Caffeine downregulates inflammatory pathways involved in autoimmunity

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Running title: Caffeine downregulates autoimmune response

Key words: Caffeine, autoimmunity, inflammation, cytokine, TNF, rheumatoid, lupus

Conflict of interest: None of the authors has any financial conflict of interest to disclose

ABSTRACT

Objectives: Caffeine is a widely consumed pharmacologically active product. In the present study, we focused on characterizing immunomodulatory effects of caffeine on peripheral blood mononuclear cells (PMBCs).

Methods: The effect of caffeine on gene expression profiles was initially evaluated using RNA sequencing data. Validation experiments were performed to confirm the results and examine dose-dependent effects of caffeine on PBMCs from healthy subjects. Gene expression levels were measured by real-time quantitative PCR, and cytokine production was determined using a multiplex cytokine assay.

Results: Caffeine at high doses showed a robust downregulatory effect of immune-related genes in PBMCs. Functional annotation analysis of downregulated genes revealed significant enrichment in cytokine activity and in genes related to several autoimmune diseases including lupus and rheumatoid arthritis. Dose-dependent validation experiments showed significant downregulation at the mRNA levels of key inflammatory genes including STAT1, TNF, and PPARG. TNF and PPARG were suppressed even with the lowest caffeine dose tested, which corresponds to the serum concentration of caffeine after administration of one cup of coffee. Cytokine levels of IL-8, MIP-1β, IL-6, IFN-γ, GM-CSF, TNF, IL-2, IL-4, MCP-1, and IL-10 were decreased significantly with caffeine treatment.

Conclusion: Our findings indicate potential downregulatory effects of caffeine on key inflammatory genes and cytokines, which play important role in autoimmunity. Further studies exploring therapeutic or disease-modulating potential of caffeine in autoimmune diseases and exploring the mechanisms involved are warranted.

INTRODUCTION

Systemic autoimmune diseases result from dysregulation of the immune response which usually causes antigen-presenting cells to trigger autoreactive lymphocytes, and are often associated with chronic inflammation involving numerous cytokines. Persistent T cell activation and the production of inflammatory cytokines lead to tissue damage. The etiopathogenesis of autoimmune diseases is not completely understood. Many different combinations of genetic and environmental factors are thought to promote the development and progression of autoimmunity. The inheritance of epigenetic susceptibility loci can cause diversity in disease prevalence and severity between different ethnic and geographical populations [1, 2].

Previous studies have shown that epigenetic factors, including DNA methylation changes, are involved in the pathogenesis of autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus [3]. Being a source of external factors responsible for some epigenetic changes, diet has been shown to affect the severity of autoimmune diseases in animal models [4]. Moreover, the study of gene-environment interactions might help clarify the pathogenic consequences of both genetic and environmental influences in autoimmunity.

Caffeine is one of the most widely consumed pharmacologically active products in the world. Hence, its pharmacological properties, physiological effects, and potential treatment doses have been a subject of interest for years. Even though chocolates and cocoa containing foods store some amount of caffeine, the main source of caffeine in daily life is coffee or other caffeinated beverages such as energy drinks, tea, and carbonated soft drinks [5]. Caffeine has several effects on the body, including central nervous system stimulation. In addition, caffeine is a

member of the methylxanthine family and has been used in neonatal intensive care units to treat apnea of prematurity. It has been studied for its effects on different diseases and was recently shown to reduce the incidence of bronchopulmonary dysplasia in neonates [6]. Previous studies suggested that caffeine is associated with reduced risk for some cancer types, chronic liver disease, Parkinson's disease, Alzheimer's disease, and diabetes [7, 8].

Studies using animal models suggest that caffeine has modulatory effects on elevated serum aminotransferase enzymes and the migration of inflammatory cells [8]. It also decreases serum inflammatory cytokine levels by suppressing their mRNA expression levels, suggesting an anti-inflammatory effect of caffeine [7, 9]. When studied with rats, chronic treatment with caffeine (10 and 30 mg/kg) has been found to ameliorate experimental autoimmune encephalomyelitis [10]. Methylxanthines, which include pentoxifylline and caffeine as members, mediate their effects by inhibiting cAMP-specific phosphodiesterase activity, thus, increasing levels of intracellular cAMP which has immunomodulatory effects on lymphocytes and monocytes/macrophages [11]. Cross-sectional studies have reported an association between higher coffee consumption and lower plasma concentrations of several markers of inflammation and endothelial dysfunction [12].

In this study, we focused on examining the immunomodulatory effects of caffeine using PBMCs obtained from healthy individuals. Our purpose was to evaluate the effect of caffeine on genes and pathways related to autoimmunity.

RESULTS

To characterize the effect of caffeine on gene expression patterns in human PBMCs, we first used RNA sequencing data publically available from a recent study that examined gene expression profiles in multiple cell types, including primary human PBMCs, in response to a large number of environmental factors for the purpose of understanding gene-environment interactions [13]. Gene expression profiles in PBMCs from 3 healthy individuals stimulated with PHA (2.5ug/ml) with and without caffeine treatment (1.16 mM for 6 hours) were extracted. Differential expression was determined using a fold difference ≥1.5 and a p value ≤0.05 after correction for multiple testing using false discovery rate. We identified 3,437 upregulated and 2,811 downregulated genes with caffeine treatment (**Supplementary Tables 1 and 2**).

Gene ontology and pathway analysis revealed far more significant functional enrichment categories in genes downregulated compared to genes upregulated with caffeine treatment. Downregulated genes are clearly enriched in immune-related categories such as “cytokine activity” (GO:0005125) and “immune response” (GO:0006955). Likewise, when we examined disease enrichment patterns in gene downregulated with caffeine treatment we noticed downregulation of genes involved in multiple autoimmune diseases including systemic lupus erythematosus and rheumatoid arthritis with caffeine treatment (**Table 1**). Gene ontology analysis in upregulated genes pointed out “phosphotransferase activity” (GO:0016773) and “kinase activity” (GO:0016301), among other enriched gene ontologies (**Table 2**).

As we detected a strong evidence for downregulating pathways involved in many immune-related genes with caffeine treatment, we performed validation experiments in a selected number of relevant downregulated genes to examine the

dose-dependent effect of caffeine concentrations. We focused on the potential downregulatory effects of caffeine on autoimmunity and related inflammatory pathways. We selected genes within the most downregulated functional categories including “Cytokine Activity” (GO:0005125), “Immune Response” (GO:0006955), “Cytokine Signaling in Immune System” (ID:1269310), “Cytokine-cytokine Receptor Interaction” (ID: 83051) and “Lupus Erythematosus, Systemic” (ID: C0024141). We investigated the mRNA expression of MX1, STAT1, IRF5, IFNG, PPARG and TNF in PBMCs isolated from healthy normal blood donors, with and without low, intermediate, and high caffeine concentrations. These concentrations correspond to the mean value of Cmax in serum when a person drinks one cup of coffee, the maximum therapeutic dose of caffeine, and the dose used in the previous RNA sequencing experiment for the purpose of understanding gene-environment interactions, respectively.

We observed significant downregulation of STAT1, TNF, and PPARG with caffeine treatment in PBMCs (**Table 3**). A dose-dependent reduction in the expression STAT1 was observed with intermediate ($p=0.0384$) and high ($p=0.0001$) caffeine concentrations. Expression levels of TNF and PPARG were significantly reduced at all caffeine concentrations, with a dose-response effect also observed (**Figure 1**). In contrast to STAT1, mRNA expression levels of TNF and PPARG were also suppressed at low caffeine concentration (0.019 mM), which is approximately equivalent to the serum caffeine concentration after administration of one cup of coffee.

Because of enrichment of cytokine genes and cytokine activity in functional enrichment analysis of genes downregulated with caffeine treatment, we measured cytokine production levels in cell culture media from PBMCs treated with and without

caffeine. We observed significant reduction in the production of multiple inflammatory cytokines, validating gene expression patterns we observed. IL-8 levels were significantly reduced with any caffeine treatment, in a dose-dependent manner. Significant reduction in the levels of IL-6, IFNγ, TNF, MCP-1 and IL-10 was also observed at medium and high caffeine concentrations (**Figure 2**, **Table 4**).

DISCUSSION

Many autoimmune diseases accompany chronic inflammatory state which involves multiple organs and systems. According to National Institute of Allergy and Infectious Diseases, there are more than 80 autoimmune diseases ranging from mild to severe multiorgan involving forms and affecting patients' quality of life and mental status [14]. In severe forms, they can be related to increased mortality by causing end-organ failure.

Heritable genetic alterations, immune system abnormalities, and environmental elements contribute to the development and progression of many autoimmune diseases [15]. Indeed, environmental factors can influence disease susceptibility by means of epigenetics mechanisms such as DNA methylation and histone modification processes. In this regard, it is not unexpected that diet might have an important role. Recent studies have demonstrated the influence of dietary factors on autoantibody levels and disease severity in mouse models of autoimmunity [4, 16].

The present study questioned and demonstrated the *in vitro* downregulatory effects of a commonly consumed product, caffeine, on inflammation and autoimmunity related genes, pathways, and inflammatory cytokine levels.

TNF, being an important inflammatory cytokine in many immune-mediated diseases, is target of several therapeutics [17]. Previous studies indicated that TNF is involved in the release of other inflammatory cytokines such as IL-1 and IL-6 [18]. We demonstrate suppression of TNF production both at the mRNA and protein levels with low to intermediate caffeine concentrations. Indeed, caffeine

concentrations corresponding to serum levels after the consumption of one cup of coffee was associated with significant suppression of TNF production in PBMCs.

Cytokines are known to have a key role in autoimmune diseases, and several pro-inflammatory cytokines were reduced with caffeine treatment in our study. IL-6 and IL-10 which are both significantly elevated in the serum with increased disease activity in lupus patients can be downregulated with caffeine. Indeed, IL-10 is produced in increased amounts by lupus PBMCs and IL-10 levels have been shown to correlated with disease activity in lupus patients [19].

Previous studies have demonstrated higher levels of IFNγ mRNA in peripheral blood T cells from patients with lupus compared to normal controls after CD28 costimulation [20]. Moreover, in lupus monocytes IFNγ signaling has been found to be more effective in inducing STAT1 phosphorylation compared with monocytes from healthy individuals [21]. It has been also reported that lupus patients have elevated STAT1 and IFNγ expression in PBMCs and that this elevation correlates with disease activity [20, 21]. Cytokines that signal through STATs are crucial in host defense and inflammation, and overactivation of STATs can induce immune pathologies [22]. Our data demonstrate significant down regulation of both STAT1 and IFNγ with caffeine.

In summary, we demonstrate that caffeine, even with low concentrations, can suppress gene expression of pro-inflammatory genes and cytokines that play key roles in autoimmune diseases. Further work to characterize the mechanisms involved in the anti-inflammatory effects observed and whether caffeine supplementation might have beneficial effects in patients with autoimmune diseases are worthy of investigation.

METHODS

RNA sequencing data

RNA sequencing data derived from PMBCs with and without treatment with caffeine were extracted from a publically available dataset. These data were generated using PBMCs isolated from 3 healthy individuals. PBMCs were activated with PHA and then treated with or without caffeine for 6 hours at a concentration of 1.16 mM prior to RNA expression and sequencing [13].

Isolating PBMCs and cell culture

PBMCs were isolated using density gradient centrifugation (Ficoll) from buffy coats isolated from whole blood of 11 healthy blood donors and stored in liquid nitrogen. PBMCs were thawed and suspended in warm RPMI (RPMI+L-glutamine+10% FBS)/benzonase. Following resuspension, cells were centrifuged at 300xg for 10 minutes, suspended, then centrifuged again at 100xg for 10 minutes. PBMCs were then cultured in RPMI without benzonase and transferred to 6-well plates to rest overnight at 37 degrees Celsius with 5.0% CO_2. Cells were then centrifuged at 300xg for 10 minutes, then plated at 1×10^6 cells/mL in RPMI with L-Glutamine and 0.1% charcoal-stripped FBS (1-2 million cells in 1ml media in a 12-well plate). Cells were activated with PHA (2.5µg/mL) and incubated with or without caffeine for 6 hours. Three different concentration of caffeine were used: Low (0.019 mM), Intermediate (0.102 mM), and high (1.16 mM). The low dose corresponds to the mean value of Cmax in serum when a person drinks one cup of hot coffee containing 160 mg of caffeine [23]. For the intermediate dose, we used caffeine at the maximum therapeutic concentration according to Mayo Medical Laboratories (www.mayomedicallaboratories.com). The high dose was the dose used in a

previous experiment for the purpose of understanding gene-environment interactions [13]. Only the highest dose was within what is considered toxic levels of serum caffeine concentration (Mayo Medical Laboratories).

Preparation of RNA and qPCR analysis

Cells were centrifuged at 300xg for 10 minutes, and cell culture media were stored at -80 degrees Celsius for subsequent cytokine analysis. The cells were washed with PBS then TRIzol Reagent (Thermo Fisher Scientific) was added. RNA was extracted using Direct-zol RNA Isolation Kit (Zymo Research). cDNA was prepared using the Verso cDNA Synthesis Kit (Thermo Fisher Scientific). Quantitative reverse transcriptase PCR was performed using primers for human MX1, STAT1, IRF5, IFNG, PPARG, TNF and β-actin. Power SYBR Green PCR master mix (Applied Biosystems) was used for qPCR, which was run by a ViiA™ 7 Real-Time PCR System. Primers were obtained from KiCqStart® SYBR® Green Primers from Sigma and QuantiTect Primer Assays from Qiagen.

Bioinformatics analysis

Functional enrichment analysis of genes upregulated and downregulated with caffeine treatment was performed using ToppGene to identify Gene Ontology (Molecular Function and Biological Process), Pathway, and Disease annotations [24].

Cytokine analysis

Cytokines were measured using Bio-Plex Pro™ Human Cytokine 17-Plex Assay which includes MIP-1β, IL-6, IFNγ, IL-5, GM-CSF, TNF, IL-2, IL-1β, IL-13, IL-4, MCP-1, IL-8, IL-10, G-CSF, IL-7, IL-12, and IL-17.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.00 software. To compare the differences between groups, we used ANOVA with post-hoc test, with statistical significance at a P value <0.05.

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Figure Legends

Figure 1: Quantitative real time RT-PCR results for TNF, PPARG, and STAT1 in PBMCs with and without caffeine treatment *in vitro* demonstrates a dose-dependent downregulation in gene expression level. **A)** TNF mRNA expression (p=0.023, p=0.0015, and p=0.0001 for low, medium, and high caffeine concentrations compared to untreated cells, respectively) (One-way ANOVA p<0.0001). **B)** PPARG mRNA expression (p=0.0105, p=0.0007, and p=0.0002 for low, medium, and high caffeine concentrations compared to untreated cells, respectively) (One-way ANOVA p=0.0002). **C)** STAT1 mRNA expression p=0.15, p=0.0384, and p=0.0001 for low, medium, and high caffeine concentrations compared to untreated cells, respectively) (One-way ANOVA p<0.0001). **D)** IFNG mRNA expression p=0.9, p=0.63, and p=0.0054 for low, medium, and high caffeine concentrations compared to untreated cells, respectively) (One-way ANOVA p=0.011).

Figure 2: Cytokine levels measured in the supernatants of PBMCs with and without caffeine treatment. *, p-value <0.05.

Figure 1

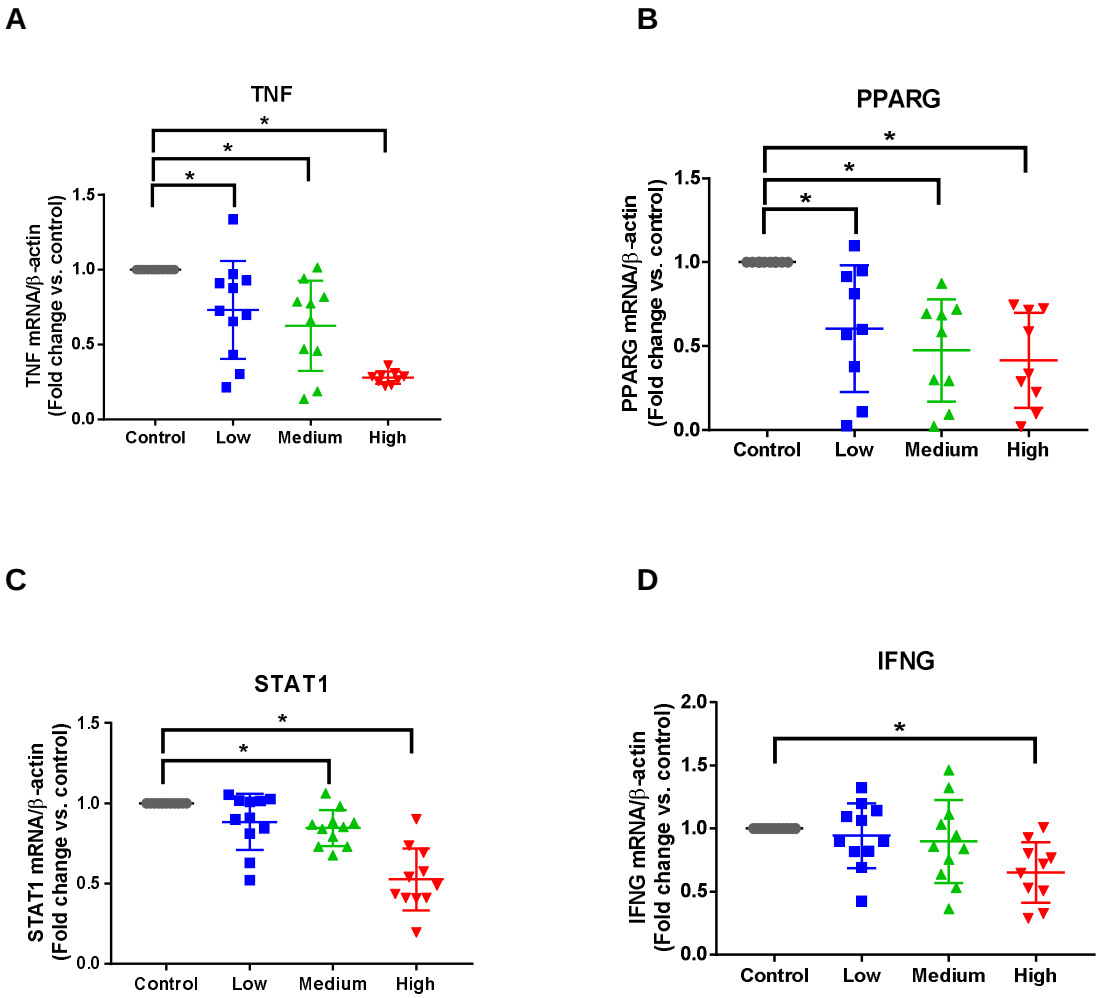

Figure 2

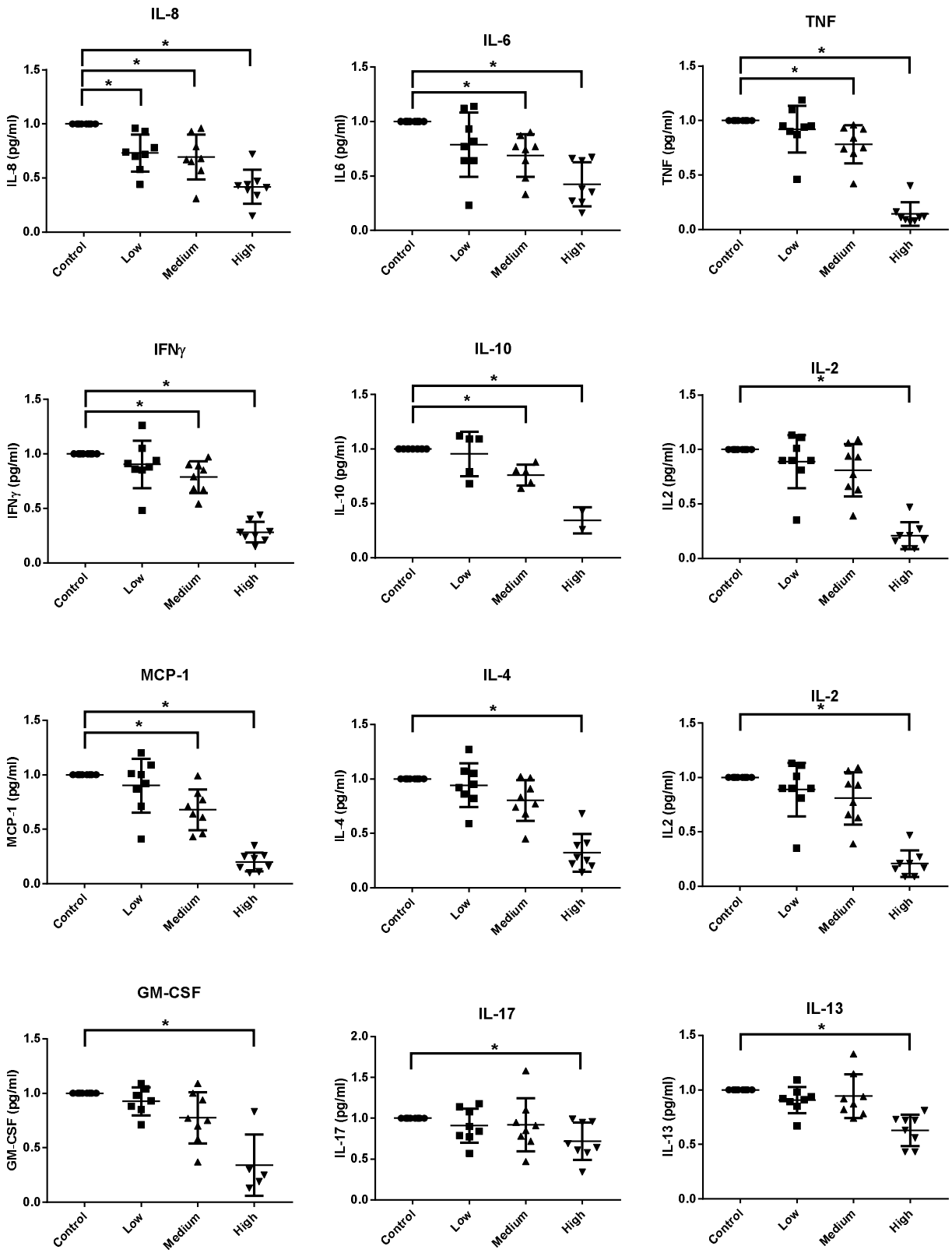

Table 1: Gene Ontology-Biological Process, Gene Ontology-Molecular Function, Pathway, and Disease enrichment analysis in genes downregulated in PBMCs with caffeine treatment *in vitro*. Only the top 10 annotations in each category are shown.

Number	ID	Name	p-value	FDR (B&H)	Genes from Input	Genes in Annotation
Gene Ontology: Biological Process						
1	GO:0006955	immune response	2.22E-27	1.96E-23	292	1572
2	GO:0006952	defense response	8.14E-26	3.59E-22	298	1651
3	GO:0042254	ribosome biogenesis	3.97E-24	1.17E-20	97	322
4	GO:0022613	ribonucleoprotein complex biogenesis	8.49E-23	1.87E-19	120	468
5	GO:0034097	response to cytokine	1.22E-22	2.15E-19	175	825
6	GO:0071345	cellular response to cytokine stimulus	1.66E-22	2.44E-19	158	713
7	GO:0045087	innate immune response	1.30E-20	1.64E-17	173	846
8	GO:0009607	response to biotic stimulus	2.51E-19	2.77E-16	195	1027
9	GO:0019221	cytokine-mediated signaling pathway	3.61E-19	3.53E-16	126	553
10	GO:0034470	ncRNA processing	1.98E-18	1.75E-15	100	399
Gene Ontology: Molecular Function						
1	GO:0003723	RNA binding	1.37E-45	3.29E-42	347	1632
2	GO:0005125	cytokine activity	3.64E-20	4.36E-17	72	222
3	GO:0005126	cytokine receptor binding	1.93E-15	1.54E-12	76	289
4	GO:0003725	double-stranded RNA binding	1.73E-11	1.04E-08	28	68
5	GO:0048020	CCR chemokine receptor binding	2.98E-11	1.42E-08	20	37
6	GO:0051082	unfolded protein binding	3.24E-09	1.29E-06	32	103
7	GO:0042379	chemokine receptor binding	5.82E-09	1.99E-06	23	60
8	GO:0008009	chemokine activity	1.05E-08	2.87E-06	20	48
9	GO:0030515	snoRNA binding	1.08E-08	2.87E-06	15	28
10	GO:0005102	receptor binding	3.46E-07	8.28E-05	221	1601
Pathway						
1	1269310	Cytokine Signaling in Immune system	1.06E-20	3.03E-17	170	763
2	1383087	rRNA modification in the nucleus and cytosol	4.26E-15	6.09E-12	32	63
3	83051	Cytokine-cytokine receptor interaction	9.60E-15	9.15E-12	75	270
4	1269311	Interferon Signaling	1.45E-14	1.04E-11	62	202
5	1269312	Interferon alpha/beta signaling	5.30E-12	3.03E-09	30	69
6	SMP00023	Steroid Biosynthesis	1.07E-10	5.10E-08	15	21
7	1270037	Cholesterol biosynthesis	1.27E-10	5.18E-08	16	24
8	142269	superpathway of cholesterol biosynthesis	3.16E-10	1.13E-07	16	25
9	PW:0000454	cholesterol biosynthetic	1.60E-09	5.08E-07	13	18
10	132956	Metabolic pathways	2.48E-09	7.08E-07	205	1272

Disease						
1	C0019693	HIV Infections	2.05E-26	2.06E-22	177	799
2	C0024299	Lymphoma	2.02E-24	1.02E-20	236	1253
3	C0021400	Influenza	5.76E-23	1.93E-19	141	610
4	C0004096	Asthma	1.20E-22	3.01E-19	218	1155
5	C0019196	Hepatitis C	1.79E-22	3.61E-19	161	751
6	C0026764	Multiple Myeloma	5.03E-20	8.42E-17	222	1241
7	C0003873	Rheumatoid Arthritis	1.59E-18	2.29E-15	268	1640
8	C0524910	Hepatitis C, Chronic	3.18E-18	4.00E-15	93	367
9	C0024141	Lupus Erythematosus, Systemic	2.01E-17	2.24E-14	176	951
10	C0023418	leukemia	2.45E-17	2.46E-14	290	1854

Table 2: Gene Ontology-Biological Process, Gene Ontology-Molecular Function, Pathway, and Disease enrichment analysis in genes upregulated in PBMCs with caffeine treatment *in vitro*. Only the top 10 annotations in each category are shown.

Number	ID	Name	p-value	FDR (B&H)	Genes from Input	Genes in Annotation
Gene Ontology: Biological Process						
1	GO:0048646	anatomical structure formation involved in morphogenesis	6.49E-09	5.61E-05	201	1299
2	GO:0000902	cell morphogenesis	2.49E-08	1.08E-04	203	1337
3	GO:0032989	cellular component morphogenesis	1.25E-07	3.38E-04	211	1430
4	GO:0060271	cilium assembly	1.81E-07	3.38E-04	57	269
5	GO:0044782	cilium organization	1.95E-07	3.38E-04	56	263
6	GO:0030030	cell projection organization	3.15E-07	4.54E-04	208	1423
7	GO:0030031	cell projection assembly	4.69E-07	5.80E-04	80	436
8	GO:0051270	regulation of cellular component movement	6.25E-07	6.75E-04	136	860
9	GO:0072359	circulatory system development	1.07E-06	9.26E-04	160	1058
10	GO:0072358	cardiovascular system development	1.07E-06	9.26E-04	160	1058
Gene Ontology: Molecular Function						
1	GO:0016773	phosphotransferase activity, alcohol group as acceptor	2.09E-07	2.81E-04	127	781
2	GO:0016301	kinase activity	2.46E-07	2.81E-04	136	853
3	GO:0035004	phosphatidylinositol 3-kinase activity	5.37E-07	4.09E-04	24	77
4	GO:0004674	protein serine/threonine kinase activity	1.49E-06	8.52E-04	80	452
5	GO:0052813	phosphatidylinositol bisphosphate kinase activity	2.30E-06	1.05E-03	22	72
6	GO:0004672	protein kinase activity	8.82E-06	3.35E-03	103	649
7	GO:0046934	phosphatidylinositol-4,5-bisphosphate 3-kinase activity	1.06E-05	3.45E-03	19	62
8	GO:0008092	cytoskeletal protein binding	2.13E-05	6.06E-03	131	886
9	GO:0004115	3',5'-cyclic-AMP phosphodiesterase activity	2.95E-05	7.48E-03	10	22
10	GO:0052742	phosphatidylinositol kinase activity	5.75E-05	1.31E-02	17	58
Pathway						
1	1269384	GAB1 signalosome	1.59E-07	3.80E-04	34	128
2	1269188	PIP3 activates AKT signaling	2.81E-07	3.80E-04	33	125

3	1269473	PI3K/AKT activation	5.10E-07	4.59E-04	33	128
4	1268879	PI3K/AKT Signaling in Cancer	7.86E-07	4.86E-04	26	90
5	1269302	Role of LAT2/NTAL/LAB on calcium mobilization	8.98E-07	4.86E-04	34	137
6	921162	FoxO signaling pathway	3.22E-06	1.45E-03	32	132
7	1268880	Constitutive Signaling by Aberrant PI3K in Cancer	1.96E-05	7.17E-03	19	65
8	1269191	Negative regulation of the PI3K/AKT network	2.12E-05	7.17E-03	24	94
9	83048	MAPK signaling pathway	2.81E-05	7.60E-03	48	255
10	868085	Ras signaling pathway	2.88E-05	7.60E-03	44	227
Disease						
1	C3495559	Juvenile arthritis	4.73E-10	4.59E-06	63	283
2	C1458155	Mammary Neoplasms	6.13E-09	2.98E-05	267	1953
3	C0175754	Agenesis of corpus callosum	5.10E-07	1.65E-03	55	280
4	C0686619	Secondary malignant neoplasm of lymph node	6.42E-06	1.26E-02	174	1283
5	C0009319	Colitis	7.74E-06	1.26E-02	89	569
6	C0040100	Thymoma	7.80E-06	1.26E-02	41	204
7	C0282160	Aplasia Cutis Congenita	9.92E-06	1.38E-02	39	192
8	C0598766	Leukemogenesis	1.42E-05	1.73E-02	108	734
9	C0023467	Leukemia, Myelocytic, Acute	1.98E-05	2.13E-02	212	1646
10	C4277690	Ciliopathies	2.22E-05	2.16E-02	23	92

Table 3: Quantitative real time RT PCR results in genes downregulated with caffeine by RNA sequencing data and selected for confirmation using different caffeine concentrations. The fold difference ± standard deviation is listed relative to untreated cells and the P values were adjusted using Dunnett's multiple comparisons test.

	Expression levels with caffeine treatment compared to untreated cells					
	Low		Medium		High	
Gene	Fold change	Adjusted p value	Fold change	Adjusted p value	Fold change	Adjusted p value
TNF	0.731±0.326	**0.023**	0.625±0.302	**0.0015**	0.279±0.042	**0.0001**
PPARG	0.605±0.377	**0.011**	0.474±0.303	**0.0007**	0.415±0.283	**0.0002**
STAT1	0.885±0.175	0.15	0.845±0.112	**0.038**	0.527±0.193	**0.0001**
MX1	1.151±0.238	0.54	1.033±0.249	0.99	1.336±0.524	**0.043**
IRF5	0.769±0.24	0.14	0.896±0.34	0.7	0.949±0.343	0.94
IFNG	0.944±0.256	0.9	0.898±0.328	0.63	0.651±0.24	**0.0054**

Table 4: Cytokine levels measured in the supernatants of PBMCs, using different caffeine concentrations. The fold difference ± standard deviation is listed relative to untreated cells and the P values were adjusted using Dunnett's multiple comparisons test.

Cytokine levels with caffeine treatment compared to untreated cells						
	Low		**Medium**		**High**	
	fold change	p value	fold change	p value	fold change	p value
MIP-1β	0.98±0.14	0.96	0.88±0.08	0.056	0.41±0.09	**0.0001**
IL-6	0.79±0.28	0.11	0.69±0.18	**0.013**	0.42±0.19	**0.0001**
IFNγ	0.9±0.21	0.39	0.79±0.14	**0.013**	0.28±0.09	**0.0001**
IL-5	1±0.14	1.00	0.88±0.11	0.26	_	
GM-CSF	0.93±0.12	0.77	0.77±0.22	0.055	0.34±0.25	**0.0001**
TNF	0.92±0.20	0.58	0.78±0.17	**0.018**	0.14±0.10	**0.0001**
IL-2	0.89±0.23	0.48	0.810.22	0.11	0.21±0.12	**0.0001**
IL-1β	0.72±0.22	0.18	0.78±0.34	0.34	0.79±0.39	0.38
IL-13	0.94±0.11	0.39	1.15±0.19	0.75	0.43±0.13	**0.0001**
IL-4	0.94±0.19	0.81	0.8±0.18	0.053	0.32±0.16	**0.0001**
MCP-1	0.9±0.23	0.48	0.68±0.18	**0.0012**	0.2±0.08	**0.0001**
IL-8	0.73±0.16	**0.0049**	0.69±0.20	**0.0015**	0.42±0.15	**0.0001**
IL-10	0.95±0.18	0.85	0.76±0.09	**0.01**	0.35±0.09	**0.0001**
G-CSF	0.96±0.15	0.95	1.04±0.22	0.95	1.11±0.17	0.51
IL-7	0.95±0.18	0.89	1.15±0.24	0.24	1.01±0.11	1.00
IL-17	0.91±0.20	0.76	0.92±0.30	0.82	0.72±0.22	**0.049**