. Interferon-γ (INF-γ) concentration

Main effects of treatment ($p=0.05$) and time ($p=0.03$) and interactions between treatment and time ($p=0.001$) were found (Figure 6). According to the pairwise comparisons, INF-γ concentration was decreased due to pomegranate juice consumption in Day 120 in the BMT patients, compared to the control ($p<0.001$). INF-γ concentration was also decreased in Day 120 compared to Day 1 both in patients under methadone ($p=0.04$) and buprenorphine ($p<0.001$) after pomegranate juice consumption.

Discussion

According to the main findings of this clinical trial that is part of the NUTRIDOPE study, pomegranate juice consumption decreased the levels of cortisol, the so-called hormone of stress, and the concentrations of pro-inflammatory parameters in the blood of patients with OUDs who are under MAT with methadone and buprenorphine, compared to the control group. To our knowledge, this is the first study to investigate and finally reveal the beneficial role of the applied nutritional intervention on inflammatory response of opioid-addicted patients being in the process of rehabilitation. Opioid use, as a chronic stressful condition, appears to activate inflammatory response by release of biomarkers including hormones (cortisol), growth factors, and cytokines [43]. Towards this direction, it has been shown that IFN-γ, IL-8, MCP-1 and cortisol are crucial blood biomarkers of inflammatory response in OUDs patients under MAT [9].

Recent experimental evidence has demonstrated that cortisol is considered a physiological indicator of HPA activity and stress state [44]. Chronic administration of opioids, including methadone or buprenorphine, is related to the disruption of HPA axis function that may affect a systematic response to stress [45]. Even though the biochemical mechanism is still unclear, it has been suggested that altered dopaminergic activity through GABA mediation in the hippocampus area is probably associated with disruption of HPA function [46,47]. It has been shown that patients with OUDs under MAT have higher

cortisol levels and are closely related to more severe depression and anxiety symptoms compared to healthy controls [44]. Patients with OUDs under MAT experience chronic stress due to factors such administration of their medication to an everyday basis, unemployment and broken family bonds that lead to negative emotions and anxiety resulting to HPA activation and increase of cortisol concentration [44,48]. Concerning cortisol levels, the present study reports that they were decreased after 120 days of pomegranate juice consumption in both MMT and BMT patients compared to Day 1. Even though relevant evidence in humans is unavailable, our finding is in consistency with previous preclinical results according to which pomegranate juice exhibits anxiolytic effects by regulating GABA expression, obviously because of the phytochemicals i.e., (ellagic acid, kaempferol, luteolin, naringenin, p-coumaric acid, rutin) it contains [28]. GABA is synthesized from glutamic acid activated by the enzyme glutamic acid decarboxylase (GAD). Stressful events or anxiety symptoms activate GAD through Ca^{+2} increase resulting in GABA dysregulation [28]. The anxiolytic effect of pomegranate juice is mainly attributed to GAD regulation in the hippocampus of Wistar rats [49]. Notwithstanding, the activation of HPA axis in patients under MAT is followed by inflammation, and pomegranate juice seems to play a crucial role in the normalization of that biochemical phenomenon [49].

Apart from cortisol, the concentrations of all pro-inflammatory biomarkers measured in this study were decreased after 120 days of pomegranate juice consumption. Pomegranate extracts, punicalagin and ellagic acid have been shown to improve the concentrations of IL-6, IL-1β, tumor necrosis factor-alpha (TNF-α), and the antioxidant glutathione (GSH), not in humans but in the brain of mice with memory impairment and neuropathies promoting modifications in the GABAergic system [50,51]. It is worth mentioning that the constituents of pomegranate comprise polyphenolic compounds such as flavonoids, ellagitannins, punicalagin, punicalin, pedunculagin, ellagic acid, anthocyanins, phenolic acids (chlorogenic, ferulic, coumaric, gallic, and caffeic). Pomegranate also contains non-phenolics with malic acid, oxalic acid, and ascorbic acid being among them, which are involved in its anti-inflammatory and cytoprotective properties through deactivation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) function. NF-κB is closely associated with generation of pro-inflammatory agent production and activation of Delta-FosB transcription factor that seems to be crucial in the addiction behavior, and in paraventricular nucleus of the hypothalamus, hence, in the HPA axis [52-54]. Furthermore, the anti-inflammatory effects of pomegranate are responsible for the inhibition of pro-inflammatory cytokine and chemokine production, including IL-6, IL-8, MCP-1, and IFN-γ through their activity on sirtuin 1 (SIRT-1) that has strong anti-inflammatory effects, directly inhibiting NF-κB transcription activity [55]. SIRT-1 is downregulated in inflammatory diseases as a result of NF-κB activation, thus the SIRT-1/NF-κB axis might be a possible target of pomegranate leading to its anti-inflammatory activity [56]. Punicalagin, as an active compound of pomegranate, seems to mediate, through SIRT-1 upregulation, in the downregulation of mitogen-activated protein kinase (MAPK), which is also involved in the release of pro-inflammatory agents and in the induction of oxidative stress in patients under MAT [23,57].

Moreover, it has been recently revealed that pomegranate juice improves blood redox status of patients with OUDs under MAT, probably due to the activation of the nuclear factor erythroid 2-related factor 2

(Nrf-2) [23]. Nrf-2, as a transcription factor, seems to play an important role in negative regulatory process of cytokine generation since it is activated through SIRT-1 and NF-κB [58]. Previous studies have confirmed that Nrf-2 deficiency promotes NF-κB expression and increases the expression of inflammation mediators [55]. Even though the mechanism through which this pathway is activated in patients with OUDs under MAT is still vague, pomegranate via its bioactive compounds seems to upregulate anti-inflammatory agents, such as SIRT-1, and Nrf-2 leading to downregulation of inflammatory cytokines and chemokines. Making a comparison of the effects of pomegranate juice consumption on the measured inflammatory biomarkers between patients under MMT and BMT, it appears that a more extended reduction in cortisol and MCP-1 concentrations were observed in the group of patients under buprenorphine. According to previous findings, methadone, as a full MORs agonist induces more severe inflammatory response compared to buprenorphine, that is followed by extended release of proinflammatory agents and oxidative molecules, thus it is more difficult for a nutritional compound to face them [11,59].

Finally, concerning the limitations of this study, it is obvious that, as is the case for every non-pragmatic randomized controlled trial, it lacks generalizability, even though the number of participants was based on statistical power calculation. Furthermore, the number of female patients included was lower than that of male volunteers since this holds true for the patients under OUDs who attend MAT programs. All patients who participated in the trial were under stabilized dose schemes, thus the MAT dose was not a factor that was studied, although the existing literature supports the positive correlation between MAT doses and inflammatory response [9].

Conclusion

The present clinical trial reveals for the first time the beneficial effects of pomegranate juice consumption on the reduction of inflammation in the blood of patients with OUDs under MAT with methadone and buprenorphine. This finding is supported by previous experimental evidence that uncovered the crucial role of the consumption of this pomegranate juice on oxidative stress, a biochemical symptom/cause of inflammation. Given that every kind of stress is a common health symptom of patients with OUDs, the current findings highlight that pomegranate juice contributes to confrontation of opioid addiction. Concurrently, it could be considered as a highly promising nutritional approach, in parallel with MAT, for those patients who make their effort towards rehabilitation.

Declarations

Author contributions: CRediT

Christonikos Leventelis: conceptualization, investigation, methodology, project administration, writing – original draft, writing – review and editing; Antzouletta Kampitsi: data curation, investigation, writing – original draft; Michopoulos Alexandros: data curation, methodology; Kosmidis Dimitrios: data curation;

Manomenidis Georgios: data curation; Panagiotis Papadopoulos: investigation; Kalliopi Liadaki: formal analysis; Aristidis S. Veskoukis: investigation, methodology, writing – review and editing, supervision.

Declaration of competing interests

Authors have no competing interest to declare.

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Data statement

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

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References

1. Hofford RS, Russo SJ, Kiraly DD (2019) Neuroimmune mechanisms of psychostimulant and opioid use disorders. Eur J Neurosci 50(3):2562–2573. 10.1111/ejn.14143
2. Karimi-Haghighi S, Chavoshinezhad S, Mozafari R, Noorbakhsh F, Borhani-Haghighi A, Haghparast A (2023) Neuroinflammatory Response in Reward-Associated Psychostimulants and Opioids: A Review. Cell Mol Neurobiol 43(2):649–682. 10.1007/s10571-022-01223-6
3. Langlois LD, Nugent FS (2017) Opiates and Plasticity in the Ventral Tegmental Area. ACS Chem Neurosci 8(9):1830–1838. 10.1021/acschemneuro.7b00281
4. Kleykamp BA, De Santis M, Dworkin RH et al (2019) Craving and opioid use disorder: A scoping review. Drug Alcohol Depend 205:107639. 10.1016/j.drugalcdep.2019.107639
5. Moeini M, Esmaeil N, Mokhtari HR, Eskandari N, Banafshe HR (2020) Neuro-Immuno-Endocrine Interactions in Early Life Stress and Heroin Withdrawal Timeline. Eur Addict Res 26(1):28–39. 10.1159/000503880
6. Morcuende A, Navarrete F, Nieto E, Manzanares J, Femenía T (2021) Inflammatory Biomarkers in Addictive Disorders. Biomolecules 11(12):1824. 10.3390/biom11121824
7. Butelman ER, Goldstein RZ, Nwaneshiudu CA et al (2023) Neuroimmune Mechanisms of Opioid Use Disorder and Recovery: Translatability to Human Studies, and Future Research Directions. Neuroscience 528:102–116. 10.1016/j.neuroscience.2023.07.031

8. Franchi S, Moschetti G, Amodeo G, Sacerdote P (2019) Do All Opioid Drugs Share the Same Immunomodulatory Properties? A Review From Animal and Human Studies. Front Immunol 10:2914. 10.3389/fimmu.2019.02914
9. Leventelis C, Veskoukis AS, Rojas Gil AP, Papadopoulos P, Garderi M, Angeli A et al (2025) Methadone and Buprenorphine as Medication for Addiction Treatment Diversely Affect Inflammation and Craving Depending on Their Doses. Pharm (Basel) 13(2):40. 10.3390/pharmacy13020040
10. Carlsen SL, Lunde LH, Torsheim T (2020) Opioid and Polydrug Use Among Patients in Opioid Maintenance Treatment. Subst Abuse Rehabil 11:9–18. 10.2147/SAR.S221618
11. Leventelis C, Goutzourelas N, Kortsinidou A, Spanidis Y, Toulia G, Kampitsi A et al (2019) Buprenorphine and Methadone as Opioid Maintenance Treatments for Heroin-Addicted Patients Induce Oxidative Stress in Blood. Oxid Med Cell Longev 2019:9417048. 10.1155/2019/9417048
12. Leventelis C, Barmpas PT, Nellas I, Tasoulis S, Veskoukis AS, Tsironi M (2024) COVID-19 Pandemic Detrimentally Affects Craving and Quality of Life in Patients Under Medication-Assisted Treatment with Buprenorphine and Methadone: The Issue of Medication Dose. Psychiatry Int 5(4):867–882. https://doi.org/10.3390/psychiatryint5040059
13. Perez-Alvarez S, Cuenca-Lopez MD, de Mera RM et al (2010) Methadone induces necrotic-like cell death in SH-SY5Y cells by an impairment of mitochondrial ATP synthesis. Biochim Biophys Acta 1802(11):1036–1047. 10.1016/j.bbadis.2010.07.024
14. Fatseas M, Denis C, Massida Z, Verger M, Franques-Rénéric P, Auriacombe M (2011) Cue-induced reactivity, cortisol response and substance use outcome in treated heroin dependent individuals. Biol Psychiatry 70(8):720–727. 10.1016/j.biopsych.2011.05.015
15. Lu RB, Wang TY, Lee SY, Chen SL, Chang YH, See Chen P et al (2019) Correlation between interleukin-6 levels and methadone maintenance therapy outcomes. Drug Alcohol Depend 204:107516. 10.1016/j.drugalcdep.2019.06.018
16. Heilig M, MacKillop J, Martinez D, Rehm J, Leggio L, Vanderschuren LJMJ (2021) Addiction as a brain disease revised: why it still matters, and the need for consilience. Neuropsychopharmacology 46(10):1715–1723. 10.1038/s41386-020-00950-y
17. Spanou C, Veskoukis AS, Stagos D, Liadaki K, Anastasiadi M, Haroutounian SA et al (2011) Effects of grape extracts on the in vitro activity of enzymes involved in oxidative stress regulation. In Vivo, ;25(4):657 – 62, 2011
18. Veskoukis AS, Tsatsakis AM, Kouretas D (2012) Dietary oxidative stress and antioxidant defense with an emphasis on plant extract administration. Cell Stress Chaperones 17(1):11–21. 10.1007/s12192-011-0293-3
19. Veskoukis AS, Kyparos A, Nikolaidis MG, Stagos D, Aligiannis N, Halabalaki M et al (2012) The antioxidant effects of a polyphenol-rich grape pomace extract in vitro do not correspond in vivo using exercise as an oxidant stimulus. Oxid Med Cell Longev 2012:185867. 10.1155/2012/185867

20. Veskoukis AS, Vassi E, Poulas K, Kokkinakis M, Asprodini E, Haroutounian S et al (2020) Grape stem extracts from three native greek vine varieties exhibit strong antioxidant and antimutagenic properties. Anticancer Res 40(4):2025–2032. 10.21873/anticanres.14159
21. Farnia V, Tatari F, Alikhani M, Shakeri J, Taghizadeh M, Karbasizadeh H et al (2017) Rosa Damascena oil improved sexual function and testosterone in male patients with opium use disorder under methadone maintenance therapy-results from a double-blind, randomized, placebo-controlled clinical trial. Drug Alcohol Depend 176:117–125. 10.1016/j.drugalcdep.2017.02.008
22. Farnia V, Alikhani M, Ebrahimi A, Golshani S, Sadeghi Bahmani D, Brand S (2019) Ginseng treatment improves the sexual side effects of methadone maintenance treatment. Psychiatry Res 276:142–150. 10.1016/j.psychres.2019.05.004
23. Leventelis C, Makri S, Ververi A, Papageorgiou K, Tentolouri A, Mountzouridi E et al (2025) Pomegranate juice ameliorates craving and oxidative stress on patients under medication for opioid addiction treatment with methadone and buprenorphine: A ranzomised controlled trial. Clin Nutr ESPEN 66:34–45. 10.1016/j.clnesp.2025.01.025
24. Adnan LHM, Mohamad N, Che Mat K, Abu Bakar NH, Hashim SN, Shariff MM et al (2018) Attenuation of morphine-induced cAMP overshoot by thymoquinone in opioid receptor expressing cells (U87 MG) mediated by chronic morphine treatment. J Eng Appl Sci 13:8906–8911
25. Pérez-Jiménez J, Agnant K, Lamuela-Raventós RM, St-Onge MP (2023) Dietary polyphenols and sleep modulation: Current evidence and perspectives. Sleep Med Rev 72:101844. https://doi.org/10.1016/j.smrv.2023.101844
26. Sani IH, Sulaiman I, Zubairu I, Abdussalam US, Adzim MKR (2018) Antioxidant Potential of Phoenix dactylifera Linn Extract and its Effects on Calcium Channel Antagonist in the Treatment of Withdrawal Syndrome in Morphine Dependent Rats. Trop J Nat Pro Res 2(7):309–313. https://doi.org/10.26538/tjnpr/v2i7.2
27. Wang P, Zhang Q, Hou H, Liu Z, Wang L, Rasekhmagham R et al (2020) The effects of pomegranate supplementation on biomarkers of inflammation and endothelial dysfunction: A meta-analysis and systematic review. Complement Ther Med 49:102358. 10.1016/j.ctim.2020.102358
28. Flores-Bazán T, Betanzos-Cabrera G, Guerrero-Solano JA, Negrete-Díaz JV, German-Ponciano LJ, Olivo-Ramírez D (2023) Pomegranate (Punica granatum L.) and its phytochemicals as anxiolytic; an underreported effect with therapeutic potential: A systematic review. Brain Res 1820:148554. 10.1016/j.brainres.2023.148554
29. Seeram NP, Aviram M, Zhang Y, Henning SM, Feng L, Dreher M et al (2008) Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agric Food Chem 56(4):1415–1422. 10.1021/jf073035s
30. Zhaleh H, Mahdizadeh H, Khoshkhoy S (2023) Punica granatum Seed Essential Oil Suppressed Methadone-Induced Cell Death by Natural Antioxidant Activity. Asian Pac J Cancer Prev 24(3):801–810. 10.31557/APJCP.2023.24.3.801

31. Leventelis C, Tasoulis S, Kouretas D, Metsios SG, Veskoukis SA (2023) Pomegranate juice consumption by patients under medication for addiction treatment as regulator of craving and blood redox status: The study protocol of a randomized control trial (The NUTRIDOPE Study). Int J Clin Skills 17(9):133–143. https://doi.org/10.37532/1753-0431.2023.17(7).316
32. Danesi F, Ferguson LR (2017) Could Pomegranate Juice Help in the Control of Inflammatory Diseases? Nutrients 9(9):958. 10.3390/nu9090958
33. Mattick RP, Breen C, Kimber J, Davoli M (2014) Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. Cochrane Database Syst Rev 2014(2):CD002207. 10.1002/14651858.CD002207.pub4
34. Strain EC, Bigelow GE, Liebson IA, Stitzer ML (1999) Moderate- vs high-dose methadone in the treatment of opioid dependence: a randomized trial. JAMA 281(11):1000–1005. 10.1001/jama.281.11.1000
35. Saxon AJ, Hser YI, Woody G, Ling W (2013) Medication-assisted treatment for opioid addiction: methadone and buprenorphine. J Food Drug Anal 21(4):S69–S72. 10.1016/j.jfda.2013.09.037
36. Schroder K, Hertzog PJ, Ravasi T, Hume DA (2004) Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol 75(2):163–189. 10.1189/jlb.0603252
37. Kerkis I, da Silva ÁP, Araldi RP (2024) The impact of interleukin-6 (IL-6) and mesenchymal stem cell-derived IL-6 on neurological conditions. Front Immunol 15:1400533. 10.3389/fimmu.2024.1400533
38. Dixit N, Simon SI (2012) Chemokines, selectins and intracellular calcium flux: temporal and spatial cues for leukocyte arrest. Front Immunol 3:188. 10.3389/fimmu.2012.00188
39. Callewaere C, Banisadr G, Rostène W, Parsadaniantz SM (2007) Chemokines and chemokine receptors in the brain: implication in neuroendocrine regulation. J Mol Endocrinol 38(3):355–363. 10.1677/JME-06-0035
40. Deshmane SL, Kremlev S, Amini S, Sawaya BE (2009) Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 29(6):313–326. 10.1089/jir.2008.0027
41. Dennis BB, Samaan MC, Bawor M, Paul J, Plater C, Pare G, Worster A et al (2014) Evaluation of clinical and inflammatory profile in opioid addiction patients with comorbid pain: results from a multicenter investigation. Neuropsychiatr Dis Treat 10:2239–2247. 10.2147/NDT.S72785
42. Reece AS (2012) High-sensitivity CRP in opiate addiction: relative and age-dependent elevations. Cardiovasc Toxicol 12(2):149–157. 10.1007/s12012-012-9154-2
43. Pahan P, Xie JY (2023) Microglial inflammation modulates opioid analgesic tolerance. J Neurosci Res 101(9):1383–1392. 10.1002/jnr.25199
44. Yang J, Li J, Xu G, Zhang J, Chen Z, Lu Z et al (2016) Elevated Hair Cortisol Levels among Heroin Addicts on Current Methadone Maintenance Compared to Controls. PLoS ONE 11(3):e0150729. 10.1371/journal.pone.0150729
45. Coluzzi F, LeQuang JAK, Sciacchitano S, Scerpa MS, Rocco M, Pergolizzi J (2023) A Closer Look at Opioid-Induced Adrenal Insufficiency: A Narrative Review. Int J Mol Sci 24(5):4575. 10.3390/ijms24054575

46. Kalamarides DJ, Singh A, Wolfman SL, Dani JA (2023) Sex differences in VTA GABA transmission and plasticity during opioid withdrawal. Sci Rep 13(1):8460. 10.1038/s41598-023-35673-9
47. Lin SH, Chen WT, Chen KC, Lee SY, Lee IH, Chen PS et al (2013) Associations among hypothalamus-pituitary-adrenal axis function, novelty seeking, and retention in methadone maintenance therapy for heroin dependency. J Addict Med 7(5):335–341. 10.1097/ADM.0b013e31829da040
48. Zojaji F, Khalesi A, Bahrami A, Ebrahimi SA, Ganjifard M (2023) Evaluation of Serum Cortisol Levels and Response to Cosyntropin Test in Methadone-treated Opium Addicts. Anesth Pain Med 13(3):e135206. 10.5812/aapm-135206
49. Motaghi S, Moghaddam Dizaj Herik H, Sepehri G, Abbasnejad M, Esmaeli-Mahani S (2022) The anxiolytic effect of salicylic acid is mediated via the GABAergic system in the fear potentiated plus maze behavior in rats. Mol Biol Rep 49(2):1133–1139. 10.1007/s11033-021-06939-0
50. Jain V, Pareek A, Bhardwaj YR, Sinha SK, Gupta MM, Singh N (2022) Punicalagin and ellagic acid containing *Punica granatum L.* fruit rind extract prevents vincristine-induced neuropathic pain in rats: an *in silico* and *in vivo* evidence of GABAergic action and cytokine inhibition. Nutr Neurosci 25(10):2149–2166. 10.1080/1028415X.2021.1954293
51. Kim YE, Hwang CJ, Lee HP, Kim CS, Son DJ, Ham YW et al (2017) Inhibitory effect of punicalagin on lipopolysaccharide-induced neuroinflammation, oxidative stress and memory impairment via inhibition of nuclear factor-kappaB. Neuropharmacology 117:21–32. 10.1016/j.neuropharm.2017.01.025
52. Chocyk A, Czyrak A, Wedzony K (2006) Acute and repeated cocaine induces alterations in FosB/DeltaFosB expression in the paraventricular nucleus of the hypothalamus. Brain Res 1090(1):58–68. 10.1016/j.brainres.2006.03.045
53. Emami Kazemabad MJ, Asgari Toni S, Tizro N, Dadkhah PA, Amani H, Akhavan Rezayat S et al (2022) Pharmacotherapeutic potential of pomegranate in age-related neurological disorders. Front Aging Neurosci 14:955735. 10.3389/fnagi.2022.955735
54. Ruffle JK (2014) Molecular neurobiology of addiction: what's all the (Δ)FosB about? Am J Drug Alcohol Abuse 40(6):428–437. 10.3109/00952990.2014.933840
55. Huang WC, Liou CJ, Shen SC, Hu S, Chao JC, Huang CH et al (2024) Punicalagin from pomegranate ameliorates TNF-α/IFN-γ-induced inflammatory responses in HaCaT cells via regulation of SIRT1/STAT3 axis and Nrf2/HO-1 signaling pathway. Int Immunopharmacol 130:111665. 10.1016/j.intimp.2024.111665
56. De Gregorio E, Colell A, Morales A, Marí M (2020) Relevance of SIRT1-NF-κB Axis as Therapeutic Target to Ameliorate Inflammation in Liver Disease. Int J Mol Sci 21(11):3858. 10.3390/ijms21113858
57. Yang Y, Liu Y, Wang Y, Chao Y, Zhang J, Jia Y et al (2022) Regulation of SIRT1 and Its Roles in Inflammation. Front Immunol 13:831168. 10.3389/fimmu.2022.831168
58. Ren Z, He H, Zuo Z, Xu Z, Wei Z, Deng J (2019) The role of different SIRT1-mediated signaling pathways in toxic injury. Cell Mol Biol Lett 24:36. 10.1186/s11658-019-0158-9

59. Arezoomandan M, Zhiani R, Mehrzad J, Motavalizadehkakhky A, Eshrati S, Arezoomandan R (2022) Inflammatory, oxidative stress and cognitive functions in patients under maintenance treatment with methadone or buprenorphine and healthy subjects. J Clin Neurosci 101:57–62. 10.1016/j.jocn.2022.04.018

Table 1

Table 1 is available in the Supplementary Files section.

Figures

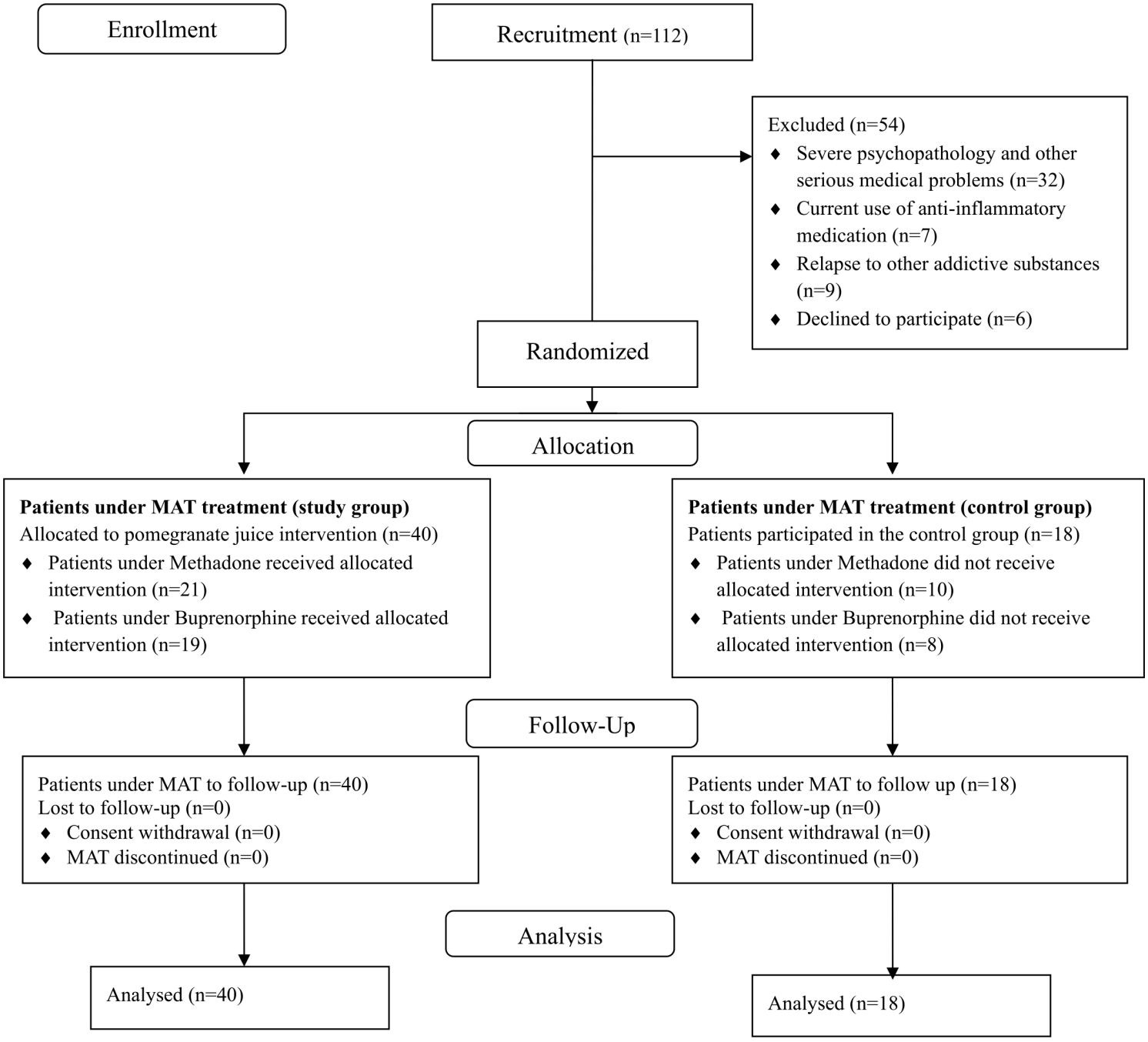


Figure 1

The Consort flowchart of the study.

MAT: Medication for addiction treatment

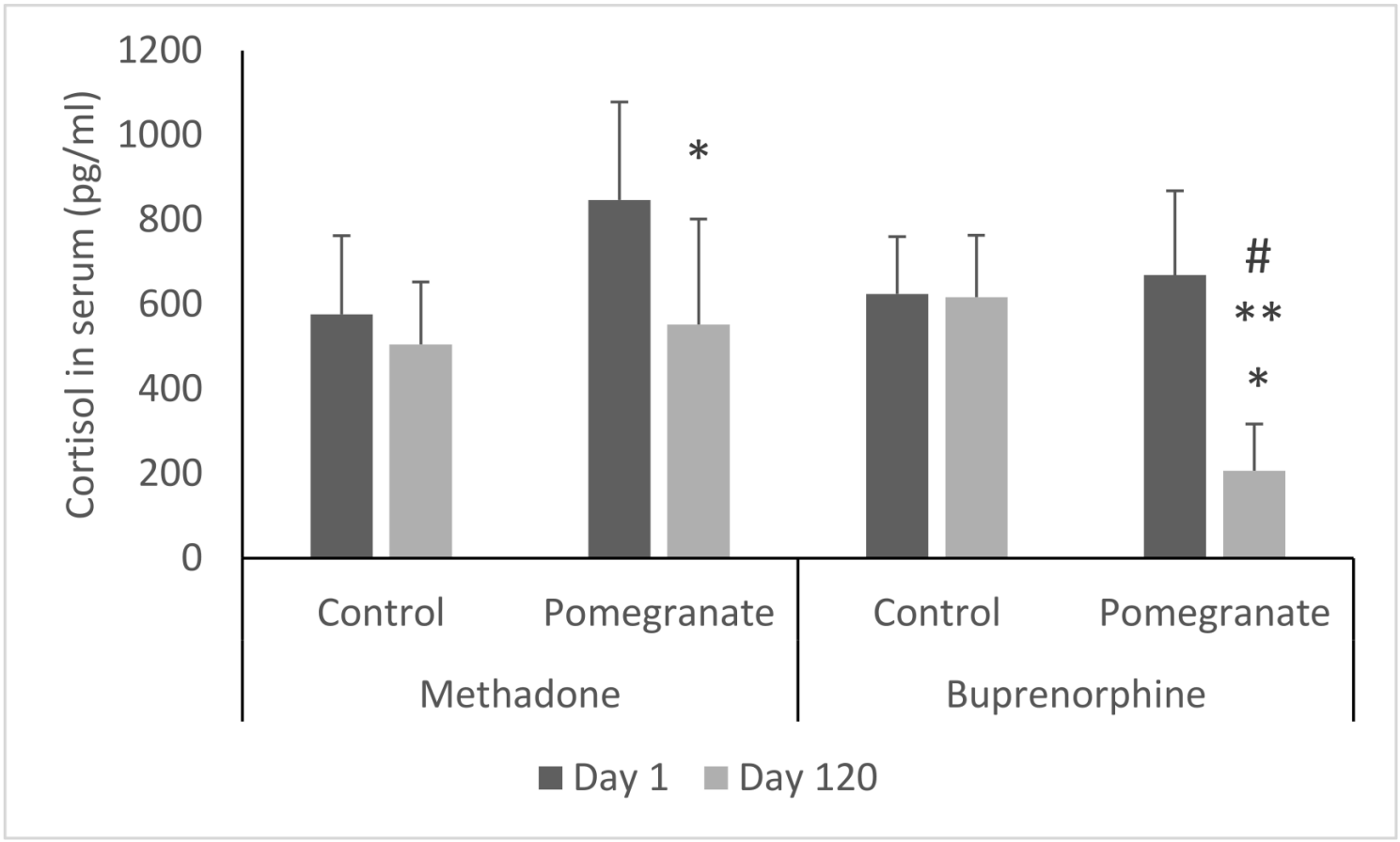


Figure 2

The effects of pomegranate juice consumption and methadone/buprenorphine medication-assisted treatment on cortisol concentration measured in serum. #: Statistically significant difference compared to the control in the same MAT in the same time point. **: Statistically significant difference between MAT in the same group and in the same time point. *: Statistically significant difference compared to Day 1 in the same group.

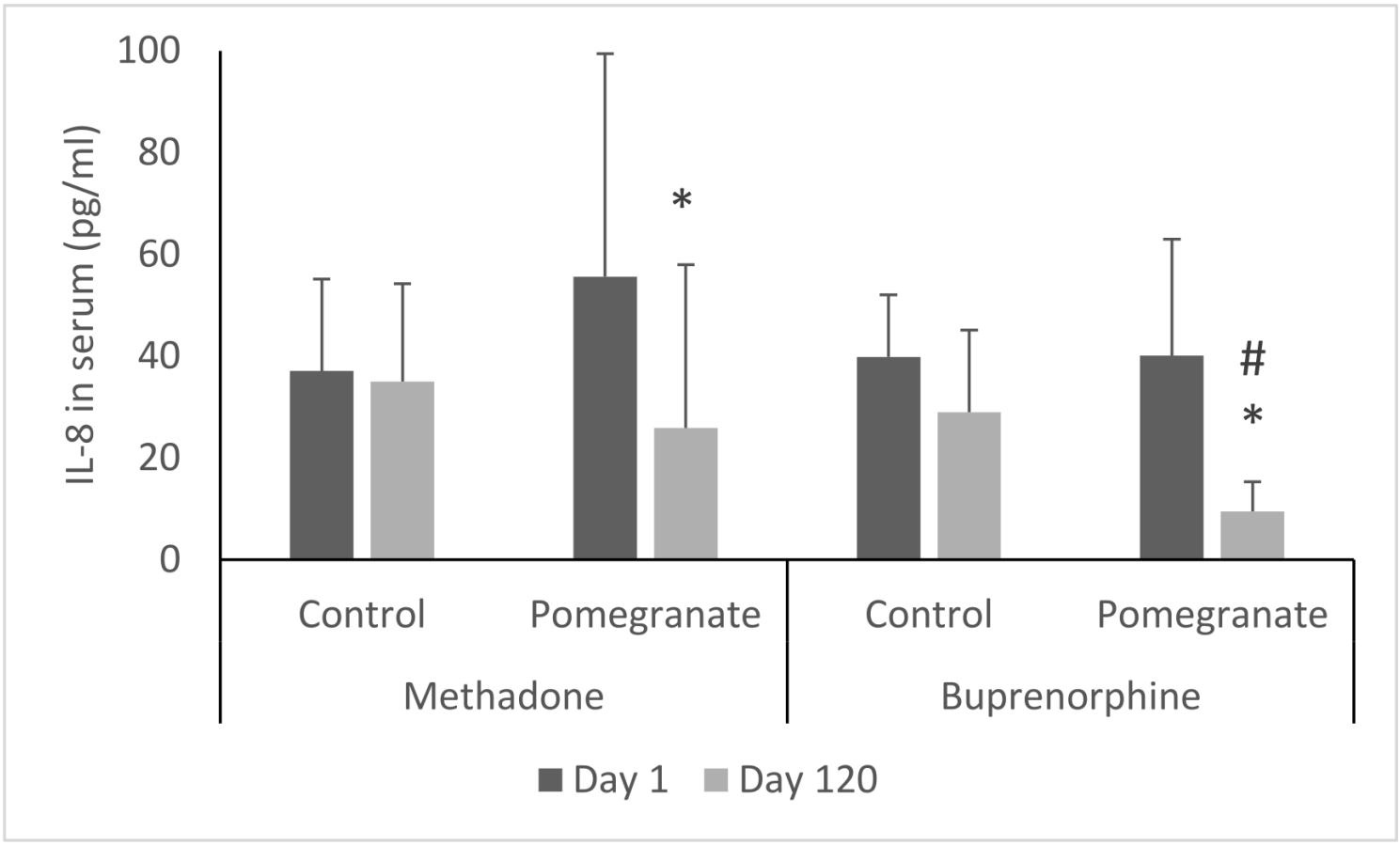


Figure 3

The effects of pomegranate juice consumption and methadone/buprenorphine medication-assisted treatment on interleukin-8 (IL-8) concentration measured in serum. #: Statistically significant difference compared to the control in the same medication in the same time point. *: Statistically significant difference compared to Day 1 in the same group.

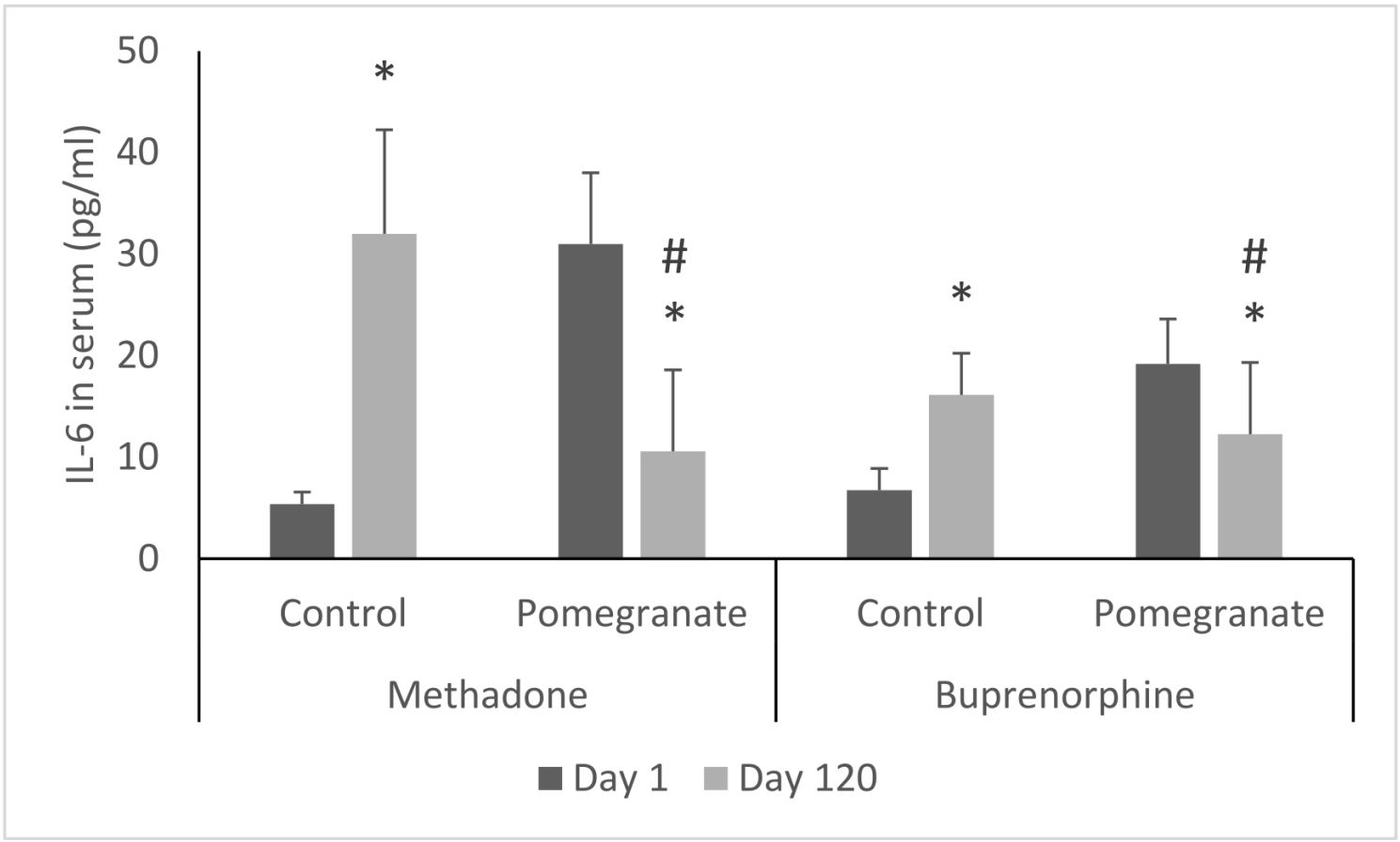


Figure 4

The effects of pomegranate juice consumption and methadone/buprenorphine medication-assisted treatment on interleukin-6 (IL-6) concentration measured in serum. #: Statistically significant difference compared to the control in the same medication in the same time point. *: Statistically significant difference compared to Day 1 in the same group.

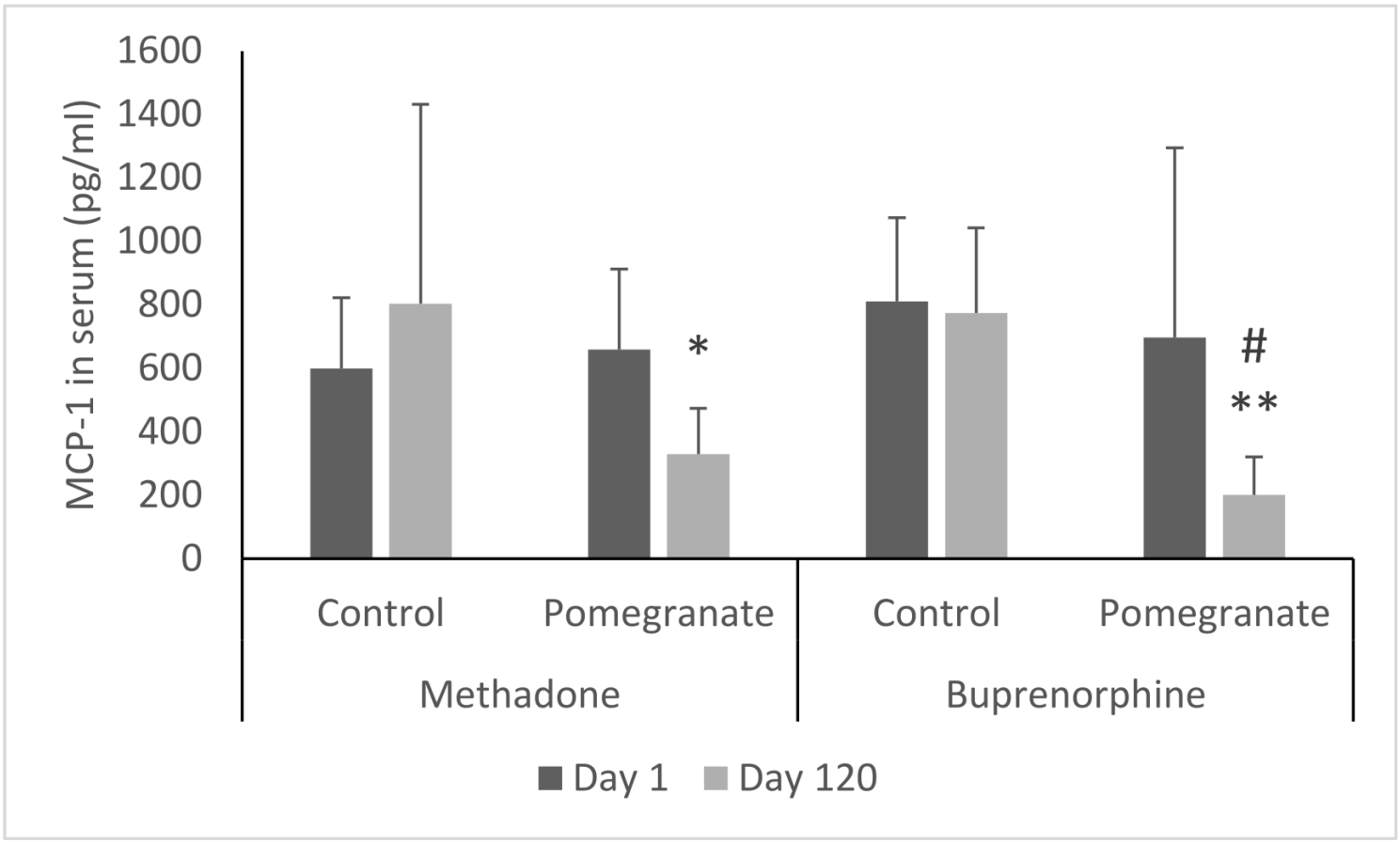


Figure 5

The effects of pomegranate juice consumption and methadone/buprenorphine medication-assisted treatment on monocyte chemoattractant protein-6 (MCP-1) concentration measured in serum. #: Statistically significant difference compared to the control in the same medication in the same time point. **: Statistically significant difference between medications in the same group and in the same time point. *: Statistically significant difference compared to Day 1 in the same group.

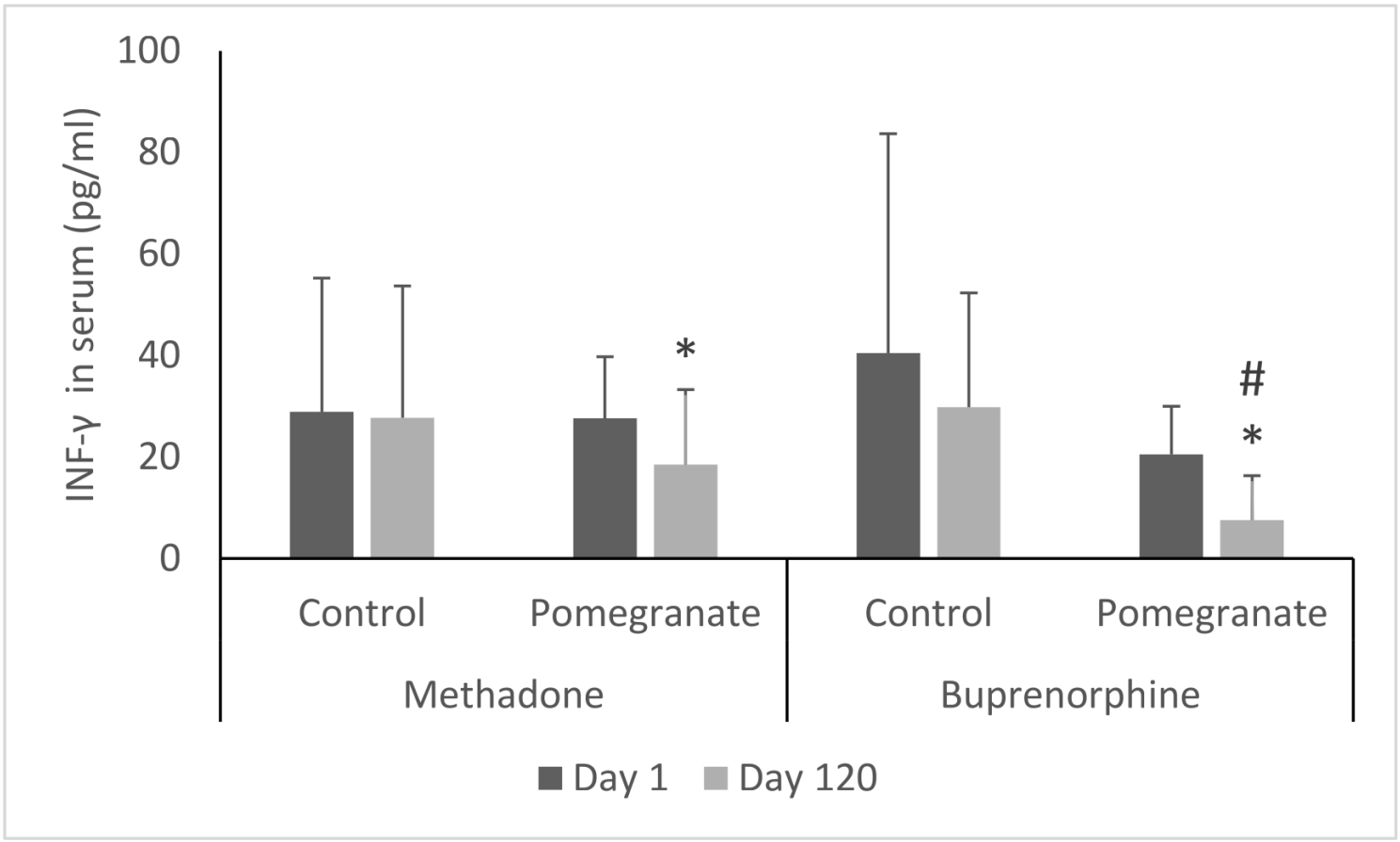


Figure 6

The effects of pomegranate juice consumption and methadone/buprenorphine medication-assisted treatment on interferon-γ (INF-γ) concentration measured in serum. #: Statistically significant difference compared to the control in the same medication in the same time point. *: Statistically significant difference compared to Day 1 in the same group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ExcelPomegranateCortisolCytokines.xlsx
- Table1.docx